Bmp signalling in filiform tongue papillae development

Katsushige Kawasaki[a,b,e], **Thantrira Porntaveetus**[a,e], **Shelly Oommen**[a], **Sarah Ghafoor**[a], **Maiko Kawasaki**[a,c], **Yoko Otsuka-Tanaka**[a], **James Blackburn**[a], **John A. Kessler**[d], **Paul T. Sharpe**[a], and **Atsushi Ohazama**[a,*]

[a]Department of Craniofacial Development, GKT Dental Institute, King's College, Guy's Hospital, London Bridge, London SE1 9RT, UK

[b]Division of Pediatric Dentistry, Department of Oral Health Science, Course for Oral Life Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[c]Division of Bio-Prosthodontics, Department of Oral Health Science, Course for Oral Life Science, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan

[d]Department of Neurology, Northwestern University, Feinberg Medical School, Chicago, IL 60611, USA

Abstract

Objective—Tongue papillae are critical organs in mastication. There are four different types of tongue papillae; fungiform, circumvallate, foliate, and filiform papillae. Unlike the other three taste papillae, non-gustatory papillae, filiform papillae cover the entire dorsal surface of the tongue and are important structures for the mechanical stress of sucking. Filiform papillae are further classified into two subtypes with different morphologies, depending on their location on the dorsum of the tongue. The filiform papillae at the intermolar eminence have pointed tips, whereas filiform papillae with rounded tips are found in other regions (anterior tongue). It remains unknown how the shape of each type of filiform papillae are determined during their development. Bmp signalling pathway has been known to regulate mechanisms that determine the shapes of many ectodermal organs. The aim of this study was to investigate the role of Bmp signalling in filiform papillae development.

Design—Comparative in situ hybridization analysis of six *Bmps* (*Bmp2–Bmp7*) and two *Bmpr* genes (*Bmpr1a* and *Bmpr1b*) were carried out in filiform papillae development. We further examined tongue papillae in mice over-expressing Noggin under the keratin14 promoter (*K14-Noggin*).

Results—We identified a dynamic temporo-spatial expression of Bmps in filiform papillae development. The *K14-Noggin* mice showed pointed filiform papillae in regions of the tongue normally occupied by the rounded type.

Conclusions—Bmp signalling thus regulates the shape of filiform papillae.

*Corresponding author at: Department of Craniofacial Development, Floor 27, Guy's Tower, GKT Dental Institute, Guy's Hospital, London Bridge, London SE1 9RT, UK. Tel.: + 44 20 7188 8038; fax: + 44 20 7188 1674. atsushi.2.ohazama@kcl.ac.uk (A. Ohazama)..
[e]Authors have contributed equally to this work

Competing interests
None declared.

Ethical approval
Not required.

Keywords

Tongue; Filiform papillae; Anterior tongue; Intermolar eminence

1. Introduction

The dorsal surface of the tongue is covered by papillae that play critical roles in the mastication and gustatory system. Inrodents, there are four different types of tongue papillae; fungiform, circumvallate, foliate, and filiform. The fungiform, circumvallate and foliate papillae contain taste buds and are referred to as taste papillae, whereas filiform papillae contain no taste buds (non-gustatory papillae). These papillae are distributed in a specific pattern over the tongue. A single circumvallate papilla is located in the centre of the terminal sulcus, which divides the tongue from the pharynx. Fungiform papillae are present on the anterior tongue in a pattern of longitudinal rows, bracketing a median furrow. Unlike the taste papillae, non-gustatory filiform papillae cover the entire dorsal surface of the tongue (Fig. 1A).[1] To date, the molecular mechanisms of filiform papillae development remain largely unknown, whereas it has been well studied in gustatory papillae development.[2–9]

The filiform papillae are important structures for the mechanical stress of sucking.[10,11] In rodents, filiform papillae are further classified into two subtypes with different morphologies, depending on their location on the dorsum of the tongue. In the intermolar eminence, the filiform papillae have pointed tips in both the anterior–posterior and the left-right axis (pointed type; Fig. 1B).[12] The pointed type filiform papillae are frequently bifurcated. Unlike the intermolar eminence, in other parts of the tongue surface (anterior tongue), the filiform papillae have rounded tips in the left-right axis, whereas their spines point posteriorly in the anterior–posterior axis (rounded type; Fig. 1B).[12] It is unclear how the shape of each type of filiform papillae is determined during their development.

