Animal Models of Temporomandibular Joint Osteoarthritis: Classification and Selection

Yuqing Zhao¹,²†, Yanxin An³†, Libo Zhou⁴†, Fan Wu², Gaoyi Wu², Jing Wang⁵,⁶* and Lei Chen¹*  

¹Department of Orthodontics, School and Hospital of Stomatology, Cheeloo College of Medicine, Shandong University & Shandong Key Laboratory of Oral Tissue Regeneration & Shandong Engineering Laboratory for Dental Materials and Oral Tissue Regeneration, Jinan, China, ²School of Stomatology, Hefei Hefei Key Lab of Oral Biomedicine Materials and Clinical Application & Experimental Center for Stomatology Engineering, Jiamusi University, Jiamusi, China, ³Department of General Surgery, The First Affiliated Hospital of Xi’an Medical University, Xi’an, China, ⁴School of Basic Medicine, Hefei Hefei Key Lab of Oral Biomedicine Materials and Clinical Application & Experimental Center for Stomatology Engineering, Jiamusi University, Jiamusi, China, ⁵Department of Oral Implants, School of Stomatology, National Clinical Research Center for Oral Diseases & State Key Laboratory of Military Stomatology & Shaanxi Key Laboratory of Stomatology, The Fourth Military Medical University, Xi’an, China, ⁶Key Laboratory of Shaanxi Province for Craniofacial Precision Medicine Research, College of Stomatology, Xi’an Jiaotong University, Xi’an, China

Temporomandibular joint osteoarthritis (TMJOA) is a common degenerative joint disease that can cause severe pain and dysfunction. It has a serious impact on the quality of lives of patients. Since mechanism underlying the pathogenesis of TMJOA is not fully understood, the development of effective tools for early diagnosis and disease-modifying therapies has been hindered. Animal models play a key role in understanding the pathological process of diseases and evaluating new therapeutic interventions. Although some similarities in disease processes between animals and humans are known, no one animal model is sufficient for studying all characteristics of TMJOA, as each model has different translatability to human clinical conditions. For the past 4 decades, TMJOA animal models have been studied by numerous researchers and can be broadly divided into induced, naturally occurring, and genetically modified models. The induced models can be divided into invasive models (intra-articular injection and surgical induction) or non-invasive models (mechanical loading, high-fat diet, and sleep deprivation). Different types of animal models simulate different pathological expressions of TMJOA and have their unique characteristics. Currently, mice, rats, and rabbits are commonly used in the study of TMJOA. This review sought to provide a general description of current experimental models of TMJOA and assist researchers in selecting the most appropriate models for different kinds of research.

Keywords: temporomandibular joint, osteoarthritis, animal models, induced models, naturally occurring models, genetically modified models

INTRODUCTION

Osteoarthritis (OA) is a chronic degenerative condition that often affects the stress-bearing joints, such as the knee, spine, hips, and fingers (Kloppenburg and Berenbaum, 2020). The temporomandibular joint (TMJ), one of the most common and complex joints in the human body, can also be affected by OA. Temporomandibular joint osteoarthritis (TMJOA) is the most
common form of arthritis occurring in TMJs due to its high clinical prevalence and consequences on TMJ (Wang et al., 2015). Patients with TMJOA usually have joint pain, swelling and stiffness that leads to activity limitations and even reduced quality of life. Therefore, numerous studies are needed to better understand the development and progression of TMJOA.

The etiology of TMJOA is complex and multifactorial, which is generally considered to be associated with mechanical overloading, abnormal occlusion, trauma, and psychological stress (Tanaka et al., 2008; de Souza et al., 2012). However, the causes of impaired cartilage and subchondral bone of TMJ remain unclear. Currently, the treatments of TMJOA mainly aim to reduce pain, restore TMJ function, and improve the quality of life of patients (Tanaka et al., 2008; Al-Moraissi et al., 2020). Although many clinical studies have investigated the effect of various treatments, no clinically approved therapeutics are currently available to restore the TMJ structure, given the limited understanding of its pathogenesis and the limited blood supply of the cartilage (Huynh et al., 2012; Wang et al., 2015). Since obtaining clinical samples from patients with TMJOA is difficult and clinical symptoms often occur late in the disease process, animal models of TMJOA play a key role in understanding the pathogenesis of diseases and evaluating new therapeutic approaches (Vapniarsky et al., 2018; Liu et al., 2021). As various animal models of TMJOA have been developed over the past four decades, a major challenge lies in selecting the “best” model when designing a study. Animal models for TMD research and mouse genetic models for TMJ preclinical research have been reviewed elsewhere (Suzuki and Iwata, 2016; Ghassemi Nejad et al., 2017; Almarza et al., 2018; Bhatti et al., 2021; Xiang et al., 2021). This review serves to systematically summarize the usefulness, histopathological changes, and scope of application of each model and current animals used in TMJOA research. We hope to provide an evidence-based reference for researchers to deepen their understanding and to select appropriate TMJOA animal models.

**CHARACTERISTICS OF TEMPOROMANDIBULAR JOINT/ TEMPOROMANDIBULAR JOINT OSTEOARTHRITIS**

**Anatomy and Physiology of the Temporomandibular Joint**

TMJ, a joint that connects the mandible to the skull and regulates mandibular movement, is composed of the mandibular condyle, articular disc, and the articular eminence and glenoid fossa. The cartilage layer on the mandibular condyle is from the superficial layer downward and composed of several layers: the fibrous, proliferative, hypertrophic and calcified cartilage layers (Thilander et al., 1976). Instead of being covered by the hyaline cartilage, the articular surface of the mandibular condyle is covered with a layer of mature fibrous tissue, consisting of a mass of collagen fibers (Toller, 1974). The hyaline cartilage is mainly composed of type II collagen, whereas the fibrocartilage is mainly composed of type I collagen (Vos et al., 2014). The orientation of the fibers on the condylar surface is a wavy interlacing of collagen fibers, which makes most fibers tangent to the surface (Toller and Wilcox, 1978). This property allows the TMJ to better withstand shear forces, whereas the hyaline cartilage is more resistant to compressive loading. Unlike the articular cartilage of the knee, the condylar cartilage has a different embryonic origin, which is derived from cranial neural crest cells (Shen and Darendeliler, 2005). Moreover, the most intriguing biological aspect of the condylar cartilage that differs from other cartilages lies in its ability to remodel in response to the changes in condylar repositioning, articular functioning, and mechanical loading (Nakano et al., 2003). Possibly, these essential structural differences in the TMJ significantly modify the clinical expression of its pathological changes.

**Radiographical Features of Temporomandibular Joint Osteoarthritis**

Several imaging techniques are available for TMJ visualization, including panoramic radiography, plain radiography, computed tomography (CT), magnetic resonance imaging (MRI), and high-resolution ultrasonography. Currently, CT and MRI are the most used imaging techniques (Talmaceanu et al., 2018). The radiographic manifestations of TMJOA include flattening of the anterior surface of the condyle, erosions, and irregularities of the joint surfaces, flattening of the articular surface of the temporal eminence, generalized sclerosis, subchondral cysts, osteophytes, and idiopathic condyle resorption (Zhao et al., 2011; Nah, 2012). Several studies suggest that erosive lesions may indicate acute or active changes, whereas sclerosis and flattening osteophytes may indicate a later and relatively stable stage (Ahmad et al., 2009).

**Histopathologic Features of Temporomandibular Joint Osteoarthritis**

The main manifestations of TMJOA are articular cartilage damage and degeneration, as well as repair of periacicular tissues and hyperplastic changes of the synovial membrane. Cartilage damage is characterized by irregular thinning and fibrillation of the fibrous layer, and reduced proteoglycan content of the cartilage matrix. Chondrocytes are frequently arranged in small groups or clusters, and many degenerated and necrotic chondrocytes are observed (de Bont et al., 1985a; de Bont et al., 1985b). In the superficial layers, the density of the collagen fibrils is diminished. The collagen fibrils show a loose and disordered arrangement. The calcified cartilage layer shows an irregular border adjacent to the fibrous layer and subchondral bone. Exposure of subchondral bone, hyperplasia, sclerosis, osteophyte formation and osteoblast activity can be found in subchondral bone (Toller, 1977) (Figure 1).

The synovial membrane of the TMJ may initially undergo synovial intima hyperplasia and cell hypertrophy, and subsequently result in deposition of fibrous material in the intima matrix. Subintimal fibroblast activity increases, and
subintima elastic fibers are present (Dijkgraaf et al., 1997). Neovascularization of the fossa cartilage and articular disc frequently occur. The joint capsule is usually thickened in the TMJ. Adhesions to the lateral TMJ structures, including the synovial membrane, articular disc, and articular eminence, are often found in the latter stages of TMJOA (Dijkgraaf et al., 1995).

