Published in final edited form as:

Dev Dyn. 2011 November ; 240(11): 2466–2473. doi:10.1002/dvdy.22748.

Tissue interaction is required for glenoid fossa development during temporomandibular joint formation

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Abstract

The mammalian temporomandibular joint (TMJ) develops from two distinct mesenchymal condensations that grow towards each other and ossify through different mechanisms, with the glenoid fossa undergoing intramembranous ossification while the condyle being endochondral in origin. In this study, we used various genetically modified mouse models to investigate tissue interaction between the condyle and glenoid fossa during TMJ formation in mice. We report that either absence or dislocation of the condyle results in an arrested glenoid fossa development. In both cases, glenoid fossa development was initiated, but failed to sustain, and became regressed subsequently. However, condyle development appears to be independent upon the presence of the forming glenoid fossa. In addition, we show that substitution of condyle by Meckel's cartilage is able to sustain glenoid fossa development. These observations suggest that proper signals from the developing condyle or Meckel's cartilage are required to sustain the glenoid fossa development.

Keywords

TMJ formation; glenoid fossa development; condyle; tissue interaction; Sox9

INTRODUCTION

The temporomandibular joint (TMJ) is a unique synovial joint that is essential for movement and function of the jaw in mammals. It is composed of a fibrous capsule that encloses the mandibular condyle, the glenoid fossa of the temporal bone, and an articulating disc separating the two articular facets lined with fibrocartilage instead of hyaline cartilage (Sperber, 2001). The glenoid fossa forms a deep concavity in the temporal bone, receiving the mandibular condyle to make the hinge of TMJ function. Associated with the TMJ are also the tendon of the pterygoid muscle and various surrounding ligaments. The TMJ disorders affect a large population in human beings, causing chronic myofascial pains and difficulty in chewing function. While the structures and functions of the TMJ have been well documented, TMJ development remains poorly understood in terms of underlying cellular and molecular mechanisms in contrast to a wealth of information regarding the formation of synovial joints in the developing limb.

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The TMJ develops from two distinct and separate mesenchymal condensations, the temporal and condylar blastemas. These two blastemas grow towards each other and undergo different ossification processes, forming the glenoid fossa and condyle, respectively. Despite that it is classified as synovial joint, the formation of TMJ differs significantly from the limb joints that develop by the cleavage or segmentation within a single skeletal condensation. In mice, TMJ development begins at embryonic day 13.5 (E13.5) as evidenced by the formation of the condylar condensation and the expression of *Sox9* in the condensed mesenchyme (Gu et al., 2008; Shibata and Yokohama-Tamaki, 2008). At E14.5, the condensation of the glenoid fossa becomes visible as a triangle structure and begins to express *Runx2* at the dorsal-caudal aspect of the condylar condensation. Subsequently, these two mesenchymal condensations grow towards each other, narrowing down the distance between them and assuming their position at E16.5. At the same time, the articular disc also begins to form, which develops from a separate flat-shaped mesenchymal condensation located between the apex of condyle and the glenoid fossa (Frommer, 1964). By E17.5 all the major components of the TMJ are formed. The glenoid fossa has encompassed the apex of the definite condyle, and the upper and lower synovial cavities, which are separated by the compact articular disc, become discernible at this stage (Gu et al., 2008).

The mandibular condyle undergoes endochondral ossification and represents a major growth site in the mandible contributing to the elongation of the mandibular ramus (Sibbermann and Frommer, 1972). Although the condylar cartilage is classified as the secondary cartilage (Beresford 1975), it grows through the chondrogenic processes similar to the primary cartilage and expresses many genes that are important for the primary cartilage growth and differentiation in the long bones (Fukada et al., 1999; Rabie and Hägg, 2002; Kuboki et al., 2003; Ogawa et al., 2003; Watahiki et al., 2003; Tang et al., 2004; Shibata et al., 2006; Shibata and Yokohama-Tamaki, 2008; Shibukawa et al., 2007; Purcell et al., 2009). For example, *Sox9* is expressed in all chondroprogenitors and differentiated chondrocytes, and is required for cartilage formation, including both the primary and secondary cartilages including the condyle (Bi et al., 1999; Akiyama et al., 2002; Mori-Akiyama et al., 2003). While distinct sets of genes are expressed in the developing limb joints and the TMJ, respectively, several genes known to be critical for joint formation are indeed expressed in these two types of synovial joints (Purcell et al., 2009). Among these genes is *Ihh*, a signaling molecule required for regulation of chondrocyte proliferation, maturation, and ossification. *Ihh*-deficient in embryonic mouse leads to digit joint ablation as well as defective TMJ lacking the articular disc (St-Jacques et al., 1999; Shibukawa et al., 2007). Mice deficient in *Gli2*, a downstream effector of the hedgehog signaling, exhibit similar TMJ defect (Purcell et al., 2009). In addition, ablation of *Ihh* in cartilages of neonatal mice produces dysplastic TMJ associated with partial disc ankylosis (Ochiai et al., 2010), mimicking the phenotype in *Shox2* mutant mice in which *Ihh* expression is down-regulated in the developing condyle (Gu et al., 2008).