It is known that multiple signalling pathways such as Bmp play critical roles in regulating the shape of ectodermal organs, including taste tongue papillae and teeth.[2,4,12–15] However, the role of Bmp signalling in filiform papillae development remains unknown.

We show here that Bmp signalling is activated in filiform papillae development. Bmp ligands and their receptors show a dynamic temporo-spatial expression pattern in filiform papillae development. Mice over-expressing Noggin under the keratin14 promoter (*K14-Noggin*) develop abnormal pointed filiform papillae in the anterior tongue where the rounded type are normally located. This result provides evidence of a role for Bmp signalling in regulating the morphogenesis of filiform papillae.

2. Materials and methods

2.1. Production and analysis of transgenic mice

K14-Noggin transgenic mice were produced as described by Guha et al.[16] Five *K14-Noggin* mice (five month-old) and twenty mice (E14.5, E16.5 and newborn) of the CD-1 strain were used. Noon of the day on which the plugs were detected was considered as E0.5. To accurately assess the age of embryos, somite pairs were counted and the stage confirmed using morphological criteria, e.g., relative sizes of maxillary and mandibular primodia, extent of nasal placode invagination, and the size of limb buds.

2.2. In situ hybridization

Embryo heads were fixed in 4% buffered paraformaldehyde, wax embedded, and serially sectioned at 7 μm. Sections were split over three to ten slides and prepared for histology or radioactive in situ hybridization. In situ hybridization with [^{35}S]UTP-labelled riboprobes was carried out as described previously.[17]

2.3. Scanning electron microscope (SEM) analysis

Tongues were fixed with 2% glutaraldehyde in 0.1 M Nacacodylate buffer and 0.1 M sucrose, followed by post-fixation with 1% osmium tetroxide in 0.1 M Na-cacodylate buffer. After critical point drying, the samples were coated with gold and photographed using scanning electron microscopy.

2.4. Immunohistochemistry

After deparaffinization, sections were treated by proteinase K and then incubated with antibody to phosphorylated Smad1, Smad5 and Smad8 (p-Smad1/5/8; 1:100; Cell signalling Technology; #9511). As a negative control, normal rabbit serum was used instead of primary antibody. Secondary antibodies conjugated with biotin (Abcam; ab6828) were used at dilution of 1:100. Tyramide signal amplification system was performed (Perkin Elmer Life Science; NEL701) for detecting the antibody. DAPI (4 ,6-diamidino-2-phenylindole dihydrochloride; Sigma) was used for nuclear staining. Pictures were taken with same exposure between control wild-type and mutant mice.

3. Results

3.1. Expression of Bmps and Bmp receptors in filiform papillae development

At 11.5 days gestation (E11.5) the tongue emerges as a swelling on the floor of the developing mandible, with a spatulate tongue being observed at E12.5. At E13.5, the tongue has a distinctive topography. The well-formed taste bud papillae can be detected by E13.5. Unlike taste papillae development, filiform papillae start to develop later, at around E15.5.[18] In common with other ectodermal appendages, such as hair and tooth, the first morphological sign of tongue papillae development is localized thickenings of the epithelium and condensation of the underlying mesenchymal cells. The subsequent formation of epithelial arch-like structures is proceeded by their vertical elongation into a cornified stratified squamous epithelium.[19] Filiform papillae seem to develop rapidly during the few days before birth.[19] At birth, filiform papillae show a similar distribution to those seen in the adult.[1]

In order to investigate whether Bmp signalling is involved in filiform papillae development, we examined Bmp signalling in filiform papillae development by immuno his to chemistry using anti-phosphorylated Smad1/Smad5/Smad8 (p-Smad1/5/8) antibody. Smad1, Smad5 and Smad8 are phosphorylated by Bmp receptors following binding of ligands.[20] P-Smad1/5/8 positive cells could not be detected in either tongue epithelium or underneath mesenchyme at E13.5, although they were observed in developing tongue muscle (Fig. 2A). P-Smad1/5/8 was localized in entire tongue epithelium at E14.5 and E16.5, whereas they were found in basal epithelial cells at birth (Fig. 2B–I). These suggested that Bmp signalling is activated in filiform papillae development from E14.5.In addition to filiform papillae, p-Smad1/5/8 positive cells were observed in fungiform papillae (Fig. 2D). Most of p-Smad1/5/8 was localized in nuclear of filiform papillae epithelial cells (Fig. 2J–L).