In summary, TMJOA is a chronic disease characterized by degenerative changes in the cartilage, accompanied by repair of surrounding tissues. Notably, TMJOA is different from OA in other synovial joints. Numerous elastic fibers, giant collagen fibrils, prominent nuclear fibrous lamina, and mineral-containing matrix vesicles are found in the degenerated condylar cartilage, which are not found in knee joint OA. Moreover, the inflammatory infiltrate is less often present in the osteoarthritic synovial membrane of the TMJ than in other synovial joints (Roy and Meachim, 1968; Meachim and Sheffield, 1969; de Bont et al., 1985b). The etiology and treatment of TMJOA are different because of differences in the structure and origin of cells that give rise to TMJ structures. Therefore, special animal models are needed to study TMJOA.

CLASSIFICATION OF ANIMAL MODELS IN TEMPOROMANDIBULAR JOINT OSTEOARTHRITIS

Although some similarities in the disease processes between animals and humans are known, no one animal model is sufficient for studying all features of TMJOA. The translatability of animal models mainly depends on how well they correspond to human conditions. Therefore, we systematically summarized the existing animal models of TMJOA (Figure 2). Animal models used to study TMJOA are broadly divided into induced, naturally occurring, and genetically modified models, depending on whether the animals are treated with or without intervention. The induced models can be divided into invasive models (intra-articular injection and surgical...
Induction) or non-invasive models (mechanical loading, high-fat diet, and sleep deprivation). Different modeling approaches mimic different etiologies of TMJOA. Selection of an appropriate animal model in studying TMJOA may be challenging. Therefore, we summarized the advantages, disadvantages, and indications of TMJOA animal models (Table 1).

**Induced Models**

**Invasive Models**

Invasive TMJOA models mainly work by producing joint destabilization, altered articular surface contact forces, and intra-articular inflammation in TMJs of animals. The procedures include injection and surgical approaches, which are related to high technique sensitivity. Therefore, improvement in the technical stability of researchers by long-term practice is the key to creating invasive models.

**Intra-Articular Injection Models**

Intra-articular injection is a well-characterized preclinical model of OA in the knee joint and TMJ. It causes disease by inducing intra-articular inflammation, cytotoxicity, or direct matrix damage in articular cartilage. Chondrocytes are the only cell type responsible for producing extracellular matrix and maintaining the homeostasis of cartilage (Dijkgraaf et al., 1995). Death of the chondrocytes, which results from necrosis or apoptosis, is a major feature of cartilage degeneration in OA (Aigner and Kim, 2002; Liu et al., 2021). Chemical drug injection can cause rapid death of many chondrocytes and destroy the homeostasis of chondrocytes, thereby creating joint damage and pain.

The commonly used drugs include monosodium iodoacetate (MIA) (Wang X. D. et al., 2012; Cledes et al., 2006; Guler et al., 2011), complete Freund's adjuvant (CFA) (Rotpenpian et al., 2011; Xu et al., 2016; Xu et al., 2017), collagenase (Li W. et al.,...
2021; Li W. et al., 2014; Wu et al., 2015), papain (Molinet et al., 2020) and vascular endothelial growth factor (VEGF) (Shen et al., 2015) (Table 2). The first four drugs are common drugs in animal models of knee OA, which can cause different types of inflammation. The most frequently used drug among these drugs is MIA. However, a transcriptome study reported that ≤4% of total gene overlap occur between MIA-induced model and human OA (Barve et al., 2007). Despite this challenge, intra-articular injection still has the advantages of ease of induction and reproducibility. Additionally, the rate of disease progression and severity of joint lesions can be adjusted by changing drug concentration (Wang X. D. et al., 2012), which can provide acute disease model for researchers to design short-term studies.

Mice, rats, and rabbits are widely used in intra-articular injection models. The most common animal models are rats (Wang X. D. et al., 2012; Li W. et al., 2014; Xu et al., 2016; Xu et al., 2017), because rats are easily managed and require low maintenance costs. Using radio-opaque dye, Hutchins et al. have

### TABLE 1 | Common TMJOA models and their basic characteristics.

| Model                          | Pros                                      | Cons                                      | Indications                                      |
|-------------------------------|-------------------------------------------|-------------------------------------------|-------------------------------------------------|
| Intra-articular injection models | Easy to operate                           | Pathogenic mechanism is different from human TMJOA | Mainly used for study of pain and inflammatory response |
| Surgical induction models     | Induce TMJOA quickly                      | May affect other part of the joint         | Mimic post-traumatic TMJOA                       |
| Mechanical loading models     | Present little risk for animals            | Complex process of model-building         | Mimic TMJOA caused by occlusal factors           |
| High-fat diet models          | Easy to operate                           | Mld lesions                               | Mimic TMJOA affected by obesity factors          |
| Sleep deprivation models      | High repeatability                        | Sleep of rodent animals are naturally different from that of human | Mimic TMJOA under psychological stress |
| Naturally occurring models    | No external interventions and induction required | Slow process of disease | Mimic primary TMJOA |
| Genetically modified models   | Develop disease naturally                  | Only act on specific genes | Study the function of a specific gene in TMJOA pathogenesis |

### TABLE 2 | Intra-articular injection models of TMJOA animal models.

| Drugs | Species                  | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms                                      |
|-------|--------------------------|-------------------------------|-------------------------------|----------------------------------------------------------|
| MIA   | Rat (Wang et al., 2012c) | Cartilage matrix degradation | Subchondral bone degradation | † Mmp-3, Mmp-13, Admats-5, Trf-α, Fas, Fasl, Bax, Caspase-8, Poma, α-SMA in whole condyle; MMP-3, CASPASE-3, α-SMA in hypertrophic layer; Aggreccan, Col1a1, Col2a1 and Timp2 in whole condyle |
|       | Rabbit (Cledes et al., 2006; Guler et al., 2011) | Fibritation | Synovial hyperplasia | Disc perforation; Glonid fossa degradation; Aggreccan in whole condyle; Col1a1, Col2a1 and Timp2 in whole condyle |
|       |                          | Chondrocyte apoptosis | Subchondral bone degradation | Synovial hyperplasia; Disc perforation; Glonid fossa degradation; Aggreccan in whole condyle; Col1a1, Col2a1 and Timp2 in whole condyle |
| CFA   | Mouse (Rotpenpian et al., 2021) | Cartilage defects | Subchondral bone degradation | † RANKL, OCN, MMP-13, COLX, ADAMTS-5 in whole condyle; IHH, PTH1 in hypertrophic layer; SMO, GLI1 in hypertrophic and mineralized layer; OPG in whole condyle; Collagenase and IHH in whole condyle |
|       | Rat (Xu et al., 2016; Xu et al., 2017) | Cartilage matrix degradation | Bone remodeling | Synovial hyperplasia; Disc perforation; Glonid fossa degradation; Aggreccan in whole condyle; Col1a1, Col2a1 and Timp2 in whole condyle |
|       |                          | Endochondral ossification increased chondrocyte synthesis | Subchondral bone degradation | Synovial hyperplasia; Disc perforation; Glonid fossa degradation; Aggreccan in whole condyle; Col1a1, Col2a1 and Timp2 in whole condyle |
|       |                          | Cartilage matrix degradation | Bone remodeling | Synovial hyperplasia; Disc perforation; Glonid fossa degradation; Aggreccan in whole condyle; Col1a1, Col2a1 and Timp2 in whole condyle |
| Collagenase | Mouse (Li et al., 2014a) | Cartilage matrix degradation | Chondroid metaplasia Articular capsule hyperplasia | † Cox-2, P65, Mmp-1, Mmp-13, SOX-9, ADAMTS-5, MMP-9, COL II in whole condyle; CD44 in subchondral bone |
|       | Rabbit (Wu et al., 2015) | Cartilage matrix degradation | Articular cartilage degradation | Timp-3, Col2a1 in whole condyle |
| Papain | Rabbit (Molinet et al., 2020) | Cartilage matrix degradation | Articular disc degeneration | — |
|        |                          | Fibrillation                  | Articular capsule degeneration | — |
|        |                          | Chondrocyte apoptosis         | Decreased lower joint space | — |
| VEGF  | Mouse (Shen et al., 2015) | Cartilage matrix degradation | Subchondral bone degradation | † MMP-9 and MMP-13 in hypertrophic layer; VEGFR2 in all cartilage layers; RANKL in subchondral bone |
|       |                          | Fibritation                  | Subchondral bone sclerosis | — |
|       |                          | Chondrocyte apoptosis         | — | — |
Surgical Induction Models