Studies on TMJ development have been primarily focusing on condyle growth and disc formation, little is known regarding the mechanistic basis of glenoid fossa formation. It is widely accepted that growth factor-mediated tissue interaction regulates growth, differentiation, and patterning of many organs during vertebrate embryogenesis. In the present study, we asked a fundamental question as whether there exists tissue interaction between the developing condyle and glenoid fossa during TMJ formation. We used various knockout/transgenic mouse models to examine the influence of the condyle on the development of the glenoid fossa. Our results show that absence or dislocation of the developing condyle arrested the development of the glenoid fossa. In addition, substitution of the condyle by Meckel's cartilage could sustain glenoid fossa development.

RESULTS

Inactivation of *Sox9* in the cranial neural crest leads to absent condyle and arrested glenoid fossa development

It was reported previously that ablation of *Sox9* in the cranial neural crest resulted in a complete elimination of cartilages and endochondral bones derived from the cranial neural crest cells; however, all the mesoderm-derived skeletal elements and intramembranous bones were essentially not affected (Mori-Akiyama et al., 2003). Although both the condyle and glenoid fossa derive from the cranial neural crest cells (Gu et al., 2008), the glenoid fossa condensation does not express *Sox9*, as shown in Fig. 1, and develops through intramembranous ossification. We thus took advantage of the *Wnt1Cre*-mediated *Sox9* gene inactivation approach to examine the development of the glenoid fossa in the absence of the condyle.

To create *Wnt1Cre;Sox9^{fl/f}* mice, we first generated *Wnt1Cre;Sox9^{fl/+}* mice. While *Sox9* haploinsufficiency results in perinatal lethality due to a cleft palate defect (Bi et al., 2001), it was reported that most *Wnt1Cre;Sox9^{fl/+}* mice were viable and developed into fertile mice with normal lifespan, despite the formation of the cleft secondary palate in a small percentage of these mice (Mori-Akiyama et al., 2003). In our studies, we found that about 75% *Wnt1Cre;Sox9^{fl/+}* mice developed a cleft palate defect. Those *Wnt1Cre;Sox9^{fl/+}* mice that survived perinatal lethality appeared and behaved almost indistinguishable from their littermate controls. Skeletal preparation of *Wnt1Cre;Sox9^{fl/f}* mice at postnatal day 0 (P0) indeed demonstrated a complete absence of the cranial neural crest-derived cartilages and endochondral bones, including the condyle (Fig. 2A, 2B; and data not shown), as reported previously (Mori-Akiyama et al., 2003). In situ hybridization assays revealed absent *Sox9* expression in the site where the condyle supposed to form in *Wnt1Cre;Sox9^{fl/f}* embryos (Fig. 1A-D), indicating successful inactivation of *Sox9* in the cranial neural crest-derived cells.

We next examined the development of the glenoid fossa by histological analyses. At E14.5 when the glenoid fossa condensation became visible in the wild type control, the glenoid fossa condensation with the similar shape was also observed in the mutant (Fig. 2C, 2D). This observation was further confirmed by *Runx2* expression in the glenoid fossa condensation at this stage (Fig. 1E, 1F). At E15.5 and E16.5, the glenoid fossa grew towards the developing condyle and became ossified in the controls (Fig. 2E, 2G); however, the mutant glenoid fossa showed a slight delay in ossification and exhibited a regression in its lateral branch (Fig. 2F, 2H). At E18.5, while the glenoid fossa has encompassed the apex of the condyle in the wild type control (Fig. 2I), the mutant glenoid fossa has completely regressed, leaving a residual bone of the lateral branch (Fig. 2J). This halted development and regression of the lateral branch of glenoid fossa in the mutants was further confirmed by the down-regulation of *Runx2* in the glenoid fossa condensation at E15.5 (Fig. 1H). Interestingly, a disc-like structure seemed to form at the apex side of the mandibular ramus in the mutant TMJ (Fig. 2J). However, this structure is less compact and the cell shape appears irregular, as compared to the control (Fig. 2K, 2L), suggesting the formation of an abnormal disc-like structure. These observations demonstrate that in the absence of the condyle, the glenoid fossa is able to initiate its developmental process, as evidenced by the appearance of mesenchymal condensation and the expression of *Runx2*. However, the glenoid fossa fails to sustain its developmental program, and becomes regressed eventually.

