The Effects of Age and Sex on the Expression of Aromatase in the Rat Temporomandibular Joint

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Aims: *To investigate the expression of aromatase in temporomandibular joints(TMJs) of male and female rats of different ages.* ***Methods:*** *The expression of aromatase in terms of protein and mRNA was examined by immunohistochemistry, Western blot, and in situ hybridization in the TMJs of Sprague-Dawley rats. The expression level of aromatase protein was also quantified by the density of aromatase-positive chondrocytes in TMJ condylar cartilage. A tritiated water assay was used to analyze the activity of aromatase in cartilage chondrocytes.* ***Results:*** *Strong aromatase protein and mRNA hybridization signals were detected in hypertrophic and mature chondrocytes in all the cases examined. The density of aromatase-positive chondrocytes was relatively stable at higher levels before 8 weeks of age. The density decreased gradually in females after 8 weeks of age but not in males.* ***Conclusion:*** *These results demonstrate that aromatase is expressed intensely in condylar cartilage and suggest that it may play an important role in pathophysiological mechanisms in condylar cartilage.* J OROFAC PAIN 2006;20:156–165

Key words: aromatase, cartilage, chondrocytes, estrogen, temporomandibular joint

Estrogen is a circulating sex hormone that also occurs in peripheral tissues, where it is synthesized by the catalytic action of the enzyme aromatase, which belongs to the XIX subfamily of cytochrome P450 (CYP19). This heme protein is responsible for binding of the C19 androgenic steroid substrate and for catalyzing the series of reactions leading to formation of the phenolic A ring characteristic of estrogens.[1–3] In mammals, a number of tissues and cells have the capacity to express aromatase and hence synthesize estrogens. These include the ovaries, testes, placenta, fetal liver, adipose tissue, chondrocytes, osteoblasts, smooth muscle, and numerous sites in the brain.[1–3] In situ estrogen biosynthesis by aromatase has also been demonstrated to influence various estrogen-related functions.[4] Several studies have indicated that aromatase is closely related to female-predilecting diseases such as breast cancer, endometriosis, and endometrial cancer.[5–9]

Temporomandibular disorders (TMD) is a term encompassing a spectrum of clinical signs and symptoms which involve the masticatory musculature, the temporomandibular joint (TMJ), and associated structures. Due to a poor understanding of the etiology of TMD, and the lack of definitive diagnostic or therapeutic methods, TMD patients often have to tolerate their symptoms, which substantially affects their quality of life.[10,11] Most epidemiologic

studies show that the predilection of TMD in women is striking. In the general population, TMD are 2 to 5 times more prevalent in women than in men, whereas the differential prevalence is even greater in patient populations.[10–15] Furthermore, unlike the similar diseases of the joints that also have a greater female predilection but occur postmenopausally, a large proportion of women with TMD are between 18 and 45 years of age. The reasons for this marked sexual dimorphism and age distribution remain unclear. Some investigators have focused on the potential role of female reproductive hormones, particularly estrogen, in the etiology of TMD.[12,14–18] However, most of these studies have focused on either the relationship between serum estrogen levels and symptoms of TMD or on the expression of estrogen receptors in the TMJ. The studies conducted thus far have produced conflicting results.[12,17–19] For example, it was reported that subjects with TMD showed significantly higher serum estrogen levels compared to a group of healthy controls.[17,18] Yet TMD pain in women is highest when estrogen levels are at their lowest.[12] It is possible that estrogen synthesized locally, instead of that from serum, has a profound effect on TMJ tissues. Therefore, the present study was carried out to investigate the expression of aromatase, which is closely associated with the biosynthesis of estrogen, in the TMJs of rats of different ages and sexes.

Materials and Methods

Animals and Tissue Preparation

Forty Sprague-Dawley (SD) rats were provided by the animal center of the Fourth Military Medical University. Thirty-six rats were divided into 12 groups (2 weeks, 4 weeks, 8 weeks, 4 months, 12 months, and 18 months old; 1 group of 3 rats per age and sex). These rats were used for immunohistochemical and in situ hybridization experiments. The remaining 4 rats, 4-week-old males, were used for Western blot analysis. The study was approved by the Animal Research Committee of the Fourth Military Medical University.

The animals for immunohistochemistry and in situ hybridization were sacrificed under deep anesthesia and perfused transcardially with 200 mL of 0.1 mol/L phosphate buffer (PB, pH 7.4) followed by 400 mL of 4% paraformaldehyde (pH 7.4). All deionized water used had been treated with diethylpyrocarbonate (DEPC, Sigma). The TMJs were dissected free of most adherent tissues and post-fixed with the same fixative overnight at 4°C. The TMJs were then decalcified in Krinstense fluid (a solution containing sodium formate and formic acid). All processes of fixation and decalcification were performed at 4°C. The TMJs were then dehydrated in a series of ethanols of varying strength, cleared by xylene, and impregnated in paraffin. The paraffin-embedded TMJs were cut into 5-μm middle-sagittal sections and mounted on clean poly-L-lysine precoated glass slides.