Surgery is the most widely used approach for building OA models. It can cause structural damage and abnormal articular forces to induce OA-like lesions directly by using surgical and mechanical devices. The common surgical methods include discectomy (Hinton, 1992; Lan et al., 2017; Liu X. et al., 2020; Rottenpion et al., 2021). Xu et al. (2017) suggested that IHH signaling facilitates TMJOA in CFA-induced rats by driving formation of hypertrophic chondrocytes and expression of catabolic enzymes, such as type X collagen, MMP-13, and ADAMTS-5, which may lead to degenerative changes in the articular cartilage. As an angiogenic factor, NETRIN-1 has been discovered that resident fibrocartilage stem cells (FCSCs) localized within the fibrous layer possess potent chondrogenic and osteogenic potential. Additionally, they suggested that regulation of canonical Wnt signals can sustain FCSC pool and maintain tissue homoeostasis, which provide new concepts on the development of potential therapies for TMJ regeneration. Before these regenerative strategies can be applied to humans, future studies using preclinical animal models are still required to include long-term cartilage and bone structure recovery, as well as biomechanical analyses, to verify that functional joint recovery is achieved. Therefore, surgical induction models are more suitable for osteochondral interface repair investigations, given the directly damaged TMJ structure.

One problem with many invasive models, however, is that they only operated on one side of the TMJ and use the other side as controls (Shinoda et al., 2005; Embree et al., 2015; Lemos et al., 2016). Unlike most other synovial joints, TMJ is bilaterally linked, and mastication, mouth opening, and other actions need to be completed together. Therefore, when using unilateral intervention methods, the influence on the other joint should be considered (Cohen et al., 2014). In recent years, various methods have been improved to create models while preserving as much tissue as possible in TMJ to prevent the effect of the surgical procedure on animals (Gu et al., 2006; Nguyen et al., 2020). In addition, different surgical approaches used in the same model will cause different pathological changes. Therefore, further studies on invasive models are needed in the future to achieve better model establishment.

Non-Invasive Models

Non-invasive models cause joint injury by applying external mechanical force, high fat diet, or mental stimulation without causing open trauma or articular capsule damage. In this way, the model-building process is completely sterile, and the effect of the invasive models on remaining joint tissues is eliminated. In addition, no surgical procedures are required on animals because such models mainly use mechanical devices to assist modeling.

Mechanical Loading Models

Appropriate stress stimulation can promote chondrocyte proliferation and extracellular matrix synthesis. However,
TABLE 3 | Surgical induction models of TMJOA animal models.

| Surgical Induction Models | Species | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------------------------|---------|-------------------------------|-------------------------------|----------------------|
| Discectomy                | Mouse (Liu et al., 2020a; Lan et al., 2017) | Cartilage defect, Cartilage matrix degradation | Subchondral bone degradation, Diffuse osteochondral junction | ↑ NOTCH1, HESS, TLR4, IL-1β, TNF-α, ADAMTS-5, MMP-13 in cartilage, JAGGED-1 in chondrocyte clusters; Nf-E2 P65, MyoD8 in fibrous layer | |
|                           | Rat (Kim, 1992) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation, Diffuse osteochondral junction | ↓ HES1 in whole condyle |
|                           | Rabbit (Saito et al., 2021) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Large marrow cavities | ↓ Aggrecan, Co2a1 in whole condyle |
| Partial discectomy        | Mouse (Ku et al., 2009; Le et al., 2022) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation, Diffuse osteochondral junction | ↑ RUNK2, BSP in hypertrophic layer; CD31, α-SMA in cartilage |
|                           | Rabbit (Man et al., 2009) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation | ↑ COL I, COL II in hypertrophic layer |
| Disc perforation          | Rat (Luo et al., 2020) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation, Diffuse osteochondral junction | ↑ ADAMTS-5 in hypertrophic layer; CHOP, CASPASE-3, GRP78, Caspase-12 in whole condyle |
|                           | Rabbit (Embree et al., 2015) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation | — |
|                           | Pig (Ruscillo et al., 2020) | Cartilage defect, Cartilage matrix degradation, Fibrillation | Subchondral bone degradation | — |
| Anterior disc displacement | Rat (Togni et al., 2018; Nguyen et al., 2020) | Cartilage matrix degradation, Cartilage hyperplasia | Subchondral bone degradation, Genoid fossa hyperplasia | — |
|                           | Rabbit (Ku et al., 2018) | Cartilage matrix degradation, Cartilage hyperplasia | Subchondral bone degradation, Genoid fossa hyperplasia | — |
| Injury of condylar surface | Sheep (Ishimaru and Goss, 1992; Wang et al., 2017a) | Cartilage matrix degradation, Endochondral ossification, Osteophytes | Subchondral bone degradation, Articular disc perforation | — |
| Postero-superior displacement of mandible | Rabbit (Imai et al., 2001; Liu et al., 2006) | Cartilage defect, Cartilage matrix degradation, Osteophytes | Subchondral bone degradation, Articular disc deformity, Articular eminence hyperplasia | — |

when damage caused by mechanical loading exceeds the joint’s ability to repair itself, the affected joint would suffer damage, and even develop into OA (Tanaka et al., 2008; Liu et al., 2021). The functional movement and biomechanical loading of TMJ are closely related to occlusion. Abnormal dental occlusion is one of the potential causes of TMJOA, which includes severe malocclusion and skeletal jaw asymmetry. Thus, the TMJOA models can be built by disordered occlusion, which is the TMJ-malocclusion and skeletal jaw asymmetry. Thus, the TMJOA models can be used to establish TMJOA models by affecting the metabolism of the condyle. The above models simulated TMJOA caused by occlusal factors in clinical patients (Table 4).

The most common animals used for mechanical loading models are rats (Teramoto et al., 2003; Li et al., 2013; Long et al., 2019; Yang et al., 2020; Li Y. et al., 2021; Zou et al., 2022), because rats are common rodents and can tolerate the installation of mechanical devices. However, due to the significant differences in occlusal and TMJ structure between rats and human, OA-like lesions induced by the mechanical loading method are not completely equivalent to human TMJOA lesions (Wang et al., 2015). Given the high similarity between mechanical loading models and human TMJOA caused by occlusal factor, larger animals with a more similar structure to human TMJ, such as pigs and sheep, should be used in future studies on pathogenesis.

Currently, mechanical loading models are mainly applied to study the mechanism of pathological changes in TMJOA (Jiao et al., 2009; Jiao et al., 2011; Ma et al., 2020; Ou et al., 2021), probably because this kind of model directly mimics the disease process in TMJOA patients caused by disordered occlusion. The pathogenic mechanism of TMJOA in mechanical loading models has been widely discussed. Zhang et al. (2022) revealed that elevated expression of SEMA4D in early-stage TMJOA might
TABLE 4 | Mechanical loading models of TMJOA animal models.