A comprehensive expression analysis of *Bmps* in filiform papillae development was therefore carried out between E14.5 and E18.5 using radioactive in situ hybridization to understand the significance of this gene family in filiform papillae organogenesis. In

addition to the presence of Bmp signalling, this period also encompasses development preceding the morphological appearance of filiform papillae to maturation of the organs. In order to investigate *Bmp* expression in both pointed and rounded type filiform papillae development, we examined the expression at the intermolar eminence anterior to the circumvallate papillae for the pointed type, and in the anterior part of tongue (anterior tongue) for the rounded type (Fig. 1C).

3.2. E14.5

At E14.5, the presumptive filiform papilla epithelium is composed of a few layers of cuboidal cells.[18,19]

In both the anterior tongue and the intermolar eminence, *Bmp2* was expressed in the filiform papillae epithelium (Fig. 3A and B). *Bmp4* expression was observed in mesenchymal cells facing the epithelium in the intermolar eminence, whereas very little expression was seen in the equivalent mesenchymal cells in the anterior tongue (Fig. 3E and F). In the intermolar eminence, *Bmp5* expression was found in the filiform papillae mesenchyme of the anterior portion but not seen in the posterior region (Fig. 3H). *Bmp5* expression was not observed in the anterior tongue (Fig. 3G). *Bmp6* and *Bmp7* was weakly expressed in the filiform papillae epithelium of both the anterior tongue and the intermolar eminence, with no significant difference in their expression between the two regions (Fig. 3I–L). *Bmp3* expression could not be detected in either the epithelium or mesenchyme of the filiform papillae at this stage (Fig. 3C and D). The biological activities of *Bmps* are mediated by heterodimeric receptor complexes consisting of type I *Bmp* receptors (*Bmpr1a and Bmpr1b*) and the type II receptor (*Bmpr2*). The formation of heterodimers of type I and type II receptors is essential for Bmp signal transduction.[21–23] It is possible that filiform papillae type is regulated by differences in expression of these receptors. We further examined Bmpr1a and *Bmpr1b* expression in filiform papillae development. *Bmpr1a* expression was observed in both the epithelium and mesenchyme, with a slightly weaker expression seen in the latter (Fig. 3M and N). *Bmpr1b* expression was restricted to the basal epithelial cells in both the anterior tongue and the intermolar eminence (Fig. 3O and P). There was no significant difference in the both receptor expression between in the anterior tongue and the intermolar eminence.

3.3. E16.5

At E16.5, primitive arch-like epithelial structures are found in regions of the dorsal surface of tongue.[18,19]

Bmp2 expression was only observed in the epithelial cells facing the oral cavity (Fig. 4A and B). There was no significant difference in expression between the anterior tongue and the intermolar eminence. *Bmp4* was weakly expressed in epithelium of both the anterior tongue and the intermolar eminence (Fig.4E and F). In the intermolar eminence, *Bmp5* expression was only found in the filiform papillae mesenchymal cells facing the epithelium at the anterior portion, but not seen in the posterior region (Fig. 4H). *Bmp5* expression was not observed in the anterior tongue (Fig. 4G). *Bmp7* was weakly expressed in the entire filiform papillae epithelium of both the anterior tongue and the intermolar eminence (Fig. 4K and L). *Bmp3* and *Bmp6* showed no expression in the filiform papillae epithelium or the mesenchyme at this stage (Fig.4C,D,I and J).Bmpr1a expression was observed in both the epithelium and the mesenchyme, with a slightly weaker expression in the epithelium (Fig. 4M and N). *Bmpr1b* was expressed in the basal epithelial cells of both the anterior tongue and the intermolar eminence (Fig. 4O and P). There was no significant difference in expression between the anterior tongue and the intermolar eminence.