Immunohistochemical Staining

The antibody used was anti-rat aromatase purified rabbit IgG (Wuhan Boster Biological Technology), which was obtained against the peptide sequence C-ILVTLLRRFHVKTLQ. It corresponds to residues 450 to 464 of human aromatase cytochrome P450 (P450 arom; NP112503), with 86.7% homology with residues 450 to 464 of rat cytochrome P450 arom (NP058781). The specificity of this antibody was checked previously by Western blot by Zhao et al.[20]

After being heated for 1 hour at 58°C, 120 paraffin sections (10 sections randomly selected from each group) were deparaffinized with xylene and rehydrated in a descending series of ethanols. To eliminate endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide in absolute methanol at room temperature for 10 minutes. The sections were then washed in deionized water, and 3 times in 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4). The sections were pretreated for antigen retrieval using antigen retrieval solution (Wuhan Boster Biological Technology). After being washed 3 times with PBS, the sections were then incubated with normal goat serum at 37°C for 30 minutes to prevent nonspecific binding of antibodies. They were then incubated with primary antibody (1:50) overnight at 4°C. The bound primary antibody was then localized by biotin-labeled anti-rabbit IgG (BeiJing ZhongShan Golden Bridge Biotechnology) at 37°C for 30 minutes and then an avidin-peroxidase complex at 37°C for 30 minutes. The antibody staining was performed using a peroxidase/diaminobenzidine (DAB) yellow kit (Wuhan Boster Biological Technology). The sections were lightly counterstained with hematoxylin, and then dehydrated in a series of ethanols, cleared in xylene, and coverslipped. For control experiments, the sections were incubated in a solution with a ratio of primary antibody to antigen peptide of 1:10,000, or PBS. Paraffin-embedded sections of normal male SD rat testes were stained with the same process as a positive control.

Immunofluorescence Staining

The procedure was the same as that used for immunohistochemical staining except for incubation with the primary antibody. Some sections of 4-week-old male rats were incubated with primary antibody (1:30 dilution) overnight at 4°C. The next day, sections were washed in PBS 5 times for 5 minutes at a time. Then, fluorescein isothiocyanate (FITC) -labeled anti-rabbit IgG from sheep (Wuhan Boster Biological Technology) was applied to localize the primary antibody. Subsequently, sections were washed again in 5 changes of PBS. They were then examined with a fluorescence microscope (Olympus BX-60).

Western Blot

Eight TMJs from four 4-week-old male rats were dissected and frozen (–80°C) immediately. Cartilage samples were then pulverized in liquid nitrogen and extracted with 4 mol/L guanidine hydrochloride (HCl) in 50 mmol/L Tris-HCl, pH 7.4, containing 1 mmol/L phenylmethanesulfonyl fluoride (PMSF), 2 mmol/L N-ethylmaleimide (NEM), and 0.025 mg/mL leupeptin.[21] Insoluble material was removed by centrifugation at 10,000 rpm for 10 minutes. Cartilage extracts were mixed with an equal volume of 2× SDS-PAGE [sodium dodecyl sulfate polyacrylamide gel electrophoresis] loading buffer. The total proteins were then electrophoresed in 10% SDS-polyacrylamide gel. The electrophoresed proteins were transferred onto a polyvinylidene difluoride membrane (BioRad). After the membranes were blocked in 5% fat-free dry milk in PBS, they were then incubated overnight with anti-aromatase antibody diluted 1:50. For control experiments, another membrane was incubated in a solution with a ratio of primary antibody to antigen peptide of 1:10,000. Blots were subjected to three 15-minute washes with PBS and then incubated for 2 hours with horseradish perioxidase (HRP)-labeled secondary antibody. Following extensive washes, blots were revealed by enhanced chemiluminescence (ECL) (Amersham). The exposure time was 2 minutes.

In Situ Hybridization

In humans and most other species (the pig being an exception), there appears to be only 1 gene, CYP19, that encodes P450 arom. Since the sequence for rat P450 arom is CTA CAT CTC CAG ATT CGG CAG CAA GCG TGG,[22] the sequence of the 30-base aromatase oligonucleotide probe used for in situ hybridization analysis was as follows: CCA CGC TTG CTG CCG AAT CTG GAG ATG TAG. Sense and antisense mRNA probes were synthesized with a digoxigenin labeling kit (Roche Diagnostics). For in situ hybridization, all deionized water used had been treated by DEPC.

After heating for 1 hour at 58°C, sections from 4-week-old male rats were deparaffinized with xylene and rehydrated in a descending series of ethanols. To eliminate endogenous peroxidase activity, the sections were treated with 3% hydrogen peroxide in absolute methanol at room temperature for 10 minutes, then washed in 3 times with deionized water. Sections were then incubated with 5 μg/mL proteinase K in 3% citromalic acid for 25 minutes at 37°C to expose mRNA fragments. After 3 washes of deionized water, the prepared sections were prehybridized at 42°C for 3 hours in a buffer containing 50% formamide, 2× sodium chloride/sodium citrate buffer solution (300 mmol/L NaCl and 30 mmol/L sodium citrate), 0.02% Ficoll, 10% dextran sulfate, 0.25% sodium dodecyl sulfate (SDS), 1 mg/mL herring sperm DNA, and 1mmol/L EDTA. After prehybridization, hybridization was performed at 42°C for 16 hours (hybridization mixture: 2 μg/mL aromatase oligonucleotide probe in prehybridization buffer). The next day, sections were washed in the following posthybridization solution after removing the coverslips: 2× SSC for 10 minutes at 37°C, 0.5× SSC for 10 minutes at 37°C, and 0.2× SSC twice for 15 minutes at 37°C. Then blocking buffer was applied to the sections for 30 minutes, followed by incubation with biotin-labeled mouse anti-digoxin for 1 hour at 37°C. After that, the bound complex was localized by an avidin-peroxidase complex at 37°C for 30 minutes. The staining was performed using a peroxidase/DAB blue kit (Wuhan Boster Biological Technology). The sections were lightly counterstained with eosin, and then dehydrated in a graded series of ethanols, cleared in xylene, and coverslipped. For control experiments, sections were treated with RNase A before proteinase K treatment, hybridized in an excess amount of sense probe (ratio of anti-sense probe to sense probe 1:100), or hybridized by omitting oligonucleotide probes.