| Mechanical loading models | Species | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------------------------|---------|------------------------------|-----------------------------|---------------------|
| Orthodontic tooth movement | Rat (Wang et al., 2012b; Zhang et al., 2013) | Cartilage matrix degradation | Subchondral bone degradation | ↑ CTXs in serum; RUNX-2, VEGF, CTGF, MMP-9, CHM-1, M-CSF, RANKL/OPG in hypertrophic layer; BECLIN-1, LC3-II in whole condyle |
|                           |         | Chondrocyte autophagy in hypertrophic layer | Bone remodeling | ↑ OPG, Mapk4/3 in hypertrophic layer; p-MTOR, p-PTEN S6K in whole condyle |
|                           |         | Endochondral ossification | Osteophytes | ↑ GRP78, CHOP, CASPASE-12, cleaved-CASPASE-3, Trap, Mmp-13 in cartilage |
|                           |         | Osteophytes | Osteoclastogenesis | ↑ PCNA, COL II, COL X in hypertrophic layer; CD73, Npp1 in cartilage |
| Unilateral anterior crossbite | Mouse (Lu et al., 2020a) | Cartilage matrix degradation | Subchondral bone degradation | ↑ IL-6, TH in hypertrophic layer; IHH, SMO, MMP-13, CASPASE-3 in proliferative and hypertrophic layers; GLI-1 in cartilage |
|                           | Rat (Wang et al., 2014b; Zhang et al., 2016) | Chondrocyte apoptosis in hypertrophic layer | Neoin mineralization | ↑ TH in fibrous and proliferative layers |
|                           |         | Mineral deposition | | ↑ BRDU in proliferative layer; Op, PDI, CRT, CHOP, CASPASE-3, BIP, p-BF2a in cartilage |
| Unilateral bite-raise | Mouse (Ou et al., 2021) | Fibrillation | Subchondral bone degradation | ↑ COL II, COL X, p-PIN1, TCTP, Runx2 in whole condyle |
|                           | Rat (Long et al., 2019) | Cartilage matrix degradation | Inflammation | ↑ MMP-13, CXCRI, SDF-1 in hypertrophic layer; RUNX2 in cartilage; OSX, p-S6 in subchondral bone |
| Mandibular movement restriction | Rat (Teramoto et al., 2003, Li et al., 2013) | Cartilage matrix degradation | Subchondral bone degradation | ↑ Myelofibrosis |
|                           |         | Chondrocyte apoptosis in hypertrophic layer | Local bone sclerosis | ↑ MMP-13, CXCR4, SDF-1 in hypertrophic layer; RUNX2 in cartilage; OSX, p-S6 in subchondral bone |
| Mandibular advancement | Rat (Yang et al., 2020; Li et al., 2021b) | Cartilage matrix degradation | Bone remodeling | ↑ Myelofibrosis |
|                           |         | Chondrocyte apoptosis | Bone remodeling | ↑ Mmp8, Itf1, Itf3, Itgb1, Itgb3, Itgb2, VEGF in cartilage; mNos in synovial membrane |
| Mandibular lateral deviation | Rat (Zou et al., 2022) | Cartilage defect | Subchondral bone degradation | ↑ Sox9, Itgb4 in cartilage; SOD in synovial membrane |
|                           | Rabbit (Zhao et al., 2010) | Cartilage matrix degradation | Myelofibrosis | ↑ MMP-3 in cartilage; TIMP-1 in hypertrophic layer and synovial membrane |
| Impact loading | Goat (Wang et al., 2008) | Cartilage defect | Bone remodeling | MMP-13 first increase and then decrease |
|                           |         | Cartilage matrix degradation | Exposure of subchondral bone | ↑ Articular disc defection |
|                           |         | Osteophytes | Synovial hyperplasia | Exposure of subchondral bone |
| Forced-jaw-opening | Mouse (Khurel-Ochir et al., 2021) | Fibrillation | Fibrous adhesion | ↑ MMP-1, MMP-3, MMP-9, MMP-13, IL-1β, Caspase-3, VEGF in proliferative and hypertrophic layers |
|                           | Rat (Nicol et al., 2010) | Cartilage matrix degradation | Articular disc defection | ↑ ACAN in cartilage |
|                           | Rabbit (Fujisawa et al., 2003) | Chondrocyte apoptosis | Bone remodeling | ↑ MMP-3, MMP-13 in whole condyle; Prg4 in cartilage |
| Soft diet | Mouse (Robinson et al., 2019) | Cartilage matrix degradation | Subchondral bone degradation | ↑ IL-2, IL-15, TGF-β in cartilage |
|                           | Rat (Zhang et al., 2021a) | Chondrocyte apoptosis | Decreased bite force | |

decrease the bone formation activity of osteoblasts in the subchondral bone by binding to PLEXIN-B1 expressed by osteoblasts. HIF-1, which may repress OPG expression, was activated in mature chondrocytes in mechanical loading models, resulting in osteoclastogenesis and development of TMJOA (Shirakura et al., 2010). He et al. (2018) discovered several new genes that had never been reported to be associated with TMJOA by RNA sequencing. These genes may be used as potential therapeutic genes related to TMJOA. In the future, attention should be paid to the development of therapeutic strategies that take full advantage of this model.

**High-Fat Diet Models**

Obesity is found to be associated with OA. Overweight and obesity do not only significantly increase the risk of incident hip and knee OA, but also aggravate its radiographic changes (Johnson and Hunter, 2014). Studies in experimental animals have shown that obesity increases the incidence and severity of OA (Issa and Griffin, 2012). According to the cross-sectional studies, obesity is also associated with TMJ disease (Jordani et al., 2017; Karaman and Sadry, 2021). In addition, several chewing characteristics, such as chewing speed and duration, are associated with obesity in young adolescents, and they might affect the development of the TMJ. Therefore, further investigation is needed to reveal the relationship among jaw mastication, obesity, and TMJOA.

Griffin et al. (2010) first observed loss of proteoglycans in the TMJ of C57BL/6J mice with a high-fat diet (45% kcal fat) for 45 weeks. Du et al. (2020) studied the effect of high-fat diet (60% kcal fat) on TMJ of C57BL/6 mice. Less cartilage matrix, thinner
condylar cartilage, and vertical clefs were observed in overweight mice after 12 weeks of high-fat feeding. Additionally, they found that the expression of IL-1β, MMP-3 and leptin were upregulated in condylar cartilage of high-fat-fed mice. Patients with knee OA have increased level of serum leptin and the abnormal leptin level in synovial fluid. Leptins are one of the increased proinflammatory factors in individuals with obesity (Conde et al., 2013). This research team also confirmed that statins had anti-inflammatory effects in TMJOA-like changes and a protective effect on the damaged TMJ cartilage.

Not only does diet induce obesity-increased OA joint pathology in mice but also induces anxiety and hyperalgesia, and reduces muscle function and locomotor activity (Griffin et al., 2010). Currently, studies only focus on the establishment of high-fat TMJOA model, and more investigations will be required in the future to reveal the mechanisms involved in obesity-induced TMJOA.

Sleep Deprivation Models

Osteoarthritis is one of the characteristics of premature aging in organisms. It is affected by circadian disturbances (Berenbaum and Meng, 2016). Many studies have shown that psychological factors, such as sleep disorders, mental stress, and depression, may be related to TMJ dysfunction (LeResche et al., 2007; Slade et al., 2007). Therefore, establishment of sleep deprivation models could be helpful for related studies. This model mainly applies the modified multiple platform method (MMPM) proposed by Suchecki and Tufik (2000). The principle of this technique is to take advantage of the rat’s fear of water and inability to sleep in water. A certain number of platforms with small diameters (≤6.5 cm in diameter) are placed in a tank filled with water. The rats can stand on the platform and jump between the platforms. When the rats are about to sleep, their muscles relax and their faces would touch the water, which could awake them to achieve the goal of sleep deprivation in rats.

Chen et al. (2013) first showed that the surface of fibrous layer was cracked and exfoliated in sleep-deprived rats, compared with control rats, suggesting that sleep deprivation may lead to histopathological changes in the TMJ of rats. Chen et al. (2020) then successfully built a TMJOA model by using a similar model and demonstrated that sleep deprivation could induce OA-like lesions in TMJ of rats, and the OA-like lesions may be reversible in the early stage. Additionally, they found that rhythmic gene expression dysregulation in sleep deprivation models, which further leads to MAPK/ERK signaling pathway activation and then aggravates TMJOA. Chen Y. et al. (2019) indicated that hypoxia played an important role in TMJOA and accelerated angiogenesis of condylar cartilage through the HIF-1-VEGF-Notch signaling pathway. These studies may provide new insights into the clock gene mechanism of endochondral homeostasis and the complex pathophysiological mechanism of TMJOA.

Currently, this model only applies to rats, and the therapeutic effect of low intensity pulsed ultrasound (LIPUS) on this model has been studied. Liang et al. have found that LIPUS had a good treatment effect on early TMJ injury by regulating the MMP-3/TIMP-1 and RANKL/OPG expression ratios in cartilage tissues, and have demonstrated that LIPUS treatment at an intensity of 45 mW/cm² for at least 2 weeks is the optimal regimen for TMJOA in rats (Liang et al., 2019; Liang et al., 2020). Given the difficulty of using humans as participants to advance this study, the establishment of an experimental animal model of TMJOA is necessary to further study the pathogenesis of TMJOA under psychological stress, especially in studies for testing clinical treatment and exploring better medications.