3.3.1. Newborn—At birth, the dorsal tongue epithelium is composed of a typical stratified squamous epithelium. Filiform papillae become more distinct and the connective tissue of the lamina propria penetrate the centre of each structure.[18,19]

Bmp2 was expressed in the basal epithelial cells of both the intermolar eminence and the anterior tongue (Fig. 5A and B). *Bmp4* was weakly expressed in the filiform papillae epithelium and the mesenchymal cells facing the epithelium in the anterior tongue but not in the intermolar eminence (Fig. 5E and F). In the intermolar eminence, *Bmp5* showed restricted expression only in the mesenchymal cells facing the epithelium to the anterior domain, with no expression seen in posterior region (Fig. 5H). *Bmp5* expression could not be detected in the anterior tongue (Fig. 5G). *Bmp7* was expressed in the epithelial cells in both the anterior tongue and the intermolar eminence (Fig. 5K and L). *Bmp3* and *Bmp6* expression could not be detected in the epithelium or the mesenchyme of the filiform papillae at this stage (Fig. 5C, D, I and J). No expression of *Bmpr1a* was observed in either the anterior tongue or the intermolar eminence (Fig. 5M and N). *Bmpr1b* expression was found in the basal epithelial cells in both the intermolar eminence and the anterior tongue, with no significant difference in expression between the two regions (Fig. 5O and P).

Bmp ligands and their receptors thus show a dynamic temporo-spatial expression pattern in filiform papillae development.

The expression of *Bmps* and *Bmprs* during filiform papillae development is summarized in Table 1.

3.4. Tongue papillae in K14-Noggin mice

In order to investigate the role of Bmp signalling in filiform papilla development, we examined the tongue papillae of transgenic mice over-expressing the BMP antagonist, Noggin, under the control of the Keratin 14 (K14) promotor (*K14-Noggin*). K14 is expressed in tongue epithelium from E12.5 to adult in both the anterior tongue and the intermolar eminence (Fig. 6A–D). [19,24] In the wild-type tongue, significant *Noggin* expression could not be detected in the filiform papillae within the embryonic or adult stages, whereas expression was seen in the fungiform papillae at E14.5 (Fig. 6E; data not shown).[4] Ectopic *Noggin* expression and reduced p-Smad1/5/8 positive cells were seen in the filiform papillae epithelium of *K14-Noggin* adult mice (Fig. 6F and H).

In the wild-type tongue, the pointed tips of the filiform papillae were observed in the intermolar eminence (Fig. 7C). In other regions, including the anterior part and edges of the tongue, filiform papillae have rounded tips (Fig. 7D and E). In *K14-Noggin* mice, the fungiform papillae were more prominent than those seen in wild-type mice (Fig. 7G). This supports previous reports that primitive fungiform papillae are enlarged by addition of exogenous Noggin *in vitro*.[4] In *K14-Noggin* mice, the anterior part and edge of the tongue showed abnormally pointed tips of the filiform papillae, where rounded types are usually presented (Fig. 7I and J). Significant differences could not be found in the filiform papillae in the intermolar eminence in the *K14-Noggin* mice (Fig. 7H). Unlike the left-right axis, the filiform papillae were normal in the anterior–posterior axis in *K14-Noggin* mice (data not shown).

4. Discussion

Bmps show a dynamic temporo-spatial expression pattern in filiform papillae development of both anterior tongue and intermolar eminence. Reduced Bmp signalling induces pointed type papillae formation in the anterior tongue that usually have rounded type papillae. This suggests that Bmp signalling is required for regulating the shape of filiform papillae in

anterior tongue. Unlike anterior tongue, the filiform papillae in the intermolar eminence retained their shape as pointed type in *K14-Noggin* mice. Mice with mutation of follistatin, an antagonist of a subset of TGF superfamily molecules, however show a lack of the filiform papillae only in the intermolar eminence which is believed to be caused by upregulation of Bmp7.[2] This suggests that Bmp signalling is involved in the formation of both types of filiform papillae, but that there are differences in sensitivity to the Bmp signalling between filiform papillae in the anterior tongue and the intermolar eminence.

It is also conceivable that filiform papillae development is regulated by the expression of different ligands between the anterior tongue and the intermolar eminence. In fact, the Bmp ligands and Bmp receptors examined in this study show slightly difference in expression between the anterior tongue and the intermolar eminence. *Bmp5* expression was found in the intermolar eminence but not in the anterior tongue at E14.5 and E16.5. *Bmp4* also showed slightly restricted expression pattern. In addition to these ligands, it is feasible that differences in the expression patterns of other Bmp signalling related ligands, such as *Gdf5* and *Gdf11*, could contribute to the different shapes of filiform papillae between the tongue regions.

