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ORIGINAL ARTICLE

Association between oestrogen receptors and female temporomandibular disorders

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ABSTRACT

Objective: To evaluate if temporomandibular disorders (TMDs) are associated with genetic polymorphisms in *ESR1* and *ESR2*, which are genes encoding oestrogen receptor alpha (*ERα*) and beta (*ERβ*). Also, we included an animal model to check if *ERα* and *ERβ* are expressed in the temporomandibular joint (TMJ) during adolescence.

Materials and methods: A total of 139 teenagers and 93 adults were diagnosed according to the *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMDs). The DNA was collected and the markers *ESR1* and *ERS2* were genotyped. Additionally, immunohistochemistry was performed in TMJ tissues from female *Wistar* rats during puberty. All data were submitted to statistical analysis with confidence interval of 95%.

Results: Teenagers presented more disc displacement and arthralgia than adults ($p < .05$). The genetic polymorphism *rs1256049* in *ESR2* was associated with disc displacement ($p = .040$; OR = 10.50/95%CI 1.17–98.74) and arthralgia ($p = .036$; OR = 7.20/95%CI 1.10–46.88) in adults. The *ERα* and *ERβ* are expressed in rat TMJ tissues.

Conclusions: We provide evidence that *ESR2* is associated with TMD and could be a genetic marker for this condition in adult women. Furthermore, oestrogens receptors are presented in TMJ of adolescent female rats.

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Introduction

Temporomandibular disorders (TMDs) are referred to as a set of clinical conditions classified by pain, joint click and dysfunction [1,2]. Studies suggest that the prevalence of TMD in women is 1.5–2 times higher than in men, and that 80% of treated patients are women [2,3]. The severity of symptoms is also related to age and is more prevalent in women during the reproductive years, between 20 and 40 years, and less in children, adolescents and elderly [2,4,5]. The association between sex and age for the onset of TMD suggests a possible link to female reproductive hormones, especially oestrogens [2,5,6].

Oestrogens regulate various physiological processes, including cell growth, reproduction, differentiation and development. They also consist of three functional domains: the NH_2-terminal domain, the DNA-binding domain and the COOH-terminal ligand-binding domain. Cellular signalling of oestrogens is mediated through oestrogens receptors (ERs), which have two forms: α and β [7]. The oestrogen receptor alpha (*ERα*) is codified by *ESR1* gene, while oestrogen receptor beta (*ERβ*) is codified by *ESR2* gene. The receptor *ERα* acts as a regulator of intracellular mediators and is found in cartilage tissue, intra-articular osteocytes [8,9] and in mandibular condylar fibrocartilage [10–12]. *ERα* is expressed in all cells of the mandibular condylar fibrocartilage demonstrating the possible importance that this receptor must play in oestrogen signalling in the temporomandibular joint (TMJ) [10]. The *ERβ* is found in the ovary, central nervous system, cardiovascular system, lung, male reproductive organs, prostate, colon, kidney, skeletal and immune system. *ERβ* secretes endocrine factors that support bone development or remod-

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Supplemental data for this article can be accessed here.

elling [2,13,14]. Additionally, previous studies indicated that *ERβ* mediated oestrogen's role on condylar fibrocartilage cell proliferation [15].

Some studies have shown the presence of oestrogen receptors, α and β, and a possible effect of oestrogen through these receptors in the TMJ of animal models [10–12,15] and humans [8,16–18]. However, the genetic aspects of oestrogen receptors on TMD aetiology are still poorly understood. Therefore, this study evaluated TMD in teenage girls and adult women, in order to investigate if genetic polymorphisms in *ESR1* and *ESR2* are involved in this condition. Also, we used animal model to check if ERα and ERβ are expressed in the TMJ during adolescence.

Materials and methods

Sample

This is a convenience cross-sectional study that recruited 232 females from two subsets in 2017 and 2018, in Curitiba-Brazil. One subset included 139 teenagers, age ranging from 10 to 14 years old. The second subset included 93 adults, age ranging from 18 to 50 years old. Males and syndromic individuals were not included in the study. All participants and/or legal guardians were informed about the project and read, signed and dated the informed consent document before taking part of this study. STrengthening the REporting of Genetic Association studies (STREGA) criteria were used to report the data. The project was approved by both universities committee (CAEE: 80846317.8.0000.0093 and CAEE: 2006.086).

The teenage sample was defined according to a random number generator website (www.randomizer.org), based on a population representative sample of adolescents evaluated in public schools from Curitiba-Brazil. The characteristics of this population were previously described [19]. The self-reported age of menarche was obtained through a questionnaire with the legal guardians.

The adult women sample comprised consecutive patients who sought for a treatment at the Oral and Maxillofacial Surgery Services at Positivo University and at Federal University of Paraná in Curitiba, Brazil. The characteristics of this sample were previously published [20].