Naturally Occurring Models

Some animals develop OA-like lesions with slow progression, which is very similar to the disease progression of primary OA in humans. Therefore, such models are often referred to as naturally occurring models. Studies have shown that STR/Ort (Kumagai et al., 2015; Yamashita-Futani et al., 2021), STR/IN (Dreessen and Halata, 1990), SAMP8 (Ishizuka et al., 2014), C57BL/6S (Fukuoka et al., 1993), C57BL/6J (Cui et al., 2020), C57BL/6NCrSlc (Ukita et al., 2020), ICR (Silbermann and Livne, 1979; Livne and Silbermann, 1986) mice, Dunkin-Hartley guinea pigs (Wu et al., 2016), and horses (Smyth et al., 2019) all manifest OA-like lesions with increasing age, among which multiple subtypes of SAM mice can develop OA-like lesions in TMJ (Chen et al., 1989) (Table 5). Yamashita-Futani et al. (2021) found in STR/Ort mice that the production of elastin-digested peptides was related to the upregulation of pro-inflammatory mediators, such as IL-6 and MMP-12. IL-6 induced the expression of ADAMTS-4 and ADAMTS-5 in chondrocytes, following cartilage degradation. OA lesions also appeared in articular cartilage of C57B/6S mice and were correlated to increased levels of collagen-like peptidase and prolyl endopeptidase in the serum, which indicated collagen degradation (Fukuoka et al., 1993). Ishizuka et al. revealed that a downregulation of IHH signaling accompanies the early onset TMJ degeneration changes in senescence-accelerated mice (Ishizuka et al., 2014). Naturally occurring models are ideal for studying cartilage degradation and bone remodeling in TMJOA and can provide evidence for the study of pathogenesis of TMJOA at different ages. The induced model-building methods can also be used to cause diseases in such animals, which can naturally result in TMJOA to study the effect of external stimulus during the disease course of primary TMJOA.

The naturally occurring models have slow disease progression. Like the spontaneous OA-like lesions in human TMJ, naturally occurring models do not require invasive procedures to generate the arthritis, thus eliminating many potential side effects. They are thought to be closely related to the natural progression of TMJOA in humans, which are inapplicable for simulating the development of post-traumatic TMJOA. Since obtaining the articular cartilage samples of TMJOA in humans is difficult, naturally occurring models have gradually served as important models for the study of pathogenesis of OA. The underlying mechanisms that drive the onset and progression of spontaneous TMJOA in these animals are not well defined and may reflect specific subtypes of idiopathic human TMJOA. Currently, few studies have been conducted on this type of model. Due to the extremely long study period and high cost, most of these studies focus on the etiological mechanism.
Genetically Modified Models
The use of genetically modified mice has greatly improved our understanding of the precise molecular pathophysiology and therapies of many human diseases (Little and Hunter, 2013). In the field of TMJOA, specific genetic modifications are made to the mice to reveal the role of different genes in TMJ development or disease processes. Unlike invasive animal models, genetically modified models can provide biological information for a population that is prone to developing TMJOA. Since genetic and environmental factors can be precisely controlled, this kind of model has the potential to reveal molecular pathways involved in the progressive degeneration of TMJ.

Preclinical studies of genetically modified mice have increased over the past two decades, making them the best candidate models for the study of the molecular pathway involved in TMJOA (Table 6). The genes involved in this review can be divided into three main categories. The first group mainly contains transcription factors or signaling regulators (β-catenin, Hif-1α, Opg, Smad3, Runx2, Osx, Bmp2, Tgf-β1, Axin1, Shox2), enzymes (1α(OH)ase, Dnmt3b), and receptors (Fgfr3, Bmpr1a, Ddr1, Ddr2). Genes that participate in joint inflammation are the second group, including cytokine (Il-1β), receptor (Il-1βra) and enzyme (Adams5). The third group includes genes encoding for extracellular matrix components (Col2a1, Col9a1, Col11a1, Prg4, Bgn, Fmod, Dmp1). The genes and gene products identified in genetically modified models as decreasing or increasing the severity of TMJOA-associated cartilage erosion can all be considered potential therapeutic targets. Appropriate inhibitors of these proteins and activators or recombinant versions may lead to the development of new therapies, which need to be further investigated.

Xu et al. found increased expression of DDR2 and increased proteoglycans. One of the consequences of proteoglycan degradation is to enhance the exposure of chondrocytes to inflammation in synovial membrane; this may result in the activation of chondrocyte clusters and increased proteoglycan production in the pericellular matrix have also been identified as early OA indicators. Although the mouse models cannot simulate the biomechanical function of human joints, it is a major option for molecular studies. This is due to the advances in mouse genetics, and the easy availability of genetically modified mice, allowing the evaluation of time-dependent changes in TMJOA.

| Strain of animals | Age of Onset | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|-------------------|--------------|-------------------------------|-----------------------------|---------------------|
| STR/Ort mice      | 40-week-old  | Cartilage defect              | Subchondral bone degradation| ↑ MMP-12 in cartilage; IL-6, ADAMTS-4, ADAMTS-5 in subchondral bone |
|                   |              | Cartilage matrix degradation  | Subchondral bone resorption | —                   |
|                   |              | Increased thickness of cartilage | Synovial metaplasia         | —                   |

| STR/IN mice       | 26-week-old  | Cartilage defect              | —                            | —                   |
|                   |              | Cartilage matrix degradation  | Subchondral bone degradation | —                   |
|                   |              | Increased lysosomes in chondrocytes | Decreased lower joint cavity | —                   |
|                   |              | —                             | No inflammation in synovial membrane | —                   |

| SAMP8 mice        | 16-week-old  | Cartilage matrix degradation  | —                            | —                   |
|                   |              | Increased thickness of cartilage | —                            | —                   |

| C57BL/6S mice     | 12-week-old  | Cartilage defect              | Subchondral bone degradation| ↑ Col1a1, Col2a1 in cartilage; Col10a1, Ihh, Gli1, Gli2, Ptc1, Hip in whole condyle |
|                   |              | Cartilage matrix degradation  | Synovial hyperplasia         | —                   |
|                   |              | Osteophytes                    | —                            | —                   |

| C57BL/6J mice     | 45-week-old  | Cartilage defect              | Subchondral bone degradation| ↑ MMP-13, COL X in cartilage; P16ink4a, pSMAD3, CTSK in subchondral bone |
|                   |              | Cartilage matrix degradation  | Bone remodeling              | —                   |
|                   |              | Increased thickness of cartilage | —                            | —                   |

| C57BL/6Ncr1c mice| 80-week-old  | Cartilage defect              | Subchondral bone degradation| ↑ H3K9Me1, H3K9Me2, H3K9Me3 in hypertrophic layer |
|                   |              | Cartilage matrix degradation  | —                            | —                   |
|                   |              | Osteophytes                    | —                            | —                   |

| ICR mice          | 28-week-old  | Cartilage defect              | Subchondral bone degradation| ↑ CAD-11, MMP-3 in proliferative and hypertrophic layer of cartilage |
|                   |              | Cartilage matrix degradation  | Focal ankylosis between condyle and articular disc | —                   |

| Dunkin-Hartley guinea pigs | 12-week-old | Cartilage matrix degradation | Large marrow cavities | ↑ COL II in proliferative and hypertrophic layer of cartilage |

| Horses            | —           | Cartilage matrix degradation  | Articular disc degradation | Articular disc metaplasia | —                   |
| Protein | Mice model | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------|------------|-------------------------------|-------------------------------|---------------------|
| Beta catenin associated protein | β-catenin(ex3)Flp<sup>Cre</sup> (Wang et al., 2014a) | Cartilage collapse | Subchondral bone sclerosis | COL X in hypertrophic layer; RUNX2, Mmp-13, Adamts-4, Adamts-5 in cartilage |
| | β-catenin(ex3)fl/fl<sup>CreER</sup> | Cartilage matrix degradation | Subchondral bone sclerosis | MMP-13 in fibrous, hypertrophic layer and articular disc; COL X in cartilage; ADAMTS-4, ADAMTS-5 in fibrous layer |
| | Hypoxia-inducible transcription factor 1α | Hif-1α<sup>fl/fl</sup> (Mino-Oka et al., 2017) | Cartilage matrix degradation | ↑ MMP-9, cleaved-CASPASE-3 in hypertrophic layer |
| | | Hif-1α<sup>fl/fl</sup>; ctsk cre<sup>+</sup> (Tang et al., 2023) | Cartilage matrix degradation | ↑ CASPASE-3 in cartilage; OCN in subchondral bone |
| Osteoprotegerin | Opg<sup>−/−</sup> (Chen et al., 2019a) | Cartilage matrix degradation | Subchondral bone sclerosis | COL X in cartilage |
| Mothers against decapentaplegic homolog 3 | Smad3<sup>−/−</sup> (Mori et al., 2015) | Cartilage matrix degradation | Subchondral bone sclerosis | MMP-9, MMP-13, CASPASE-3, CASPASE-9 in cartilage |
| | | Cartilage matrix degradation | Subchondral bone sclerosis | p-SMAD3 in fibrous layer; COL II, ACAN, SPHK1, S1P<sub>3</sub> in cartilage |
| Runx-related transcription factor-2 | Runx2<sup>fl/fl</sup>; Agc1-CreER<sup>T2</sup> (Liao et al., 2019) | Cartilage matrix degradation | Subchondral bone sclerosis | COL II, COL X in hypertrophic layer; VEGF, CD31, TRAF5, Ctsk in cartilage; MMP-9 in subchondral bone |
| Osterix | Osx<sup>−/−</sup>; Agc1-CreER<sup>T2</sup> (Jing et al., 2014a) | Cartilage matrix degradation | Subchondral bone sclerosis | COL X, PCNA, IHH in hypertrophic layer; Mmp-13, Col2α1, Acan in cartilage |
| Bone morphogenetic protein 2 | Bmp2<sup>−/−</sup>; Agc1-CreER<sup>T2</sup> (O’Brien et al., 2021) | Cartilage matrix degradation | Subchondral bone sclerosis | COL II, COL X, ACAN, SOX9 in hypertrophic layer |
| Transforming growth factor β1 | Tgf-β1 mutant (Jiao et al., 2014) | Cartilage matrix degradation | Subchondral bone sclerosis | VEGF, MMP-9, MMP-13, CASPASE-3 in hypertrophic |
| Axis inhibition protein 1 | Axin<sup>−/−</sup> (Zhou et al., 2019a) | Cartilage matrix collapse | Subchondral bone sclerosis | MMP-13, ADAMTS-5 in superficial layer; CATNB, Col10α1, Fgfr1, Fgfr2, Fgfr3, pERK1/2 in cartilage |
| Short stature homeobox 2 | Wnt1-Cre; pMes-stop Shox2 (Li et al., 2014b) | Cartilage dysplasia | Subchondral bone sclerosis | COL II in cartilage; IHH, GLI2 in condyle |
| | Shox2<sup>Scribble</sup> (Li et al., 2014c; Liang et al., 2016) | Cartilage matrix degradation | Subchondral bone sclerosis | MMP-9, MMP-13 in cartilage |
| 1α-hydroxylase | 1α(OH)ase<sup>−/−</sup> (Shen et al., 2013) | Cartilage matrix collapse | Subchondral bone sclerosis | COL II in cartilage |