Chondrocyte Culture and Aromatase Tritiated Water Assay

Under sterile conditions, articular cartilage was harvested from the resected TMJs, minced into 1 mm^3 pieces, and washed in PBS containing 100 mg/L penicillin and streptomycin. Primary chon-

drocytes were isolated by type II collagenase (Worthington Biochemical) digestion for 2 hours, rinsed in PBS containing $CaCl_2$, and $MgCl_2$ at pH 7.4 with 15% fetal bovine serum (FBS) (Invitrogen), and transferred to a well-defined culture medium: Dulbecco's Modified Eagle Medium (DMEM) and 4.5 g/L glucose, supplemented with 10% FBS, 1 mmol/L sodium pyruvate (Invitrogen), 50 μg/mL penicillin, 50 μg/mL streptomycin, 0.4 mmol L-proline, 0.1 mmol/L nonessential amino acids, and 10 mmol/L HEPES buffer (N-[2-hydroxyethyl]piperazine-N'-2-ethane -sulfonic acid) (Sigma).

Aromatase activity was assessed by measurement of tritiated water[23,24] released from [1β–^{3}H] androstenedione (NET926, NEN Perkin-Elmer Life Sciences). Briefly, chondrocytes were maintained in medium as already described. When chondrocytes reached 80% of confluence, the medium was changed to 10% dextran-coated charcoal-treated fetal calf serum (DCC–FCS) phenol red-free medium. One million cells per well were then seeded in 12-well dishes with fresh medium. Two days later, culture plates were washed twice with PBS. One milliliter of serum-free medium containing [1β–^{3}H] androstenedione at a concentration of 60 nmol/L as a substrate (specific activity, 25.9 Ci/mmol) was added to each well. After 6 hours of incubation at 37°C, the reaction mixture was removed and extracted with 2 volumes of chloroform to terminate the reaction and extract unused substrate and steroids. To eliminate residual steroids, the aqueous phase was then treated with an equal volume of a 5% charcoal suspension in K_2HPO_4 containing dextran (1.5%). Tritiated water was measured in a liquid scintillation counter.

Quantification of Aromatase-positive Chondrocytes and Statistical Analysis

All immunohistochemical-stained sections (10 samples in each group) were examined under an Olympus CX-31 microscope (Olympus). Image acquisition (×100) was performed with a Pixera PVC100C system. With Photoshop 7.0 software, 3 continuous regions (64 pixels × 64 pixels) in the mature and hypertrophic layers were selected in the central region of the anterior, middle, and posterior parts of the condylar cartilage (eg, Fig 1). Aromatase-positive chondrocytes in the selected regions were counted at the same threshold of staining by the same person, who was blind to the grouping of the animals. The threshold is shown in Fig 2, and chondrocytes of that staining or stronger than that would be counted as positive cells. The data were analyzed using SPSS version 10.0. The Student *t* test was used to evaluate the differences between groups in the number of aromatase-positive chondrocytes.

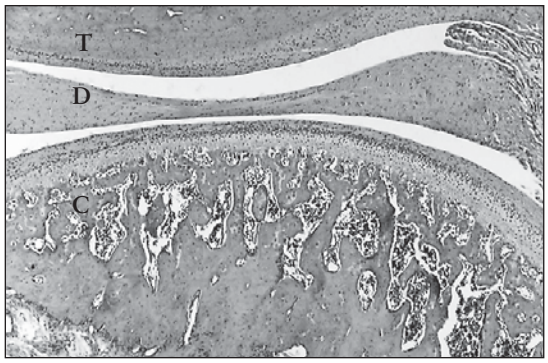

Fig 1 Regions selected for counting aromatase-positive chondrocytes. Aromatase-positive chondrocytes in 9 selected regions were counted. T = temporal bone; D = articular disc; C = mandibular condyle.

Results

Immunolocalization of Aromatase

In a control experiment, intense aromatase immunoreactivity was detected in spermatocytes and sertoli cells of the rat testis (Fig 3a), which is consistent with the findings of Carpino et al.[25] No immunoreactivity was observed in controls in which the primary antibody was either absorbed with excessive antigen or omitted (Figs 3b and 3c).