Dislocation of the developing condyle leads to arrested glenoid fossa development

To further confirm a requirement of the condyle for normal glenoid fossa development, we utilized a different transgenic mouse model in which the developing condyle becomes dislocated away from the developing glenoid fossa. In a separate study on Wnt signaling in

palate development, we compounded the *K14-Cre* allele to an exon3 floxed *Catnb* allele (*Catnb^{F(ex3)}*) (He et al., 2011). The *Catnb^{F(ex3)}* allele, up Cre-mediated recombination, produces a stabilized β -catenin, leading to ectopic activity of the canonical Wnt signaling (Harada et al., 1999). In addition to severe defects in ectodermal appendages, *K14Cre;Catnb^{F(ex3)}* mice exhibited a wide open mouth phenotype due to abnormal skin development (Närhi et al., 2008; Zhang et al., 2008). Skeletal preparations of *K14Cre;Catnb^{F(ex3)}* mice revealed an abnormal position of the mandibular arch almost perpendicular to the maxilla (Fig. 3A, B), and formation of ectopic bone that connects the maxillae to the mandible (Fig. 3D). Coronal sections of the transgenic embryos at E14.5 demonstrated an anterior shift of the developing condyle (Fig. 3E-H). In the wild type controls at this stage, the developing condyle, identified by *Sox9* and *Runx2* expression (Fig. 3E, G), is always associated with the developing glenoid fossa that undergoes condensation (Fig. 3E) and expresses *Runx2* (Fig. 3G). In contrast, in the transgenic animals, a condensed primordial glenoid fossa was not present at the same section plane of the developing condyle (Fig. 3F), but was found, as evidenced by the expression of *Runx2*, at a caudal level where the condyle was not present (Fig. 3H). Similar to the phenotype observed in *Wnt1Cre;Sox9^{fl/fl}* mice, at E16.5, the glenoid fossa failed to develop, lacking the lateral branch (Fig. 3J). These observations indicate that the presence of the developing condyle and its proximity are essential for the normal development of the glenoid fossa.

Interestingly, despite the lack of the glenoid fossa, the condyle continued to develop at its dislocated position in *K14Cre;Catnb^{F(ex3)}* mice. While slightly smaller in size, the transgenic condyle appeared to develop and differentiate normally, as assessed by histology and the expression of molecular markers, including *Ihh*, *Col II*, and *Col X*, when compared to its control counterpart (Fig. 4). However, the articular disc never formed (Fig. 4G, H). It appears that the presence of the glenoid fossa is not a prerequisite for the development and differentiation of the condyle, but is required for the formation of the articular disc, indicating a requirement for tissue interactions between the condyle and the glenoid fossa during normal TMJ formation.

Meckel's cartilage can substitute for condyle to sustain glenoid fossa development

Mice deficient in *Noggin*, a BMP antagonist, exhibit a spectrum of craniofacial defects, including a significantly enlarged Meckel's cartilage (McMahon et al., 1998; Bachiller et al., 2000; Stottmann et al., 2001; Anderson et al., 2006; He et al., 2010). A detailed analysis of the developing TMJ in *Noggin* mutants (*Nog^{-/-}*) revealed a surprising phenotype. The rapid growth and expansion of Meckel's cartilage pushed the developing condyle away from its position in the proximity of the forming glenoid fossa condensation. Skeleton preparation of P0 wild type and *Nog^{-/-}* mice revealed a direct contact of Meckel's cartilage with the temporal bone (Fig. 4A, 4B). Histological analyses showed that, at E14.5, the enlarged Meckel's cartilage has assumed a position adjacent to the glenoid fossa condensation, evidenced by its morphology and the expression of *Runx2* (Fig. 4D, 4F). At E16.5, the mutant glenoid fossa continued to grow and differentiate, encompassing the apex of the contacting Meckel's cartilage (Fig. 4H). At E17.5 when the articular disc formed in the wild type control, no such disc-like structure was observed in the Meckel's cartilage-glenoid fossa complex, indicating a true TMJ did not form (Fig. 4I, 4J). Interestingly, the size of the glenoid fossa in the mutants appeared much larger than the controls, in accord with the larger size of Meckel's cartilage. These results demonstrate that Meckel's cartilage is able to substitute for the condyle to sustain glenoid fossa development, suggesting that Meckel's cartilage and the condyle produce similar secreted factors responsible for this process. However, it cannot replace the condyle for the formation of a normal TMJ, because a true joint with the articular disc did not form.