(Continued on following page)
**TABLE 6 | (Continued) List of genes of genetically modified models.**

| Protein | Mice model | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------|------------|-------------------------------|------------------------------|---------------------|
| DNA (cytosine 5)-methyltransferase 3 beta | Dnmt3a/-, Agc1-CreERT2 (Zhou et al., 2019a) | Cartilage defection | Presence of pain | ↑ KIA7, COL X, CATNB in cartilage ↓ COL II in cartilage |
| | | Cartilage matrix degradation | ↑ KIA7, COL X, CATNB in cartilage ↓ COL II in cartilage |
| Fibroblast growth factor receptor 3 | Fgfr2ppx4 (Yasuda et al., 2012) | Cartilage defection | Subchondral bone resorption | ↑ Ihh, Ptc1, Hh4, Col2a1, Col10a1 in cartilage; Col1a1, Mmp-13 in subchondral bone; Ihh, Col1a1, Op in secondary cartilage ↓ COL X, MMP-13, ADAMTS-5 in fibrous layer; Ihh, RUNX2 in cartilage ↓ PRG4 in fibrous layer |
| | Fgfr2ppx4, Col2a1-CreER72 (Zhou et al., 2016) | Cartilage defection | Subchondral bone sclerosis | ↓ COL II, COL X, Sox9, in cartilage; OSX in subchondral bone |
| Bone morphogenetic protein receptor-1A | Bmpr1a/-, Agc1-CreER (Lam et al., 2007; Long et al., 2016) | Cartilage defection | Subchondral bone sclerosis | ↓ COL II, COL X, Col10a1, Runx2 in cartilage ↓ COL II, Nid-2, Col3a1, Acan, Sox-9 in cartilage |
| Discoidin domain receptor 1 | Ddr1Fcre (Schminke et al., 2014) | Cartilage defection | Subchondral bone degradation | ↓ COL I, COL III, Col10a1, Runx2 in cartilage ↓ COL II, Nid-2, Col3a1, Acan, Sox-9 in cartilage |
| Discoidin domain receptor 2 | Ddr2Fcre (Ge et al., 2018) | Cartilage defection | Subchondral bone degradation | ↓ COL I, COL III in hypertrophic layer; Sox9, MMP-13 in cartilage; BGLAP in subchondral bone |

**Genes encoding inflammation mediators**

| Protein | Mice model | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------|------------|-------------------------------|------------------------------|---------------------|
| Interleukin-1β | Col1-Il-1βfl/fl (Lai et al., 2006; Huang et al., 2013) | Cartilage defection | Presence of pain | ↑ COL II, MMP-9, IL-6, COX-2, TGF-β in hypertrophic layer; NGF, TRKAR in cartilage |
| | Il-1βfl/fl (Tabeian et al., 2019) | Cartilage matrix in fibrous layer first increase and then decrease | Subchondral bone degradation | — |
| A disintegrin and metalloproteinase with thrombospondin motifs 5 | Adamts5Fcre (Rogers et al., 2018; Rogers-DeCotes et al., 2021) | Cartilage matrix degradation | Subchondral bone degradation | ↓ ACAN in cartilage ↓ COL II, COL III in hypertrophic layer; Sox9, MMP-13 in cartilage; BGLAP in subchondral bone |

**Genes encoding components of the extracellular matrix**

| Protein | Mice model | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------|------------|-------------------------------|------------------------------|---------------------|
| Type II collagen | Dmmn/- (Rocks et al., 2013; Long et al., 2016) | Cartilage defection | Diffuse osteochondral junction | ↑ TGF-β1, p-SMAD2, HTRA1 in chondrocytes; MMP-13, DDR2 in cartilage |
| | Del1 mice (Rintaia et al., 1997) | Cartilage matrix degradation | Subchondral cysts | — |
| Type IX collagen | Col9a1Fcre (Lam et al., 2007; Polur et al., 2010) | Cartilage defection | Diffuse osteochondral junction | ↑ HTRA1, MMP-13 in fibrous layer; DDR2 in cartilage; MMP-derived type II collagen fragments in fibrous layer |
| Type XI collagen | Col11a1Fcre (Lam et al., 2007; Polur et al., 2010; Long et al., 2016) | Cartilage defection | | ↑ HTRA1 in fibrous layer; DDR2, MMP-13 in cartilage; TGF-β1, p-SMAD2, HTRA1 in chondrocytes; MMP-derived type II collagen fragments in superficial layer |
| Proteoglycan-4 | Prg4Fcre (Hill et al., 2014; Koyama et al., 2014) | Cartilage defection | Subchondral bone resorption | ↑ COL II, COL X in hypertrophic layer; CTSK in subchondral bone; SOX-9 in cartilage; HAS-2 in cartilage, glenoid fossa, and synovial membrane |
| | | Cartilage matrix degradation | Articular disc hyperplasia | ↑ COL II, COL X in hypertrophic layer; CTSK in subchondral bone; SOX-9 in cartilage; HAS-2 in cartilage, glenoid fossa, and synovial membrane |
| Biglycan Fibromodulin | BgnFcre; FmodFcre (Wadhwa et al., 2005; Embree et al., 2010) | Cartilage defection | Subchondral bone resorption | ↑ COL I, COL II in fibrous layer |
| Dentin matrix protein 1 | Ss9g-Dmp1 mice (Weng et al., 2017) | Cartilage defection | Osteophytes in glenoid fossa | ↓ ACAN in cartilage ↓ PCNA first decrease and then increase |
| | | Cartilage matrix degradation | Subchondral bone degradation | ↑ MMP-13, CASPASE-9 in cartilage ↓ COL I, COL II, ACAN, DCN, SOX9, PCNA. (Continued on following page) |
after specific genetic modifications. Moreover, genetically modified models have the virtue of eliminating other interferences to allow researchers directly observe how individual genes influence the process of TMJOA at the genetic level. Consequently, this kind of model facilitates the study and establishment of the molecular basis for TMJOA development. However, multiple genes are generally implicated in the pathogenesis of human TMJOA (Pitsillides and Beier, 2011), whereas genetically modified models mainly act on specific genes. Therefore, this model cannot comprehensively simulate the situation of TMJOA induced by multi-gene interaction. As with naturally occurring models, researchers need consider the lengthy experimental time and the cost of housing these animals.

**CURRENT ANIMALS USED IN TEMPOROMANDIBULAR JOINT OSTEOARTHRITIS RESEARCH**

Animal models are the primary means of testing potential therapeutic agents to determine their potential efficacy in TMJOA. However, existing animal models are inadequate to simulate complex clinical conditions. On the one hand, most animal models of TMJOA are single-factor models, which only simulate one specific pathogenic factor of clinical patients. On the other hand, most animal models are characterized by histological phenotypes and a few key molecular markers of TMJOA. Many models mimic the phenotype, but the similarity to the underlying molecular components of human TMJOA is typically not known.