Clinical assessment

The epidemiological data of patients were assessed and the patients were evaluated about the signs and symptoms of TMD using the *Research Diagnostic Criteria for Temporomandibular Disorders* (RDC/TMDs) [21]. The individuals were diagnosed according to the RDC/TMD subgroups as follows: myofascial pain with or without limited opening mouth, disc displacement and arthralgia. Patients were classified according to the presence or absence of each of these phenotypes.

Table 1. Candidate studied genes and polymorphisms.

Gene	Position	Genetic polymorphism	MAF[a]	Mutant allele
ESR1	6q25.1	*rs2234693*[b]	0.446	C
		rs9340799[c]	0.281	G
ESR2	14q23.2	*rs1256049*	0.129	G
		rs4986938	0.259	G

Obtained from database: ncbi.nlm.nih.gov.
[a]MAF means minor allele frequency.
[b]Also known as Pvull.
[c]Also known as Xbal.

DNA samples and ESR1 and ESR2 genotyping

The DNA was obtained from buccal mucosa epithelial cells by a 5 ml rinse of 3% glucose solution for two minutes and scraped the oral mucosa with a sterilized wooden spatula [22] and purified with ammonium acetate at 10 M and 1 mM EDTA [23]. The genetic polymorphisms of *ESR1* (*rs2234693* and *rs9340799*) and *ERS2* (*rs1256049* and *rs4986938*) were blinded genotyping using the real-time PCR technique StepOne™ Real-time PCR System using TaqMan™ Technology (Applied Biosystems, Foster City, CA) [24]. The studied polymorphisms characteristics are presented in Table 1.

Immunohistochemistry of ERα and ERβ in rats

All experiments were performed in accordance with animal welfare based on an approved Institutional Animal Care and Use Committee protocol (2014.1.721.58.7) from the University of São Paulo. In order to confirm if *ERα* and *ERβ* are expressed in the TMJ tissues of females undergoing to the puberty, 20 female *Wistar* rats were used as a model. The animals were euthanized at 45 days old, period related to the middle of the rat's puberty, that range from the 35° to the 55° days old [25]. Therefore, the mandibular condyles were dissected for immunohistochemical analysis. The mandibular condyles were fixed in 10% formalin for 24 hours at room temperature, in sequence, washed for four hours in running water. Then, decalcified in 4.13% ethylenediaminetetraacetic acid (EDTA) (pH 7–7.4) for 50 days. Subsequently, the samples were submitted to the histotechnical processing, they were washed in running water for two hours, dehydrated through progressive concentrations of ethanol (70% and 95% per 30 minutes each; two exchanges of 100% per 20 minutes each and two exchanges of 100% per 40 minutes each), diafined in xylene (two washes of 20 minutes and one of 40 minutes), and embedded in paraffin. Sagittal serial sections of 5 μm thickness were made of the TMJ (mandibular condyle) using a microtome (Leica RM2145; Leica Microsystems GmbH, Wetzlar, Germany).

For the immunohistochemical reactions, the slides were hydrated and treated with hydrogen peroxide (5%). For ERα and ERβ epitope retrieval, tissue specimens required pressure cooker pre-treatment in 10 mM, pH 6.0 sodium citrate buffer. Sections were successively incubated with the primary antibodies against ERα (clone 2Q418, 1:200 dilution; Santa Cruz Biotechnology Cat# sc-71064, Santa Cruz, CA) and ERβ (clone B-1, 1:200 dilution; Santa Cruz Biotechnology Cat# sc-390243, Santa Cruz, CA). After incubation with the primary antibody,

Table 2. Characteristics of both samples.

Phenotypes	Teenage girls ($n = 139$)	Adult women ($n = 93$)	p Value
Age (mean ± SD)	11.24 ± 1.12	31.73 ± 9.87	–
Mouth opening (mean ± SD)	50.52 ± 5.52	47.57 ± 7.50	.99[a]
Myofascial pain, *n* (%)	101 (72.7)	57 (61.3)	.06[b]
Without myofascial pain, *n* (%)	38 (27.3)	36 (38.7)	
Disc displacement, *n* (%)	114 (82.0)	65 (69.9)	**.03**[b]
Without disc displacement, *n* (%)	25 (18.0)	28 (30.1)	
Arthralgia, *n* (%)	125 (89.9)	75 (80.6)	**.04**[b]
Without arthralgia, *n* (%)	14 (10.1)	18 (19.4)	

Bold value indicates significance level.
OR: odds ratio; CI: confidence interval.
[a]*t* test was used.
[b]Chi-square was used.

secondary antibodies conjugated with streptavidin-biotin-peroxidase (K0690; Universal Dako LSAB®+ Kit, Peroxidase, Carpinteria, CA), with diaminobenzidine and counterstained with Carazzi's haematoxylin, were used. Negative control specimens included replacing the primary antibody with isotype-specific serum. Microscopic analysis was performed by senior and calibrated evaluator, using an AXIO IMAGER.M1 microscope (Carl Zeiss, Jena, Germany) coupled to an AXIOCAM MRc5 camera (Carl Zeiss, Jena, Germany). The results were expressed considering the presence (cellular localization) or absence of the immunomarkers.