Immunocytochemistry for aromatase demonstrated an intense immunoreaction in various cellular elements in the rat TMJ. More intense aromatase immunoreactivity was observed in chondrocytes of the condyle than in other sites (Fig 2a). In accordance with Bloom and Fawcett,[26] articular cartilage was viewed as having 5 layers, the fibrous layer, the proliferating layer, the mature layer, the hypertrophic layer, and the calcified layer. Aromatase-positive chondrocytes were abundant in the hypertrophic layer and mature layers, but only few aromatase-positive chondrocytes were found in the fibrous and proliferating layers (Figs 2a, 2b, and 2d). The cytoplasms of chondrocytes showed intense aromatase

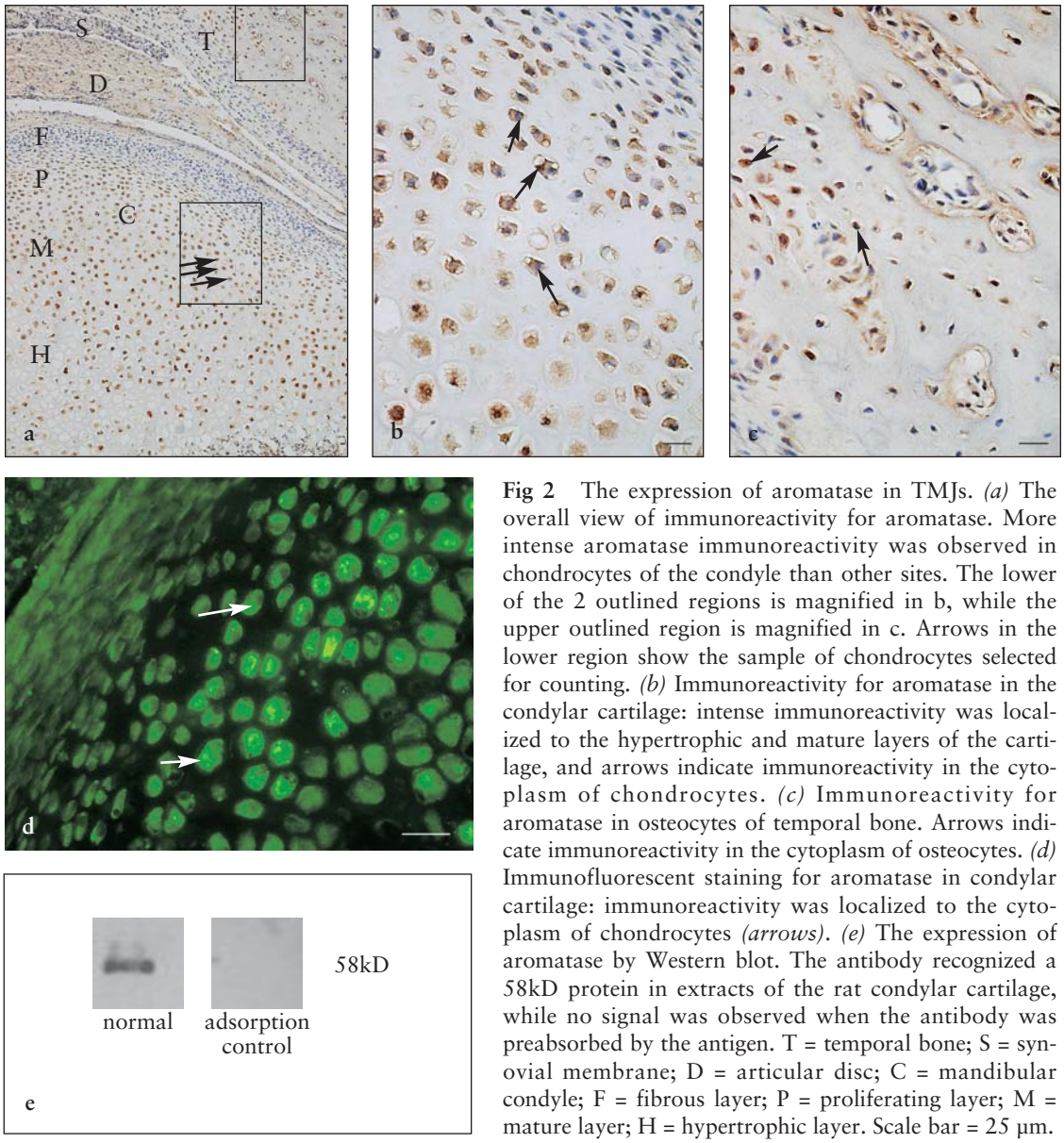

Fig 2 The expression of aromatase in TMJs. *(a)* The overall view of immunoreactivity for aromatase. More intense aromatase immunoreactivity was observed in chondrocytes of the condyle than other sites. The lower of the 2 outlined regions is magnified in b, while the upper outlined region is magnified in c. Arrows in the lower region show the sample of chondrocytes selected for counting. *(b)* Immunoreactivity for aromatase in the condylar cartilage: intense immunoreactivity was localized to the hypertrophic and mature layers of the cartilage, and arrows indicate immunoreactivity in the cytoplasm of chondrocytes. *(c)* Immunoreactivity for aromatase in osteocytes of temporal bone. Arrows indicate immunoreactivity in the cytoplasm of osteocytes. *(d)* Immunofluorescent staining for aromatase in condylar cartilage: immunoreactivity was localized to the cytoplasm of chondrocytes *(arrows)*. *(e)* The expression of aromatase by Western blot. The antibody recognized a 58kD protein in extracts of the rat condylar cartilage, while no signal was observed when the antibody was preabsorbed by the antigen. T = temporal bone; S = synovial membrane; D = articular disc; C = mandibular condyle; F = fibrous layer; P = proliferating layer; M = mature layer; H = hypertrophic layer. Scale bar = 25 μm.