As mentioned earlier, rodents are the most frequently used animals for TMJOA modeling. The primary disadvantages of these models are related to differences in anatomical structure and joint mechanics between these species and humans. The mandibular condyles of rodents extend antero-posteriorly, whereas in humans, the direction is lateromedial (Bermejo et al., 1993). The anterior-posterior axial length of the condyle is about 5 mm in rats and about 10 mm in rabbits, both of which were much smaller than that in humans (Orset et al., 2014; Monteiro et al., 2021). Moreover, the condyle axis is sagittal in rodents for propulsion movement, whereas it is transversal in humans for tridimensional motions, including opening, deduction, and propulsion (Orset et al., 2014). Some differences in disease expression between animal and human remain inexplicable. Unlike in humans, the incidence and severity of TMJOA is higher in male mice than in female mice (Silberberg and Silberberg, 1963). Even so, the advantages of small animal models include relatively low cost, ease of handling, more rapid disease progression, and availability of housing. As a result, they have been particularly popular for evaluating new therapeutic interventions and investigating the pathological process of TMJOA.

The advantage of large animal models is that they are anatomically similar to humans, particularly in joint size and cartilage thickness. Pigs have been regarded as the most suitable experimental model for human TMJ due to their similar condyles, articular disc, and mechanical properties to those of humans (Herring et al., 2002; Sun et al., 2002). However, disadvantages of large animal models are primarily related to the high costs, long maturation periods, and slow disease progression. Due to the rapid progress of TMJOA in small animals, the TMJ in small animals can be used for screening of potential therapeutics. The efficacy of drugs in small animals may not accurately reflect the efficacy observed in human TMJOA. Therefore, the TMJs from large animals, such as pigs and sheep, are still needed for preclinical studies to evaluate the clinical processes and their treatment in TMJOA. Despite various problems, animal models are still irreplaceable at least in the study of the pathology and progression of TMJOA rather than the etiology of TMJOA.

**DISCUSSION**

Animal models of TMJOA are important tools for studying the pathogenesis of TMJOA and evaluating potential therapeutic interventions. The value of these models mainly depends on how well they correspond with human disease. Various methods are used to build disease models of TMJOA, but each kind of model has its limitations. For each new study, considering the application of each model may help guide model selection. Different animal models could induce different TMJOA lesions. For example, chemical models can be used for the study of pain mechanisms; surgical models may be optimal for therapeutic study. Mechanical models may be appropriate for the study of pathogenesis; naturally occurring models would provide best models for studying aging phenotype; and genetically modified models are required for research of specific genes.

Animal models of OA can be classified into five categories: naturally occurring, genetically modified, surgically induced, chemically induced, and non-invasive animal models (Lampropoulou-Adamidou et al., 2014; McCoy, 2015). Some laboratory animals, such as certain strains of Syrian hamsters, dogs and cynomolgus macaques can develop OA spontaneously. These animals have not been used in studies of TMJOA yet. Since the anatomical structure and physiological composition of other synovial joints are different from those of the TMJ, most surgically induced models and non-invasive models cannot be

| Protein | Mice model | Changes of Condylar Cartilage | Changes in other parts of TMJ | Molecular Mechanisms |
|---------|------------|-------------------------------|-----------------------------|---------------------|
|         | Cartilage degradation | Cartilage defect | Chondrocyte apoptosis in subchondral bone | Tgfb1, Alk1, Alk5, Smad1, Smad2, Smad3, Smad5, Smad9 in cartilage |

**TABLE 6** (Continued) List of genes of genetically modified models.
applied to the modeling of TMJOA. Among them, impact loading has been applied to TMJ and successfully induced OA-like lesions in TMJ (Wang et al., 2008). In addition to the five drugs used for intra-articular injection models, quinolone and carrageenan are commonly used in other synovial joints to induce OA (McCoy, 2015). These drugs can be used to induce TMJOA in the future.

Age is one of the strongest risk factors for OA. The incidence and severity of TMJOA increase with age (Haskin et al., 1995). In contrast, most preclinical studies are conducted in young animals. For example, in the most widely used rat model of TMJOA, namely unilateral anterior crossbite (UAC), mechanical loading is usually performed on 6-8-week-old rats. However, comparison of 6-week-old and 28-week-old rats revealed that age affects the basal pattern of gene expression in joint tissues. When UAC is performed on 28-week-old rats, the ensuing TMJOA is more severe than in young rats (Zhang Y. et al., 2021). Since OA-like lesions occur spontaneously with age in naturally occurring models, various induction methods can be used on these animals to study how other stimuli and aging synergistically affect TMJ.

Reports on the prevalence of TMJOA have shown significant gender differences (Zhao et al., 2011; Li et al., 2020). Its preponderance in women and early onset during reproductive years according to epidemiological research are totally different from the epidemiological characteristics of other joints, such as in knee OA, which primarily happens to postmenopausal women (Zhao et al., 2019; Dai et al., 2020). Severe TMJOA has been reported in young females whose blood oestrogen levels were medically low (Gunson et al., 2009), thus the relationship between TMJOA and oestrogen has attracted much attention. Studies have shown that oestrogen has an important effect on bone and cartilage metabolism. Oestrogen can regulate the secretion of cytokines and affect some key metabolic pathways to regulate bone and cartilage metabolism (Wang Q. P. et al., 2012). Several animal studies have confirmed that oestrogen deficiency leads to cartilage degeneration in the condyle, and results in more severe TMJOA-like lesions in the presence of mechanical stress stimulation (Nogami et al., 2020; Zhang J. et al., 2021). More studies are needed to further explore the role of oestrogen in the pathogenesis of TMJOA and related molecular mechanisms.

Notably, TMJOA involves not only cartilage but all the joint tissues; therefore, analysis of cartilage and periarticular tissues is recommended in vivo studies. Most of the studies evaluated cartilage degeneration and bone reconstruction by histology, histomorphometry, and immunohistochemistry. Only few studies analyzed the changes in articular disc, synovial membrane, and temporal surface. Therefore, the pathological changes in the whole joint should be studied to understand the etiological mechanism of TMJOA from a comprehensive perspective.

Over the past 40 years, TMJOA animal models have undoubtedly improved our understanding of the pathophysiology of the disease and contributed to the development of disease-modifying therapies. This review presents an overview of animal models used to study TMJOA, as well as the usefulness, histopathological changes, and scope of application of each model and current animals used in TMJOA research. Although many methods are used to build disease models, no single ideal animal model has been established for the comprehensive study TMJOA. This is because current models are mostly single-factor models, which cannot fully reflect the etiology and progression of TMJOA. In the future, the modeling approach should be improved, and more multi-factor models should be established to provide more suitable animal models for further study of TMJOA.

**AUTHOR CONTRIBUTIONS**

YZ, YA and LZ contributed equally to the content of the work. All authors contributed to the article and approved the submitted version.

**FUNDING**

This work was supported by National Natural Science Foundation of China (61701520, 61871393, 81602651) and Taishan Scholar Foundation of Shandong Province (tsqn201812137).

**REFERENCES**

Ahmad, M., Hollender, L., Anderson, Q., Kartha, K., Ohrbach, R., Truelove, E. L., et al. (2009). Research Diagnostic Criteria for Temporomandibular Disorders (Rdc/Tmd): Development of Image Analysis Criteria and Examiner Reliability for Image Analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology 107, 844–860. doi:10.1016/j.tripleo.2009.02.023

Aigner, T., and Kim, H. A. (2002). Apoptosis and Cellular Vitality: Issues in Osteoarthritic Cartilage Degeneration. Arthritis Rheum. 46, 1986–1996. doi:10.1002/art.10554

Al-Moraissi, E. A., Wolford, L. M., Ellis, E., 3rd, and Neff, A. (2020). The Hierarchy of Different Treatments for Arthrogenous Temporomandibular Disorders: A Network Meta-Analysis of Randomized Clinical Trials. J. Craniomaxillofac. Surg. 48, 9–23. doi:10.1016/j.jcsm.2019.10.004

Almarza, A. J., Brown, B. N., Arzi, B., Ângelo, D. F., Chung, W., Badyal, S. F., et al. (2018). Preclinical Animal Models for Temporomandibular Joint Osteoarthritic Cartilage Degeneration. Arthritis Rheum. 46, 1986–1996. doi:10.1002/art.10554