Table 3. Genotype distribution according to TMD phenotype in teenage girls.

Gene, polymorphism and genotypes		*n* (%)	*n* (%)	*p* Value	OR (CI 95%)
		Myofascial pain	Without myofascial pain		
ESR1 rs2234693					
	CC	7 (22.6)	16 (17.8)	.184	1.12 (0.94–1.34)
	CT	18 (58.1)	42 (46.7)	.091	1.12 (0.98–1.28)
	TT	6 (19.4)	32 (35.6)		Reference
ESR1 rs9340799					
	AA	9 (36.0)	37 (51.4)	.336	0.89 (0.71–1.11)
	AG	12 (48.0)	27 (37.5)	.868	0.98 (0.78–1.23)
	GG	4 (16.0)	8 (11.1)		Reference
ESR2 rs1256049					
	AA	23 (85.2)	72 (87.8)		Reference
	AG	4 (14.8)	10 (12.2)	.731	1.03 (0.85–1.26)
	GG	0 (0%)	0 (0%)	–	–
ESR2 rs4986938					
	AA	20 (60.6)	43 (49.4)	.414	1.09 (0.87–1.37)
	AG	11 (33.3)	36 (41.4)	.811	1.02 (0.81–1.29)
	GG	2 (6.1)	8 (9.2)		Reference
		Disk displacement	Without disk displacement		
ESR1 rs2234693					
	CC	4 (20.0)	19 (18.8)	.457	1.06 (0.90–1.24)
	CT	12 (60.0)	48 (47.5)	.187	1.08 (0.96–1.22)
	TT	4 (20.0)	34 (33.7)		Reference
ESR1 rs9340799					
	AA	7 (38.9)	39 (49.4)	.459	0.92 (0.74–1.14)
	AG	8 (44.4)	31 (39.2)	.747	0.96 (0.77–1.20)
	GG	3 (16.7)	9 (11.4)		Reference
ESR2 rs1256049					
	AA	13 (81.2)	82 (88.2)		Reference
	AG	3 (18.8)	11 (11.8)	.490	1.06 (0.88–1.28)
	GG	0 (0%)	0 (0%)	–	–
ESR2 rs4986938					
	AA	14 (63.6)	49 (50.0)	.274	1.11 (0.92–1.34)
	AG	7 (31.8)	40 (40.8)	.655	1.04 (0.86–1.26)
	GG	1 (4.5)	9 (9.2)		Reference
		Arthralgia	Without arthralgia		
ESR1 rs2234693					
	CC	3 (27.3)	20 (18.2)		Reference
	CT	1 (9.1)	59 (53.6)	.099	0.89 (0.79–1.02)
	TT	7 (63.6)	31 (28.2)	.570	1.04 (0.89–1.23)
ESR1 rs9340799					
	AA	5 (83.3)	41 (45.1)	.983	0.99 (0.81–1.22)
	AG	0 (0.0)	39 (42.9)	.264	0.90 (0.74–1.08)
	GG	1 (16.7)	11 (12.1)		Reference
ESR2 rs1256049					
	AA	10 (90.9)	85 (86.7)		Reference
	AG	1 (9.1)	13 (13.3)	.697	0.697
	GG	0 (0%)	0 (0%)	–	–
ESR2 rs4986938					
	AA	5 (38.5)	58 (54.2)		Reference
	AG	6 (46.2)	41 (38.3)	.812	0.99 (0.91–1.07)
	GG	2 (15.4)	8 (7.5)	.149	1.19 (0.93–1.53)

OR: odds ratio; CI: confidence interval.

Statistical analysis

The data were analysed using the computer program IBM SPSS (Statistical Package for Social Science) (Armonk, NY), v.24.0 software. Quantitative variables were described by mean and standard deviation (SD). Qualitative variables were described by frequencies and percentages. The association between genotypes and the groups was assessed by logistic regression model or Fisher's exact test. Chi-square was used to calculate Hardy–Weinberg's equilibrium. Haplotype analysis were calculated using the PLINK program [26]. The level of significance was set at $p < .05$.

Immunohistochemical findings from the animal experiment were analysed qualitatively.

Results

Table 2 shows the sample characteristics and clinical variables distributions between teenage girls and adult women groups. Teenagers presented more disc displacement and arthralgia than adults ($p < .05$).