immunoreactions (Fig 2b), and this was further confirmed in immunofluorescence staining (Fig 2d). Moreover, some osteocytes in the temporal bone (Figs 2a and 2c) also showed intense cytoplasmic immunoreactivity.

The expression of aromatase in condylar cartilage was also verified by Western blot. As shown in Fig 2e, the antiaromatase antibody specifically recognized a 58kD protein in the extracts of rat condylar cartilage, while no positive band was observed when antibody was preabsorbed by a larger amount of antigen peptide.

Quantification of Aromatase Immunoreactivity in Condyle Cartilage

In the 12-month-old and 18-month-old groups, the density of aromatase-positive cells was significantly higher in male rats than in female rats ($P < .01$), while no such difference was found in other age groups ($P > .05$) (Fig 4).

In male rats, the density of aromatase-positive chondrocytes in the 2-week-old group was significantly higher than any other age group ($P < .05$). In addition, the density was much higher in the 4-

and 8-week-old groups than that in the 4- and 12-month-old groups ($P < .01$). However, no difference was observed between the 4-week-old group and either the 8-week-old group or the 18-month-old group ($P > .05$ in both cases). Furthermore, the density in the 18-month-old group was much higher than that in the 4- and 12-month-old groups ($P < .01$) but significantly lower than that in the 8-week-old group ($P < .01$).

In female rats, the density of aromatase-positive chondrocytes was significantly higher in the 2- and 8-week-old groups than in any other group ($P < .01$). No significant difference was found between 2- and 8-week-old groups ($P > .05$). In addition, the density was higher in the 4-week-old group than in the 4-, 12-, and 18-month-old groups ($P < .01$). Density was greater in the 4-month-old group than in the 12- and 18-month-old groups ($P < .01$). No difference was observed between 12- and 18-month-old groups ($P > .05$).

Aromatase mRNA Expression by In Situ Hybridization

In control experiments, no aromatase mRNA hybridization signals were detected in the absence of oligonucleotide probes or in an excess amount of sense probe (ratio of anti-sense probe to sense probe 1:100), or when sections were treated with RNase A before proteinase K treatment (Fig 5).

Aromatase mRNA hybridization signals were identified primarily in the condylar cartilage, articular surface of temporal bone, and articular disc (Fig 6). In condyle cartilage, chondrocytes of proliferating layer, mature layer, and superior part of hypertrophic layer showed intense signals for aromatase mRNA (Figs 6a and 6d). In contrast to the results of immunochemistry (Figs 2a and 2b), only few aromatase mRNA signals were detected in the inferior part of the hypertrophic layer, while some signals occurred in the proliferating layer (Figs 6a and 6d). It indicates that aromatase may be synthesized in immature chondrocytes and expressed in relatively mature ones. Intense signals for aromatase mRNA were also observed in both osteocytes of the temporal bone (Figs 6a and 6b) and stromal cells of the articular disc (Figs 6a and 6c).

Aromatase Activity of Chondrocytes

After 6 hours incubation at 37°C, the average radioactive level of tritiated water released in 12 wells was 2543 ± 294 (pmol/ 10^6 cell h^{-1}).