Al-Moraissi, E. A., Wolford, L. M., Ellis, E., 3rd, and Neff, A. (2020). The Hierarchy of Different Treatments for Arthrogenous Temporomandibular Disorders: A Network Meta-Analysis of Randomized Clinical Trials. J. Craniomaxillofac. Surg. 48, 9–23. doi:10.1016/j.jcsm.2019.10.004

Bermejo, A., González, O., and González, J. M. (1993). The Pig as an Animal Model for Experimental Osteoarthritis: Nerves, Circadian Clocks and beyond. Nat. Rev. Rheumatol. 12, 508–516. doi:10.1038/nrrheum.2016.93

Bermúdez, A., González, O., and González, J. M. (1993). The Pig as an Animal Model for Experimentation on the Temporomandibular Articular Complex. Oral Surg. Oral Med. Oral Pathol. 75, 18–23. doi:10.1016/0030-4220(93)90399-o

Bhattacharya, A., Chaudhuri, S., and Banerjee, S. C. (2012). Understanding Early-Stage Posttraumatic Osteoarthritis: From Knee to Temporomandibular Joint. Curr. Osteoporos. Rep. 19, 166–174. doi:10.1007/s11914-012-00661-3

Barve, R. A., Minnerly, J. C., Weiss, D. I., Meyer, D. M., Aguilar, D. J., Sullivan, P. M., et al. (2007). Transcriptional Profiling and Pathway Analysis of Monosodium Iodoacetate-Induced Experimental Osteoarthritis in Rats: Relevance to Human Disease. Osteoarthritis and Cartilage 15, 1190–1198. doi:10.1016/j.joca.2007.03.014

Berenbaum, F., and Meng, Q.-J. (2016). The Brain-Joint Axis in Osteoarthritis: A New Insight. Arthritis Res. Ther. 18, 1–9. doi:10.1186/s13075-016-1186-7

Bermejo, A., González, O., and González, J. M. (1993). The Pig as an Animal Model for Experimentation on the Temporomandibular Articular Complex. Oral Surg. Oral Med. Oral Pathol. 75, 18–23. doi:10.1016/0030-4220(93)90399-o

Bhattacharya, A., Chaudhuri, S., and Banerjee, S. C. (2012). Understanding Early-Stage Posttraumatic Osteoarthritis: From Knee to Temporomandibular Joint. Curr. Osteoporos. Rep. 19, 166–174. doi:10.1007/s11914-012-00661-3

Codd, A. M. (2007). Animal models of temporomandibular joint osteoarthritis: a review. J. Oral Maxillofac. Surg. 65, 2361–2369. doi:10.1016/j.joms.2007.06.018

Dai, L., Chen, L., Li, Y., Li, Y., and Zhao, W. (2020). Systematic Review and Meta-Analysis of Animal Study on Gender Difference in Temporomandibular Joint Osteoarthritis. J. Med. Sci. 40, 659–682. doi:10.1007/s11805-020-02701-2

Dai, L., Li, Y., Li, Y., Chen, L., and Zhao, W. (2020). Gender difference and risk factors of temporomandibular joint osteoarthritis: a systematic review and meta-analysis. J. Med. Sci. 40, 659–682. doi:10.1007/s11805-020-02701-2
Shen, G., and Darendeliler, M. A. (2005). The Adaptive Remodeling of Condylar Cartilage- A Transition from Chondrogenesis to Osteogenesis. J. Dent Res. 84, 691–699. doi:10.1177/0022034705087002

Shen, M., Luo, Y., Niu, Y., Chen, L., Yuan, X., Goltzman, D., et al. (2013). 1,25(Oh)2D Deficiency Induces Temporomandibular Joint Osteoarthritis via Secretion of Senescence-Associated Inflammatory Cytokines. Bone 55, 400–409. doi:10.1016/j.bone.2013.04.015

Shen, P., Jiao, Z., Zheng, J. S., Xu, W. F., Zhang, S. Y., Qin, A., et al. (2015). Injecting Vascular Endothelial Growth Factor into the Temporomandibular Joint Induces Osteoarthritis in Mice. Sci. Rep. 5, 16244. doi:10.1038/srep16244

Shinoda, M., Ozaki, N., Asai, H., Nagamine, K., and Sugiura, Y. (2005). Changes in the Temporomandibular Joint Development and Disorders. Int. J. Clin. Exp. Pathol. 8, 296. doi:10.3892/ijmm.2019.4446

Toler, P. A., and Wilcox, I. H. (1978). Ultrastructure of the Articular Surface of the Condyle in Temporomandibular Arthropathy. Oral Surg. Oral Med. Oral Pathol. 45, 232–245. doi:10.1016/0030-4220(78)90090-7

Ukita, M., Matsushita, K., Tamura, M., and Yamaguchi, T. (2020). Histone H3K9 Methylation Is Involved in Temporomandibular Joint Osteoarthritis. Int. J. Mol. Med. 45, 607–614. doi:10.3892/ijmm.2019.4446

Vapniarsky, N., Huwe, L. W., Arzi, B., Houghton, M. K., Wong, M. E., Wilson, J. W., et al. (2018). Tissue Engineering toward Temporomandibular Joint Disc Regeneration. Sci. Transl Med. 10. doi:10.1126/scitranslmed.aag1892

Vos, L. M., Kuijer, R., Huddleston Slater, J. J. R., Bulstra, S. K., and Stegenga, B. (2014). Inflammation Is More Distinct in Temporomandibular Joint Osteoarthritis Compared to the Knee Joint. J. Oral Maxill. Surg. 72, 35–40. doi:10.1016/j.joms.2013.08.022

Waich, S., Embree, M., Ameye, L., and Young, M. F. (2005). Mice Deficient in Biglycan and Fibromodulin as A Model for Temporomandibular Joint Osteoarthritis. Cells Tissues Organs 181, 136–143. doi:10.1159/000091375

Wang, F., Sun, Y., He, D., and Wang, L. (2017a). Effect of Concentrated Growth Factors on the Repair of the Goat Temporomandibular Joint. J. Oral Maxill. Surg. 75, 498–507. doi:10.1016/j.joms.2016.09.006

Wang, K. H., Chan, W. P., Chiu, L. H., Tsai, Y. H., Fang, C. L., Yang, C. B., et al. (2017b). Histological and Immunohistochemical Analyses of Repair of the Disc in the Rabbit Temporomandibular Joint Using A Collagen Template. Materials (Basel) 10, 924. doi:10.3390/ma10080924

Wang, M., Li, S., Li, S., Xie, W., Shen, J., Im, H.-J., et al. (2014a). Activation of Beta-Catenin Signalling Leads to Temporomandibular Joint Defects. Eur. Cell Mater 22, 223–235. doi:10.22203/ecz.2020.015

Wang, Q. F., Yang, L., Li, X. P., Xie, H., Liao, E. Y., Wang, M., et al. (2012a). Effects of 17beta-Estradiol on Adiponectin Regulation of the Expression of Osteoprogerin and Receptor Activator of Nuclear Factor-Kappab Ligand. Bone 51, 515–523. doi:10.1016/j.bone.2012.05.011

Wang, Q. Y., Dai, J., Kuang, B., Zhang, J., Yu, S. B., Duan, Y. Z., et al. (2012b). Osteochondral Angiogenesis in Rat Mandibular Condyles with Osteoarthritic-like Changes. Arch. Oral Biol. 57, 620–629. doi:10.1016/j.archoralbio.2011.12.006

Wang, X. D., Kow, X. X., He, D. Q., Zeng, M. M., Meng, Z., Bi, R. Y., et al. (2012c). Progression of Cartilage Degradation, Bone Resorption and Pain in Temporomandibular Joint Osteoarthritis Induced by Injection of Indocetate, Plac. Onc 1, E4536. doi:10.1371/journal.pone.004536

Xiang, T., Tao, Z. Y., Liao, L. F., Wang, S., and Cao, D. Y. (2021). Animal Models of Temporomandibular Joint Osteoarthritis after Indirect Trauma in Young Goats. Br. J. Oral Maxillofac. Surg. 49, 192–197. doi:10.1016/j.bjoms.2007.10.007

Xiang, T., Tao, Z. Y., Liu, L. F., Wang, S., and Cao, D. Y. (2016). Trend of Cadherin-11 Expression and its Impact on Cartilage Degradation in the Temporomandibular Joints of Guinea Pigs with Spontaneous Osteoarthritis. J. Oral Pathol. Med. 45, 534–538. doi:10.1111/opj.12403

Zhao et al. Animal Models of Temporomandibular Joint Osteoarthritis