In the teenage group, the menarche had already started in 44 (31.7%) girls. Myofascial pain, disc displacement and arthralgia were not associated with the menarche, in the comparison performed between girls who already

Table 4. Genotype distribution according to TMD phenotype in adult women.

Gene, polymorphism and genotypes		*n* (%)	*n* (%)	*p* Value	OR (95% CI)
		Myofascial pain	Without myofascial pain		
ESR1 rs2234693					
	CC	0 (0)	6 (10.7)	–	–
	CT	18 (50.0)	30 (53.6)	.082[a]	1.66 (0.81–1.82)
	TT	18 (50.0)	20 (35.7)		Reference
ESR1 rs9340799					
	AA	24 (66.7)	27 (48.2)		Reference
	AG	12 (33.3)	28 (50.0)	.075[a]	1.12 (0.55–1.05)
	GG	0 (0)	1 (1.8)	–	–
ESR2 rs1256049					
	AA	32 (88.9)	55 (98.2)		Reference
	AG	4 (11.1)	1 (1.8)	.091	6.87 (0.73–64.2)
	GG	0 (0)	0 (0)	–	–
ESR2 rs4986938					
	AA	16 (48.5)	27 (49.1)		Reference
	AG	14 (42.4)	22 (40.0)	.878	1.01 (0.86–1.18)
	GG	3 (9.1)	6 (10.9)	.825	0.97 (0.75–1.25)
		Disk displacement	Without disk displacement		
ESR1 rs2234693					
	CC	2 (7.1)	4 (6.2)		Reference
	CT	14 (50.0)	34 (53.1)	.836	0.96 (0.71–1.30)
	TT	12 (42.9)	26 (40.6)	.932	0.98 (0.72–1.33)
ESR1 rs9340799					
	AA	19 (67.9)	32 (50.0)		Reference
	AG	8 (28.6)	32 (50.0)	.059[a]	
	GG	1 (3.6)	0 (0)		
ESR2 rs1256049					
	AA	24 (85.7)	63 (98.4)		Reference
	AG	4 (14.3)	1 (1.6)	**.040**	**10.5 (1.17–98.74)**
	GG	0 (0%)	0 (0%)	–	–
ESR2 rs4986938					
	AA	18 (64.3)	25 (41.7)		Reference
	AG	8 (28.6)	28 (46.7)	.055	0.86 (0.74–1.00)
	GG	2 (7.1)	7 (11.7)	.234	0.86 (0.67–1.10)
		Arthralgia	Without arthralgia		
ESR1 rs2234693					
	CC	2 (11.1)	4 (5.4)		Reference
	CT	9 (50.0)	39 (52.7)	.446	0.89 (0.66–1.20)
	TT	7 (38.9)	31 (41.9)	.441	0.88 (0.65–1.20)
ESR1 rs9340799					
	AA	12 (66.7)	39 (52.7)		Reference
	AG	5 (27.8)	35 (47.3)	.142	0.46 (0.28–1.34)
	GG	1 (5.6)	0 (0%)	–	
ESR2 rs1256049					
	AA	15 (83.3)	72 (97.3)		Reference
	AG	3 (16.7)	2 (2.7)	**.036**	**7.20 (1.10–46.88)**
	GG	0 (0%)	0 (0%)	–	–
ESR2 rs4986938					
	AA	9 (52.9)	34 (47.9)		Reference
	AG	7 (41.2)	29 (40.8)	.870	0.98 (0.85–1.14)
	GG	1 (5.9)	8 (11.3)	.430	0.91 (0.74–1.13)

Logistic regression. Bold form indicates significance level.
OR: odds ratio; CI: confidence interval.
[a]Fisher's exact test.
[a]The bold values signify $p<0.05$.