DISCUSSION

Tissue-tissue interaction plays an essential role in the embryonic development and organ formation. It involves a series of inductive and permissive interactions, regulating multiple processes during organogenesis such as determination, differentiation, and pattern formation. It is well documented that tissue interaction is mediated by diffusible signaling molecules also known as growth factors. The TMJ develops from two distinct mesenchymal condensations, the glenoid fossa and condylar blastemas that undergo different ossification processes. Many studies have been conducted to examine gene expression and function during TMJ development, particularly in the development of the condyle and the articular disc. However, glenoid fossa development has received little attention, and its underlying cellular and molecular mechanisms are basically unknown. In the present study, we looked into the fundamental question as whether or not tissue interaction is required for glenoid fossa development during TMJ formation. We present evidence demonstrating that in the absence of the condyle, the glenoid fossa condensation fails to sustain its development, leading to the lack of a definite glenoid fossa structure. Similar developmental defect was also observed in the glenoid fossa when the developing condyle is dislocated. These results suggest that the presence and its proximity of the developing condyle are essential for the development of the glenoid fossa during TMJ formation. It appears that the initial development of the glenoid fossa is independent on the condyle, but its continuous development relies on interaction with the condyle. In *Ihh*, *Gli2*, or *Shox2* mutants, while the condyle is hypoplastic, the glenoid fossa forms anyway (Shibukawa et al., 2007; Gu et al., 2008; Purcell et al., 2009), suggesting that even a hypoplastic condyle is able to support glenoid fossa development. Since the condensations of the condyle and glenoid fossa do not make direct contact, tissue interaction between them is apparently mediated by diffusible factor(s). On the other hand, condyle development and differentiation do not rely on the presence of the glenoid fossa. One possibility is that the developing mandibular ramus, which is in a close proximity to the developing condyle, and similar to the glenoid fossa, undergoes intramembranous ossification, may provide signals to sustain condyle development.

The articular disc forms from the mesenchymal cells located between the condyle and the glenoid fossa (Frommer, 1964). In the absence of *Ihh*, a normal disc fails to form (Shibukawa et al., 2007). In *WntCre;Sox9^{f/f}* mice, although a disc-like tissue forms, its abnormal structure and cell shape exclude it a true disc, suggesting a requirement of the condyle for the formation of a normal functional disc. In contrast, despite that the condyle develops and differentiates relatively normal in the absence of the glenoid fossa in *K14Cre;Catnb^{F(ex3)}* mice, the articular disc fails to form. This is very likely due to the dislocation of the condyle away from the mesenchymal cells that form the articular disc.

Our results also show that Meckel's cartilage can substitute for the condyle to sustain glenoid fossa development in *Nog^{-/-}* mice. However, a true joint with the TMJ characteristics never formed in *Noggin* mutants. In fact, Meckel's cartilage is considered the primary cartilage, while the condyle is classified as the secondary cartilage, characterized by its rapid differentiation from progenitor cells to hypertrophic chondrocytes and its preosteoblastic characteristics (Shibermann et al., 1987; Shibata et al., 1997; Miyake et al., 1997; Fukada et al., 1999). The difference in these two types of cartilage explains why Meckel's cartilage cannot substitute for the condyle for the formation of a normal TMJ. On the other hand, the condyle and Meckel's cartilage appears to produce similar diffusible signaling molecule(s) that acts to sustain glenoid fossa development. This is supported by the fact that the condyle does not only undergoes the similar chondrogenic processes, but also expresses many genes that are also present in the primary cartilage (Fukada et al., 1999; Rabie and Hägg, 2002; Kuboki et al., 2003; Ogawa et al., 2003; Watahiki et al., 2003; Tang

et al., 2004; Shibata et al., 2006; Shibata and Yokohama-Tamaki, 2008; Shibukawa et al., 2007; Purcell et al., 2009). While the identity of the diffusible signaling molecule(s) is currently unknown, a survey for the candidate factors warrants future investigation and will be crucial for understanding the mechanisms of glenoid fossa development