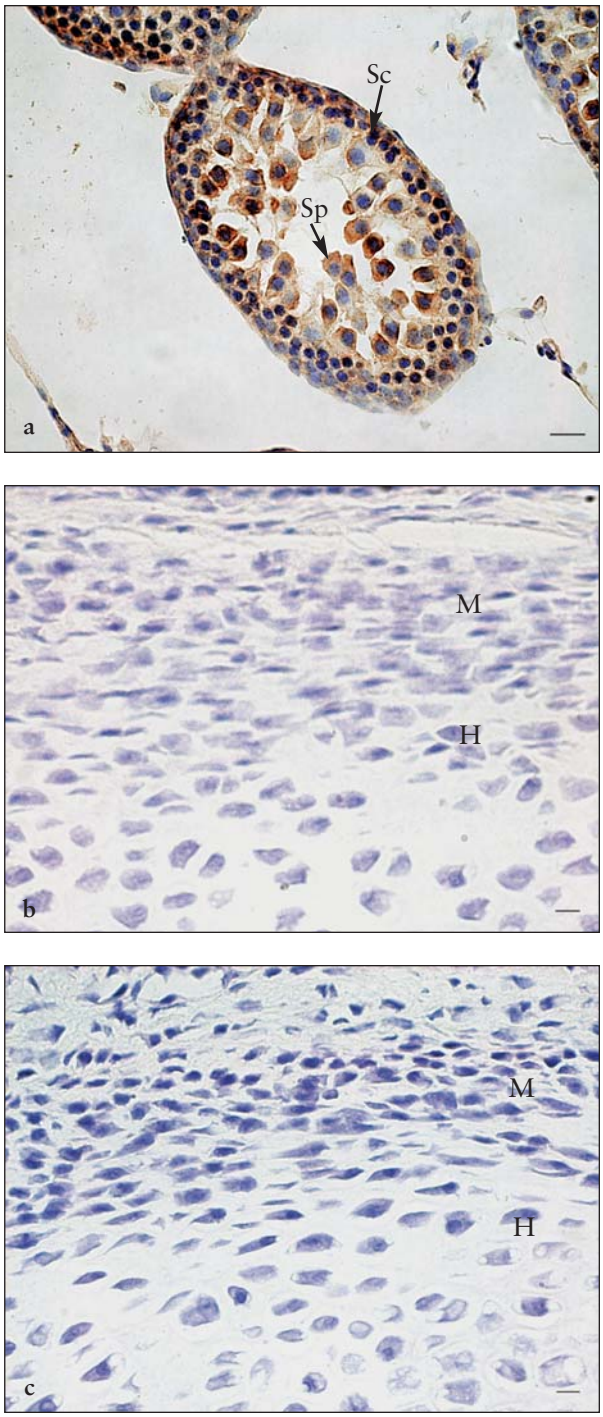

Fig 3 Immunoreactivity of aromatase in control experiments. *(a)* Aromatase positive control: strong aromatase immunoreactivity in the cytoplasm of spermatocytes and sertoli cells from a rat testis. *(b)* Adsorption control: no immunoreactivity when primary antibody was preabsorbed by the antigen. *(c)* Replacement control: no immunoreactivity when primary antibody was replaced by PBS. Sp = spermatocytes; Sc = sertoli cells; M = mature layer of condyle cartilage; H = hypertrophic layer of condyle cartilage. Scale bar = 25 μm.

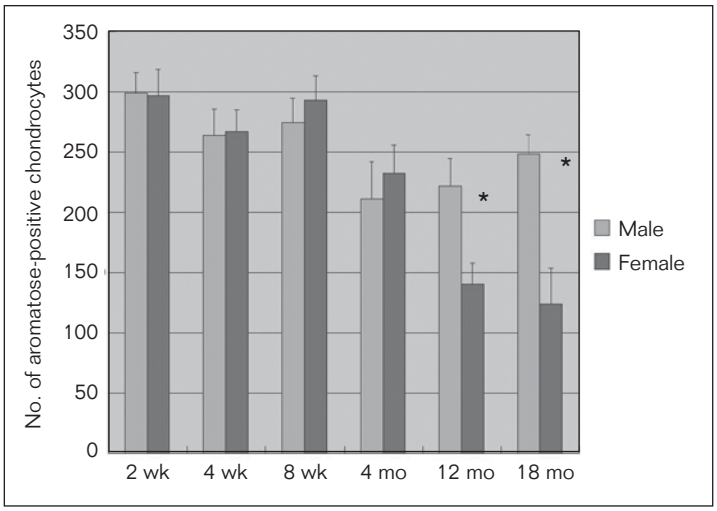

Fig 4 Density of aromatase-positive chondrocytes in condylar cartilage. Error bars represent standard error (n = 10). *Significant difference between the sexes ($P < .01$).

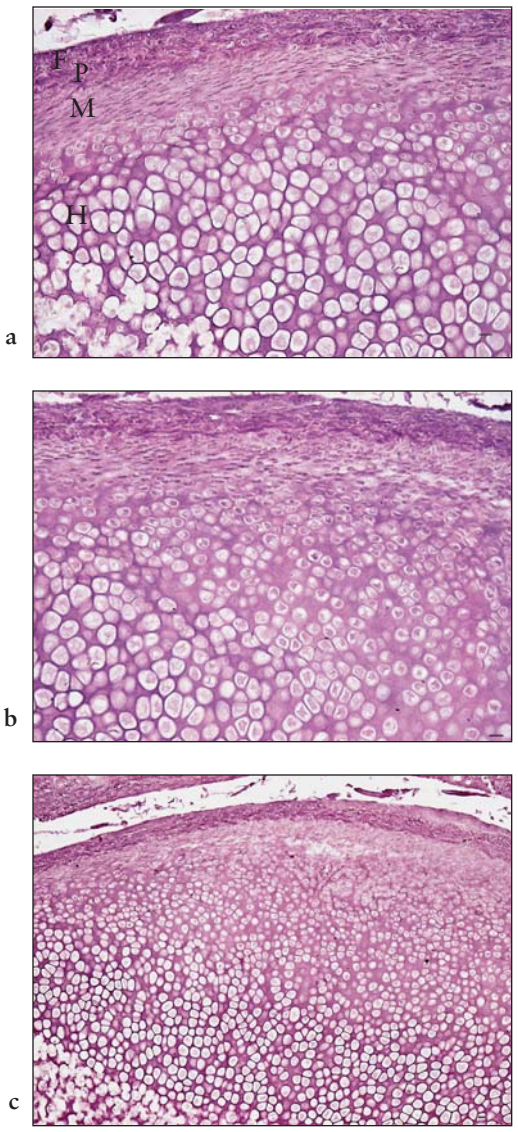

Fig 5 Negative control expression of aromatase mRNA in condylar cartilage. *(a)* Sections were treated with RNase A before proteinase K treatment. *(b)* Sections were hybridized in the presence of 100-fold amount of sense probe. *(c)* Sections were hybridized by omitting oligonucleotide probes. No mRNA signals were observed in any chondrocytes. F = fibrous layer; P = proliferating layer; M = mature layer; H = hypertrophic layer. Scale bar = 25 μm.