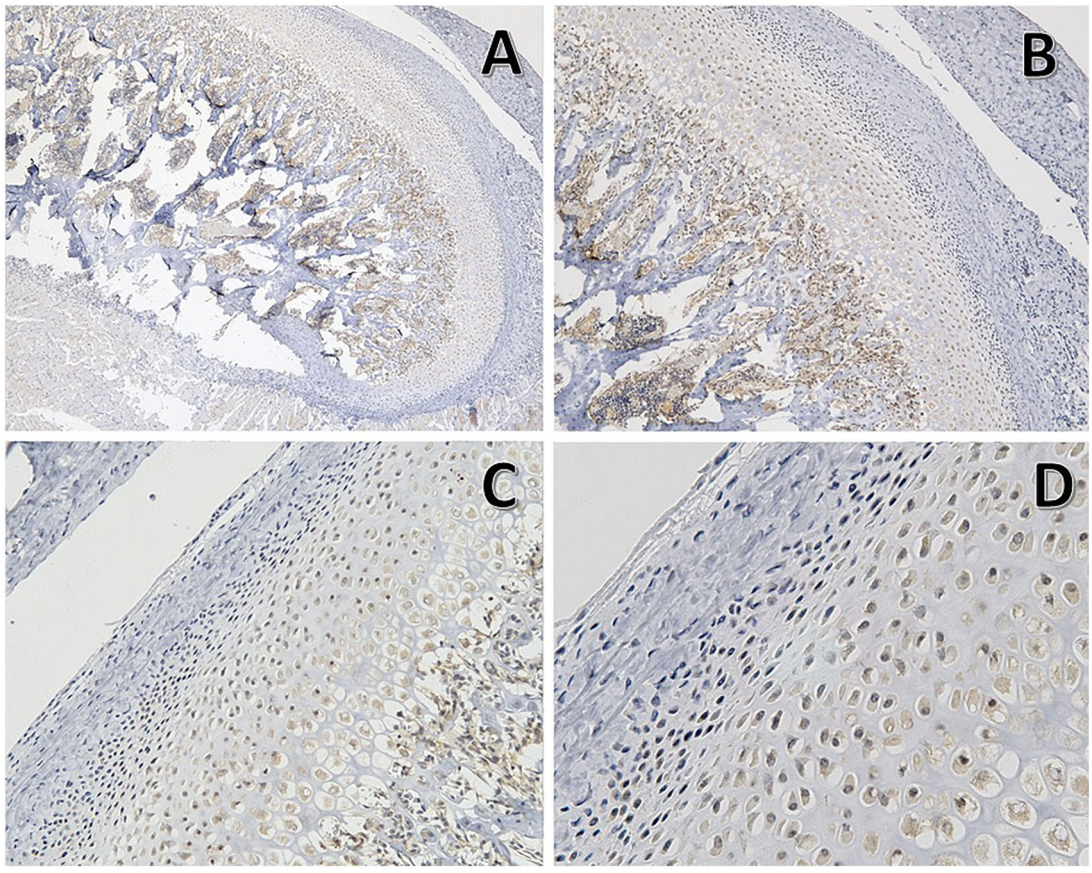

Figure 1. ERα immunohistochemistry performed on the mandibular condyles of 45-day-old female rat. Photomicrographs of the immunohistochemical findings using ERα antibody on the mandibular condyle of 45-day-old Wistar rat, in a sagittal section. (A,B) Overview of condyle under ×5 and ×10 magnification, respectively. (C) Enlargement of the image presented in (A), ×20 magnification. (D) Enlargement of the image present in the (C) evidencing ERα expression in the cytoplasm and nuclei of multiple chondrocytes and fibroblast-like cells of the articular cartilage and synovium, respectively, ×40 magnification.

experienced menarche and girls that did not experience the menarche ($p > .05$).

The genetic polymorphisms are in Hardy–Weinberg's equilibrium (data not shown). Table 3 presents the genotype distribution according to TMD phenotypes in teenagers. A statistical significance difference was not observed in none of the analysis ($p > .05$).

Table 4 presents genotypes distribution according to TMD phenotype in adults. Only the genetic polymorphism *rs1256049* in *ESR2* was associated with TMD phenotypes. To carry the mutant allele (AG genotype) increased the risk of disc displacement ($p = .040$; OR = 10.50/95%CI 1.17–98.74) and arthralgia ($p = .036$; OR = 7.20/95%CI 1.10–46.88).

The haplotype analyses in *ESR1* and *ESR2* were not statistically significant associated neither in teenagers nor in adults ($p > .05$).

In the animal model, Figure 1 demonstrates the immunohistochemical findings for *ERα*, which demonstrated immunopositivity for this marker in the TMJ tissues during adolescence. *ERα* could be observed mainly in the condylar cartilage (chondrocytes), synovium (fibroblast-like cells), on the haematopoietic cells and bone trabeculae. Figure 2 demonstrates the immunohistochemical findings for *ERβ*, which showed results similar to *ERα*.

Discussion

The overarching goal of this study was to evaluate if genetic polymorphisms in *ESR1* and *ESR2* are associated with signs and symptoms of TMD in teenagers and adult females. There are evidence for the sex predilection of TMD [2,3], which raised the possibility that oestrogen and its receptors could be involved in TMD aetiology. Also, the link between oestrogen deprivation in post-menopausal women and TMD highlights the role of oestrogen may play in homeostasis of the TMJ tissues [12]; however, in the present study, the menarche was not associated with TMD. In our study, adults presented more TMD signs and symptoms than teenagers. Another fact that corroborates with the role of oestrogen on TMD was demonstrated by a recent study with animal model, which showed that oestrogen replacement treatment promotes TMJ chondrogenesis [12,15,27]. Also, sex hormones receptors, including oestrogen receptors, were demonstrated as expressed in adult human TMJ discs [16].