Generally speaking, TMJ development is an understudied field. Many studies in this field have been focusing on analyses of gene expression and function during development of the condyle and the disc. There is very little information of molecular and cellular regulatory mechanism available on glenoid fossa development. While a number of mutations have been shown to cause abnormal TMJ formation, most of the defects were confined to the condyle and disc (Shibata et al., 2004; Shibukawa et al., 2007; Gu et al., 2008; Purcell et al., 2009). The present study represents an initial effort to investigate how tissue-tissue interactions regulate the development of the TMJ

EXPERIMENTAL PROCEDURES

Animals

The generation and genotyping of *Catnb*^{F(ex3)}, *Nog*^{+/-}, *Sox9*^{f/f}, *K14-Cre*, and *Wnt1-Cre* mice have been described previously (Danielian et al., 1998; McMahon et al., 1998; Harada et al., 1999; Akiyama et al., 2002; Andl et al., 2004). To generate *Wnt1Cre;Sox9*^{f/f} mice, *Wnt1-Cre* mice were first crossed to *Sox9*^{f/f} mice to generate *Wnt1Cre;Sox9*^{f/+} mice that were then further mated with *Sox9*^{f/f} mice. Genotypes of mice or embryos were determined by PCR based genotyping method using genomic DNA extracted from tail or extra-embryonic membranes. Embryos collected from timed pregnant mice were fixed in 4% paraformaldehyde (PFA)/PBS at 4°C for overnight prior to being processed for histology or in situ hybridization.

Skeletal preparation, histology, and in situ hybridization

Skeletal staining was conducted by using Alcian blue /Alizarin red staining for non-mineralized cartilage and bone respectively, as described previously (Zhang et al., 2000). For histological examination, paraffin sections made at 10-μm were subjected to standard hematoxylin/eosin staining or Azon red/Anilin blue staining, according to the standard protocols (Presnell and Schreiber, 1997). Non-radioactive section in situ hybridization was conducted as described before (St. Amand et al., 2000).

Acknowledgments

This work was supported by an NIH grant (R01 DE17792) to Y.P.C.

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Figure 1.

Expression of *Sox9* and *Runx2* in the developing TMJ. (A-D) In situ hybridization shows *Sox9* expression in the condylar condensation of E14.5 (A) and E15.5 (C) wild type embryo, but not in the mandibular ramus condensation of E14.5 (B) and E15.5 (D) *Wnt1Cre;Sox9^{fl/fl}* embryos, indicating efficient ablation of *Sox9* in the cranial neural crest-derived tissues. (E, F) *Runx2* expression is detected at an equal level in the glenoid fossa condensations from both E14.5 wild type (E) and mutant (F). (G, H) At E15.5, *Runx2* expression is detected in the developing condyle and glenoid fossa in the wild type control (G). In contrast, in the mutant at the same stage, while *Runx2* expression is detected in the mandibular ramus and part of the glenoid fossa, the lateral branch of the glenoid fossa exhibits a downregulation of *Runx2* expression (arrow) (H). c, condyle; gf, glenoid fossa; mr, mandibular ramus.

Figure 2.

Inactivation of *Sox9* in the cranial neural crest cells results in an absence of condylar cartilage and TMJ malformation. (A, B) Skeletal preparations of mandibles from P0 wild type control (A) and *Wnt1Cre;Sox9^{f/f}* mice (B) reveal an absence of cartilage elements in the condyle and angular process in the mutant. The mutant mandible appears shorter and also lacks the coronoid process. (C, D) Coronal sections through the TMJ forming region of E14.5 wild type (C) and *Wnt1Cre;Sox9^{f/f}* embryos (D) show formation of the glenoid fossa condensation. In the wild type control, the Meckel's cartilage and condylar condensation are evident (C), however, in the mutant, the mandibular ramus condensation is observed instead, and the Meckel's cartilage is also absent (D). (E-J) Coronal sections through the TMJ

forming site of wild type and mutant mice at various stages reveal normal processes of TMJ development (E, G, I) and regression of the glenoid fossa in *Wnt1Cre;Sox9^{f/f}* mice (F, H, J). (K) Higher magnification of the TMJ from E18.5 wild type mice shows a compact normal articular disc structure with flattened cells (arrow). (L) Higher magnification of the disc-like structure from E18.5 *Wnt1Cre;Sox9^{f/f}* mice shows a less compact tissue with irregular cell shape (arrow). c, condyle; m, Meckel's cartilage; gf, glenoid fossa; mr, mandibular ramus; agp, angular process; crp, coronoid process; lpm, lateral pterygoid muscle. Scale bars: 1-mm (A, B); 200- μ m (C-J).