Discussion

Although it has been reported that circulating estrogen in humans is largely derived from the ovaries in women, while the testes account for 15% of circulating estrogens in men,[2,27] estrogen is formed almost exclusively by conversion of adrenal androgens by the enzyme P450 arom in peripheral tissues, including bone, in men and postmenopausal women. During the past several years, aromatase has been detected in many human extragonadal tissues, including human bone. It has been considered that estrogens derived from the local aromatization of testosterone or androgens mediate many aspects of testosterone actions and exert estrogenic actions on these tissues.[2,28–30] In sex-dependent diseases, eg, breast cancer, endometrial cancer, endometriosis, and ovarian carcinoma, increased aromatase expression and/or activity in the tumor tissue has been demonstrated to be closely correlated with malignant phenotypes,[31–33] despite the fact that there has been no consistent evidence of increased serum estrogen concentration in women with these tumors,[34] which suggests that estrogen synthesized locally by aromatase plays a important role in these processes.

Recently, aromatase activity has been examined in bone tissue in which both androgens and estrogens exert their effects. Using immunocytochemistry and in situ hybridization techniques, Sasano et al[4] located aromatase expression in tibial

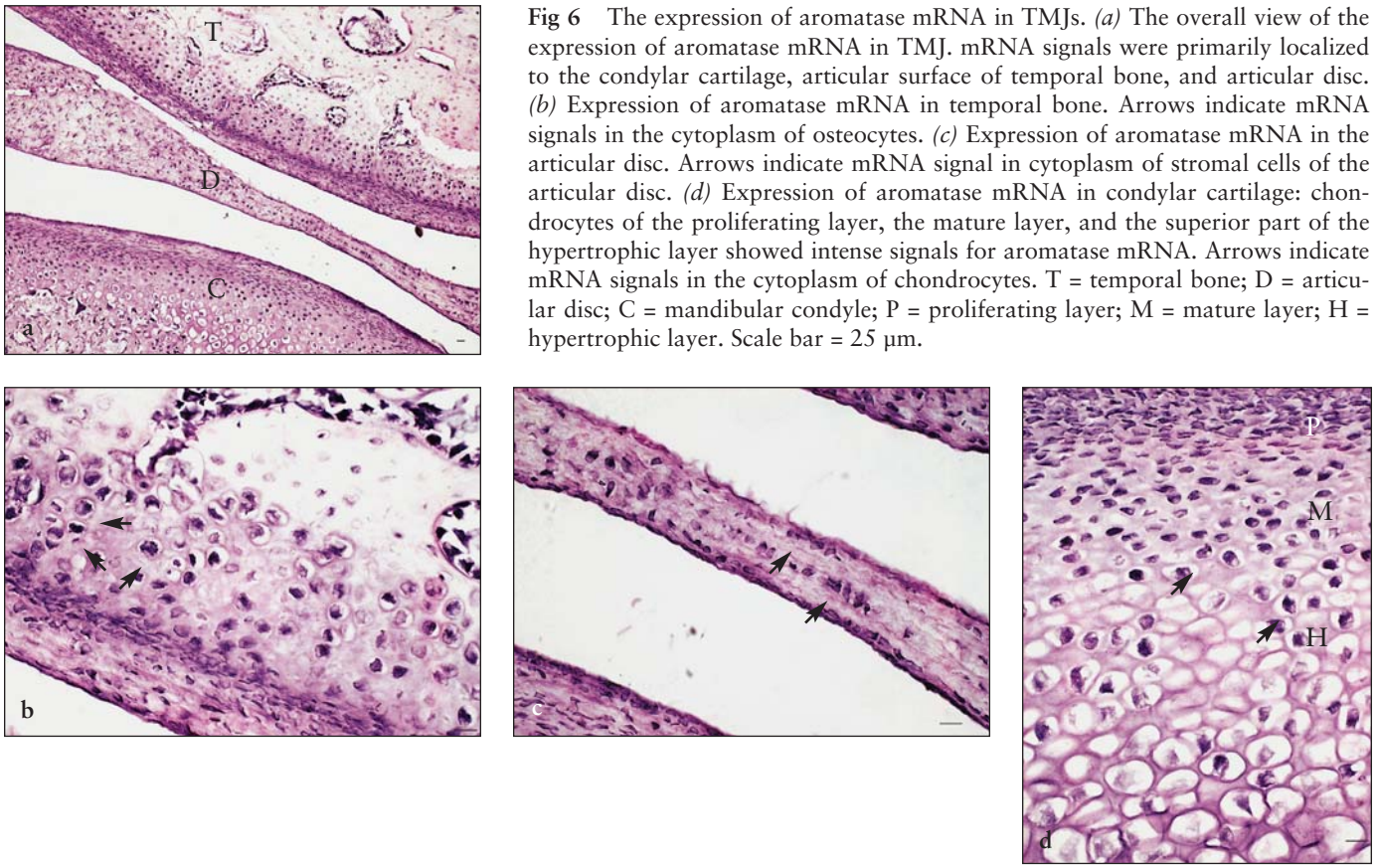

Fig 6 The expression of aromatase mRNA in TMJs. *(a)* The overall view of the expression of aromatase mRNA in TMJ. mRNA signals were primarily localized to the condylar cartilage, articular surface of temporal bone, and articular disc. *(b)* Expression of aromatase mRNA in temporal bone. Arrows indicate mRNA signals in the cytoplasm of osteocytes. *(c)* Expression of aromatase mRNA in the articular disc. Arrows indicate mRNA signal in cytoplasm of stromal cells of the articular disc. *(d)* Expression of aromatase mRNA in condylar cartilage: chondrocytes of the proliferating layer, the mature layer, and the superior part of the hypertrophic layer showed intense signals for aromatase mRNA. Arrows indicate mRNA signals in the cytoplasm of chondrocytes. T = temporal bone; D = articular disc; C = mandibular condyle; P = proliferating layer; M = mature layer; H = hypertrophic layer. Scale bar = 25 μm.

osteoblasts and chondrocytes. Several researchers also reported the presence of aromatase activity and mRNA in primary cultured osteoblast-like cells,[35–38] which suggests that local synthesis of estrogen in bone may play a key role in the modulation of bone turnover independent of circulating hormone concentrations.[37,39] Therefore, bone is not only a target of estrogen but also an extraglandular source of local estrogen, which plays an important role in bone mineral metabolism through autocrine and paracrine actions.[36] The ability to produce estrogen in bone may be important for long bone growth and development. Sylvia et al[40] and Van Der Eerden et al[41] have reported that costochondral chondrocytes can produce estrogen. Individuals or aromatase knockout mice lacking the ability to convert androgens into estrogens have exhibited delayed closure of their epiphyses, resulting in greater than normal stature, indicating that endochondral ossification is affected and providing additional evidence that local production of estrogen plays an important role in this process.[40]