Genetic polymorphisms in *ESR1* [8,14,17,18,28–32] and *ESR2* [28,31] have been investigated in some head and face painful conditions, including migraine [28,29,31]. Moreover, in the past decade, genetic polymorphisms in *ESR1* have been explored and associated with TMD conditions. Stemig et al. studied the polymorphisms *rs2234693* and *rs9340799* in

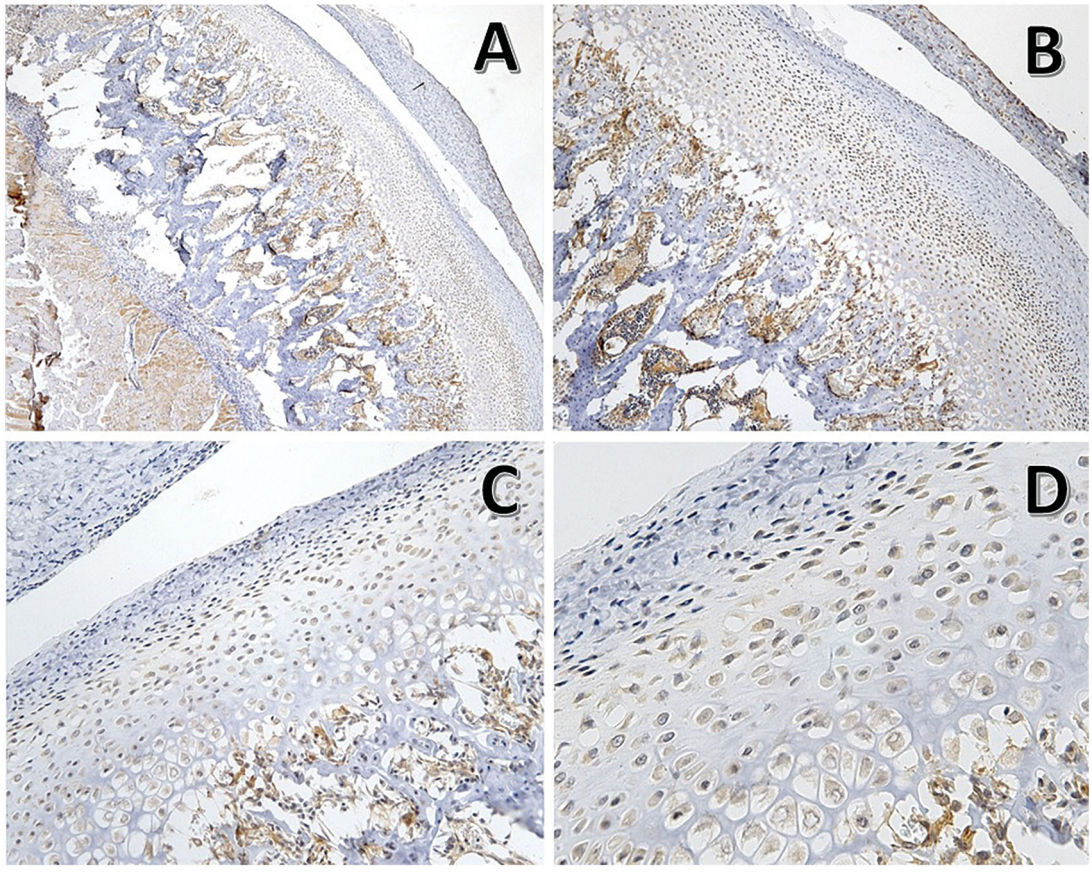

Figure 2. ERβ immunohistochemistry performed on the mandibular condyles of 45-day-old female rat. Photomicrographs of the immunohistochemical findings using ERβ antibody on the mandibular condyle of 45-day-old Wistar rat, in a sagittal section. (A, B) Overview of condyle under ×5 and ×10 magnification, respectively. (C) Enlargement of the image present in (A), ×20 magnification. (D) Enlargement of the image present in (C) evidencing ERβ expression in the cytoplasm and nuclei of multiple chondrocytes in the articular cartilage and fibroblast-like cells in the synovium, ×40 magnification.

ESR1, which are the same polymorphisms evaluated here, and found that these both polymorphisms were associated with degenerative joint disease of the TMJ [8]. The haplotype of these same polymorphisms was also associated with pain susceptibility in female symptomatic temporomandibular osteoarthritis [14] and with painful and non-painful TMD in women [17]. However, the study performed by Kim et al. was not able to confirm this haplotype as a statistically significant marker of TMD symptoms risk [9]. Although the other genetic polymorphisms studied in *ESR1* were not statistically associated with TMD in our study, a borderline association was observed for disc displacement and myofascial pain forms the polymorphism *rs223469* in adults. It is possible that a statistical significance was not observed for the polymorphism *rs2234693* and for the haplotype *rs223469-rs9340799* due to the sample size, and this could be a false negative.