Figure 3.

Dislocation of condyle in *K14Cre;Catnb^{F(ex3)}* mice. (A, B) Skeleton preparation of P0 wild type (A) and *K14Cre;Catnb^{F(ex3)}* head (B). Arrow in (B) points to the mis-positioned mandibular arch. (C) Skeleton preparation of E16.5 wild type head shows articulation of the lower and upper jaws. Note that the condyle (c) is in close proximity to the temporal bone (Tb). (D) Skeleton preparation of E16.5 *K14Cre;Catnb^{F(ex3)}* head shows dislocated mandible and ectopic bone formation (*). The condyle is located behind the ectopic bone, away from the temporal bone (Tb). (E) A coronal section of E14.5 wild type embryonic head shows the *Sox9*-expressing condyle and the glenoid fossa condensation at the same plane. (F) A coronal section of E14.5 *K14Cre;Catnb^{F(ex3)}* head shows the *Sox9*-expressing

condyle but no glenoid fossa condensation. (G) A coronal section of E14.5 wild type head shows the *Runx2*-expressing condyle and glenoid fossa condensation. (H) In situ hybridization shows the *Runx2*-positive glenoid fossa in E14.5 *K14Cre;Catnb^{F(ex3)}* head. The condyle is not seen at this plane. Asterisk marks the position where the condyle supposed to be. (I) A coronal section shows a normal developing TMJ in E16.5 wild type embryo. (J) A coronal section shows the regressed glenoid fossa in E16.5 *K14Cre;Catnb^{F(ex3)}* head. Note the lack of condyle at the position it supposed to be (marked by the asterisk). C, condyle; M, Meckel's cartilage; gf, glenoid fossa; Mx, maxillary bone; Tb, temporal bone; lpm, lateral pterygoid muscle; Man, mandible.

Figure 4. condyle develops and differentiates independently of the glenoid fossa but fails to form an articular disc in *K14Cre;Catnb^{F(ex3)}* mice. (A, C, E) In situ hybridization shows expression of *Ihh* (A), *Col II* (C), and *Col X* (E) in the developing condyle of E16.5 wild type control. (B, D, F) In situ hybridization shows similar expression patterns of *Ihh* (B), *Col II* (D), and *Col X* (F) in the developing condyle of E16.5 *K14Cre;Catnb^{F(ex3)}* mice. (G) A coronal section through an E18.5 wild type head shows normal TMJ structure. The arrow points to the articular disc. (H) A coronal section through an E18.5 *K14Cre;Catnb^{F(ex3)}* head shows the condyle and temporal bone, but the absence of glenoid fossa and articular disc. C, condyle; gf, glenoid fossa; tb, temporal bone. Scale bars: 200-μm.

Figure 5.

Substitution of the condyle by Meckel's cartilage to sustain glenoid fossa development. (A) Skeleton preparation of P0 wild type head shows contact of the condyle with the temporal bone. (B) Skeleton preparation of P0 *Nog*^{-/-} head shows direct contact of enlarged Meckel's cartilage with the temporal bone. (C, G, I) Coronal sections of E14.5 (C), E16.5 (G), and E17.5 (I) wild type heads show normal development of the TMJ. The arrow in (I) points to the articular disc. (E) In situ hybridization shows *Runx2* expression in the glenoid fossa condensation. (D, H, J) Coronal sections of E14.5 (D), E16.5 (H), and E17.5 (J) *Nog*^{-/-} heads show replacement of the condyle by Meckel's cartilage in the position proximity of the developing glenoid fossa. The size of the glenoid fossa condensation in E14.5 mutant

appears similar to the control, but becomes much larger at the late stages. Note the presence of the residual condyle in (H). (F) In situ hybridization shows initial *Runx2* expression in the glenoid fossa condensation of E14.5 mutant. C, condyle; M, Meckel's cartilage; gf, glenoid fossa; Tb, temporal bone; lpm, lateral pterygoid muscle. Scale bars: 200- μ m.