Using immunocytochemistry, Western blot, and in situ hybridization techniques, the present study provides evidence for the expression of aromatase both in terms of protein and mRNA in the rat TMJ. The most intense aromatase immunoreactivity was located in chondrocytes of the condylar cartilage. The most intense staining was observed in the cytoplasm of hypertrophic and mature chondrocytes, as in the report by Öz et al.[42] Aromatase mRNA hybridization signals were also located in hypertrophic, mature, and proliferating chondrocytes. Taken together with the demonstration of estrogen receptor expression in the TMJ,[19,43–45] the data suggest the existence of an autocrine/paracrine network with direct action of estrogens on chondrocytes in the regulation of physiologic and pathologic activities of TMJ. In addition, it is possible that local estrogens may be important for condylar cartilage in both men and women.

It has been reported by Lea et al that the levels of aromatase transcripts in the femurs of elderly people are higher at a fracture site than distal to the fracture site, where aromatase is unde-

tectable.[39] Purohit et al also showed that inflamed breast tissue, as a result of silicone injections, had significantly raised levels of P450 arom compared with those previously detected in non-neoplastic breast tissue.[46] It seems that the expression of aromatase is tissue-protective at sites of injury or inflammation. Some animal studies have indicated that estrogens may contribute to extracellular matrix catabolism in the TMJ.[10] In the present study, male and female rats expressed different levels of aromatase after 8 weeks of age. Aromatase levels were higher in the males than in the females. These results suggest that the tissue-protective capacity of condylar cartilage in older females may be lower than that in males of the same age and thereby partially explain the greater female disposition to TMD.[44,47–49] Based on these findings, the current authors speculate that aromatase may play an important role in the development of TMD by producing estrogen or directly regulating the metabolism of extracellular matrices and the functions of other cytokines. Further investigations are needed to address this possibility.

In summary, this study has demonstrated the presence of aromatase (protein and mRNA) and its activity in the rat TMJ, especially in the chondrocytes of the condylar cartilage. The density of aromatase-positive chondrocytes is relatively stable at a high level before 8 weeks of age, with no difference between males and females. However, the density decreases gradually in females after 8 weeks of age, but not in males. These data may indicate that mature females are sensitive to TMJ injury. The possible roles of aromatase in the TMJ are still not completely understood, but aromatase may represent a good marker of estrogens synthesized locally. Taking into account the presence of estrogen receptors in the TMJ, further investigations of the activities of aromatase in both physiologic and pathologic TMJ tissues will help clarify the physiologic roles of estrogens in the TMJ.

Acknowledgments

The authors thank Dr Yuhao Gao (Division of Endocrinology and Metabolism, Emory University School of Medicine, USA) for his assistance in the revision of this paper, Dr Jianliang Pang and Dr Bo Zhang for technical assistance, and Dr Shisen Liu for providing [1β–^{3} H] androstenedione. This study was supported by the National Natural Science Foundation of China (NSFC) grant no. 30471909 and Science Foundation of Shaanxi province grant no. 2005K14-62 (2).

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