Other genetic polymorphisms in *ESR1* (*rs12154178*, *rs1884051*, *rs2273206*, *rs7774230*) were also investigated in the TMD aetiology [18,32]. The genetic polymorphism *rs2273206* was associated with an increased risk to develop muscle TMJ pain in Brazilian adults [18]. The genetic polymorphism *rs1643821* in *ESR1* was also associated with postoperative TMD [32].

To the best of our knowledge, this is the first research to investigate if genetic polymorphisms in *ESR2* are associated with TMD. The genetic polymorphism *rs1256049* in *ESR2* was associated with disc displacement and arthralgia in adults and may be a marker for these conditions. The gene *ESRRB* (oestrogen related receptor beta) was associated with TMD in Brazilians [18,33]. *ERβ* can replace some of *ERα*'s roles in its absence [34]. Previous studies indicated that *ERβ* mediates oestrogen's role on mandibular condylar fibrocartilage cell proliferation but not the chondrogenic matrix effects, which suggests the role of *ERα* in oestrogen-mediated chondrogenesis [15].

It is important to highlight here that the association between *ESR1* and TMD was observed for the adult subset, but not for the teenage subset. For this reason, we decided to confirm that *ERα* and *ERβ* are expressed in the TMJ during the adolescence, and, in fact, these proteins are expressed in these tissues. Therefore, it is possible that the lack of association in the teenagers were not related to the absence of expression of these receptors in the TMJ cells. Results from previous studies showed that cells of the mandibular condylar fibrocartilage respond to oestradiol resulting in an increase in chondrogenesis [15,27]. However, it is reasonable to hypothesize that TMD has an intricate aetiology that

includes the interaction between oestrogen-oestrogens receptors-aging.

Although *ERα* and *ERβ* are expressed in the TMJ during the puberty in rats, the genetic polymorphisms in genes encoding these receptors were not associated with TMD in teenagers. The genetic polymorphism *rs1256049* in *ESR2* was associated with arthralgia and disc displacement, and could be a genetic marker for these conditions in adult women.

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Disclosure statement

The authors declare that they have no conflicts of interest.

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References

[1] Bi RY, Ding Y, Gan YH. A new hypothesis of sex-differences in temporomandibular disorders: estrogen enhances hyperalgesia of inflamed TMJ through modulating voltage-gated sodium channel 1.7 in trigeminal ganglion? Med Hypotheses. 2015;84(2):100–103.

[2] Wang J, Chao Y, Wan Q, et al. The possible role of estrogen in the incidence of temporomandibular disorders. Med Hypotheses. 2008;71(4):564–567.

[3] Alimy-Allrath T, Ricken A, Bechmann I. Expression of estrogen receptors α and β in the trigeminal mesencephalic nucleus of adult women and men. Ann Anat. 2014;196(6):416–422.

[4] Meloto CB, Bortsov AV, Bair E, et al. Modification of COMT-dependent pain sensitivity by psychological stress and sex. Pain. 2016;157(4):858–867.

[5] da Silva CG, Pachêco-Pereira C, Porporatti AL, et al. Prevalence of clinical sings of intra-articular temporomandibular disorders in children and adolescents: a systematic review and meta-analyses. J Am Dent Assoc. 2016;147(1):10–18.e8.

[6] Berger M, Szalewski L, Bakalczuk M, et al. Association between estrogen levels and temporomandibular disorders: a systematic literature review. Prz Menopauzalny. 2015;14:260–270.

[7] Jia M, Dahlman-Wright K, Gustafsson JÅ. Estrogen receptor alpha and beta in health and disease. Best Pract Res Clin Endocrinol Metab. 2015;29(4):557–568.

[8] Stemig M, Myers SL, Kaimal S, et al. Estrogen receptor-alpha polymorphism in patients with and without degenerative disease of the temporomandibular joint. Cranio. 2015;33(2):129–133.

[9] Kim BS, Kim YK, Yun PY, et al. The effects of estrogen receptor α polymorphism on the prevalence of symptomatic temporomandibular disorders. J Oral Maxillofac Surg. 2010;68(12):2975–2979.

[10] Yamada K, Nozawa-Inoue K, Kawano Y, et al. Expression of estrogen receptor alpha [ER alpha] in the rat temporomandibular joint. Anat Rec. 2003;274:934–941.

[11] Robinson JL, Cass K, Aronson R, et al. Sex differences in the estrogen-dependent regulation of temporomandibular joint remodeling in altered loading. Osteoarthritis Cartilage. 2017;25(4):533–543.

[12] Robinson JL, Gupta V, Soria P, et al. Estrogen receptor alpha mediates mandibular condylar cartilage growth in male mice. Orthod Craniofac Res. 2017;20:167–171.