Mutation of *Hoxc13* is known to lead to weaken filiform papillae, which results in break of tip of filiform papillae. *Hoxc13* mutant mice therefore show abnormal rounded filiform papillae.[25] However, any sign of broken-off filiform papillae pieces could not be detected in *K14-Noggin* mice. Unlike the left-right axis, the filiform papillae were normal shape in the anterior–posterior axis in *K14-Noggin* mice, ruling out possibility that abnormal shape of filiform papillae in *K14-Noggin* mice is caused by wearing papillae during usage. In the anterior tongue, Bmps thus only regulate the shape of filiform papillae in the left-right axis. In *Pax9* mutant mice, filiform papillae lack anterior–posterior polarity and a cornified layer is absent.[26] This suggests that the pathways regulated by *Pax9* are related to shape in the anterior–posterior polarity, whereas Bmps regulate the left-right axis morphology.

K14-Noggin mice also show significant increase in the number of trigeminal neurons in skin.[27] Innervation is known to play a critical role in morphology of tongue papillae.[28–30] It is possible that alteration of innervation lead to abnormal shape of filiform papillae formation in *K14-Noggin* mice.

Epithelium of oral mucosa including tongue papillae provides permeability barrier function. *Follistatin* mutants show defects in barrier formation in intermolar eminence, suggesting that Bmp signalling play a critical role in barrier formation.[2] In *K14-Noggin* mice, any significant signs of disruption of barrier formation such as oedema, erythema, blister and infusion of inflammatory cells could not be detected in anterior tongue where shapes of filiform papillae are altered. In common with filiform papillae shape, barrier formation is probably regulated by different molecular mechanisms between the anterior tongue and the intermolar eminence.

Similar transgenic mouse model (overexpression of *Noggin* in epithelium) has shown that strength of phenotypes correlates with transgene copy number in the mouse genome and Noggin expression level.[31] Filiform papillae phenotypes could be variable by intensity of Bmp signalling.

Acknowledgments

REFERENCES

1. Iwasaki S, Okumura Y, Kumakura M. Ultrastructural study of the relationship between the morphogenesis of filiform papillae and the keratinization of the lingual epithelium in the mouse. Cells Tissues Organs. 1999; 165(2):91–103. [PubMed: 10516422]

2. Beites CL, Hollenbeck PL, Kim J, Lovell-Badge R, Lander AD, Calof AL. Follistatin modulates a BMP autoregulatory loop to control the size and patterning of sensory domains in the developing tongue. Development. 2009; 136(13):2187–2197. [PubMed: 19474151]

3. Okubo T, Pevny LH, Hogan BL. Sox2 is required for development of taste bud sensory cells. Genes Dev. 2006; 20(19):2654–2659. [PubMed: 17015430]

4. Zhou Y, Liu HX, Mistretta CM. Bone morphogenetic proteins and noggin: inhibiting and inducing fungiform taste papilla development. Dev Biol. 2006; 297(1):198–213. [PubMed: 16828469]

5. Nie X. Apoptosis, proliferation and gene expression patterns in mouse developing tongue. Anat Embryol (Berl). 2005; 210(2):125–132. [PubMed: 16151852]

6. Jung HS, Oropeza V, Thesleff I. Shh, Bmp-2, Bmp-4 and Fgf-8 are associated with initiation and patterning of mouse tongue papillae. Mech Dev. 1999; 81(1–2):179–182. [PubMed: 10330496]

7. Kim JY, Mochizuki T, Akita K, Jung HS. Morphological evidence of the importance of epithelial tissue during mouse tongue development. Exp Cell Res. 2003; 290(2):217–226. [PubMed: 14567981]

8. Kim JY, Lee MJ, Cho KW, Lee JM, Kim YJ, Jung HI, et al. Shh and ROCK1 modulate the dynamic epithelial morphogenesis in circumvallate papilla development. Dev Biol. 2009; 325(1):273–280. [PubMed: 19014928]

9. Ota MS, Kaneko Y, Kondo K, Ogishima S, Tanaka H, Eto K, et al. Combined in silico and in vivo analyses reveal role of Hes1 in taste cell differentiation. PLoS Genet. 2009; 5(4):e1000443. [PubMed: 19343206]