[13] Böttner M, Thelen P, Jarry H. Estrogen receptor beta: tissue distribution and the still largely enigmatic physiological function. J Steroid Biochem Mol Biol. 2014;139:245–251.

[14] Kang SC, Lee DG, Choi JH, et al. Association between estrogen receptor polymorphism and pain susceptibility in female temporomandibular joint osteoarthritis patients. Int J Oral Maxillofac Surg. 2007;36(5):391–394.

[15] Chen J, Kamiya Y, Polur I, et al. Estrogen via estrogen receptor beta partially inhibits mandibular condylar cartilage growth. Osteoarthritis Cartilage. 2014;22(11):1861–1868.

[16] Abubaker AO, Raslan WF, Sotereanos GC. Estrogen and progesterone receptors in temporomandibular joint discs of symptomatic and asymptomatic persons: a preliminary study. J Oral Maxillofac Surg. 1993;51(10):1096–1100.

[17] Ribeiro-Dasilva MC, Peres Line SR, Leme Godoy dos Santos MC, et al. Estrogen receptor-alpha polymorphisms and predisposition to TMJ disorder. J Pain. 2009;10(5):527–533.

[18] Quinelato V, Bonato LL, Vieira AR, et al. Association between polymorphisms in the genes of estrogen receptors and the presence of temporomandibular disorders and chronic arthralgia. J Oral Maxillofac Surg. 2018;76(2):314.e1–314.e9.

[19] Bertoli FMP, Bruzamolin CD, Pizzatto E, et al. Prevalence of diagnosed temporomandibular disorders: a cross-sectional study in Brazilian adolescents. *PLoS One*. 2018;13(2):e0192254.

[20] Cunha A, Nelson-Filho P, Marañón-Vásquez GA, et al. Genetic variants in ACTN3 and MYO1H are associated with sagittal and vertical craniofacial skeletal patterns. Arch Oral Biol. 2018;97:85–90.

[21] Dworking SF, Lereshe L. Research diagnostic criteria for temporomandibular disorders: review, criteria, examinations and specifications, critique. J Cranio Mandib Disord. 1992;6:301–355.

[22] Trevilatto PC, Line SR. Use of buccal epithelial cells for PCR amplification of large DNA fragments. J Forensic Odontostomatol. 2000;18(1):6–9.

[23] Aidar M, Line SR. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. Braz Dent J. 2007;18(2):148–152.

[24] Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. Genome Res. 2001;11(7):1262–1268.

[25] Ojeda SR, Wheaton JE, Jameson HE, et al. The onset of puberty in the female rat: changes in plasma prolactin, gonadotropins, luteinizing hormone-releasing hormone (LHRH), and hypothalamic LHRH content. Endocrinology. 1976;98(3):630–638.

[26] Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007;81(3):559–575.

[27] Orajarvi M, Hirvonen O, Yu SB, et al. Effect of estrogen and altered diet hardness on the expression of estrogen receptor

alpha and matrix metalloproteinase-8 in rat condylar cartilage. J Orofac Pain. 2011;25(3):261–268.

[28] Oterino A, Toriello M, Cayón A, et al. Multilocus analyses reveal involvement of the ESR1, ESR2, and FSHR genes in migraine. Headache. 2008;48(10):1438–1450.

[29] Rodriguez-Acevedo AJ, Maher BH, Lea RA, et al. Association of oestrogen-receptor gene (ESR1) polymorphisms with migraine in the large Norfolk Island pedigree. Cephalalgia. 2013;33(14): 1139–1147.

[30] Smith SB, Reenilä I, Männistö PT, et al. Epistasis between polymorphisms in COMT, ESR1, and GCH1 influences COMT enzyme activity and pain. Pain. 2014;155(11):2390–2399.

[31] Coşkun S, Yücel Y, Çim A, et al. Contribution of polymorphisms in ESR1, ESR2, FSHR, CYP19A1, SHBG, and NRIP1 genes to migraine susceptibility in Turkish population. J Genet. 2016; 95(1):131–140.

[32] Nicot R, Vieira AR, Raoul G, et al. ENPP1 and ESR1 genotypes influence temporomandibular disorders development and surgical treatment response in dentofacial deformities. J Craniomaxillofac Surg. 2016;44(9):1226–1237.

[33] Bonato LL, Quinelato V, Pinheiro Ada R, et al. ESRRB polymorphisms are associated with comorbidity of temporomandibular disorders and rotator cuff disease. Int J Oral Maxillofac Surg. 2016;45(3):323–331.

[34] Lindberg MK, Movérare S, Skrtic S, et al. Estrogen receptor (ER)-beta reduces ERalpha-regulated gene transcription, supporting a "ying yang" relationship between ERalpha and ERbeta in mice. Mol Endocrinol. 2003;17(2):203–208.