10. Wojcik SM, Longley MA, Roop DR. Discovery of a novel murine keratin 6 (K6) isoform explains the absence of hair and nail defects in mice deficient for K6a and K6b. J Cell Biol. 2001; 154(3): 619–630. [PubMed: 11489919]

11. Wong P, Colucci-Guyon E, Takahashi K, Gu C, Babinet C, Coulombe PA. Introducing a null mutation in the mouse K6alpha and K6beta genes reveals their essential structural role in the oral mucosa. J Cell Biol. 2000; 150(4):921–928. [PubMed: 10953016]

12. Hirao T, Saga T, Kusukawa J, Yamaki K. Angiogenesis and developmental expression of vascular endothelial growth factor in rat lingual papillae. Kurume Med J. 2007; 54(1–2):9–24. [PubMed: 18332592]

13. Nie X, Luukko K, Kettunen P. BMP signalling in craniofacial development. Int J Dev Biol. 2006; 50(6):511–521. [PubMed: 16741866]

14. Tucker AS, Matthews KL, Sharpe PT. Transformation of tooth type induced by inhibition of BMP signaling. Science. 1998; 282(5391):1136–1138. [PubMed: 9804553]

15. Kim JY, Cho SW, Lee MJ, Hwang HJ, Lee JM, Lee SI, et al. Inhibition of connexin 43 alters Shh and Bmp-2 expression patterns in embryonic mouse tongue. Cell Tissue Res. 2005; 320(3):409–415. [PubMed: 15846511]

16. Guha U, Gomes WA, Kobayashi T, Pestell RG, Kessler JA. In vivo evidence that Bmp signaling is necessary for apoptosis in the mouse limb. Dev Biol. 2002; 249(1):108–120. [PubMed: 12217322]

17. Ohazama A, Johnson EB, Ota MS, Choi HY, Choi HJ, Porntaveetus T, et al. Lrp4 modulates extracellular integration of cell signaling pathways in development. PLoS One. 2008; 3(12):e4092. [PubMed: 19116665]

18. Jung HS, Akita K, Kim JY. Spacing patterns on tongue surface-gustatory papilla. Int J Dev Biol. 2004; 48(2–3):157–161. [PubMed: 15272380]

19. Iwasaki S, Yoshizawa H, Aoyagi H. Immunohistochemical expression of keratins 13 and 14 in the lingual epithelium of rats during the morphogenesis of filiform papillae. Arch Oral Biol. 2006; 51(5):416–426. [PubMed: 16271699]

20. Miyazawa K, Shinozaki M, Hara T, Furuya T, Miyazono K. Two major Smad pathways in TGF-beta superfamily signalling. Genes Cells. 2002; 7(12):1191–1204. [PubMed: 12485160]

21. Miyazono K. Positive and negative regulation of TGF-beta signaling. J Cell Sci. 2000; 113(Pt 7): 1101–1109. [PubMed: 10704361]

22. ten Dijke P, Korchynskyi O, Valdimarsdottir G, Goumans MJ. Controlling cell fate by bone morphogenetic protein receptors. Mol Cell Endocrinol. 2003; 211(1–2):105–113. [PubMed: 14656483]

23. Söderberg SS, Karlsson G, Karlsson S. Complex and context dependent regulation of hematopoiesis by TGF-beta superfamily signaling. Ann N Y Acad Sci. 2009; 1176:55–69. [PubMed: 19796233]

24. Iwasaki S, Aoyagi H, Yoshizawa H. Immunohistochemical detection of the expression of keratin 14 in the lingual epithelium of rats during the morphogenesis of filiform papillae. Arch Oral Biol. 2003; 48(8):605–613. [PubMed: 12828990]

25. Godwin AR, Capecchi MR. Hoxc13 mutant mice lack external hair. Genes Dev. 1998; 12:11–20. [PubMed: 9420327]

26. Jonker L, Kist R, Aw A, Wappler I, Peters H. Pax9 is required for filiform papilla development and suppresses skin-specific differentiation of the mammalian tongue epithelium. Mech Dev. 2004; 121(11):1313–1322. [PubMed: 15454262]

27. Guha U, Gomes WA, Samanta J, Gupta M, Rice FL, Kessler JA. Target-derived BMP signaling limits sensory neuron number and the extent of peripheral innervation in vivo. Development. 2004; 131(5):1175–1186. [PubMed: 14973275]

28. Oakley B, Wu LH, Lawton A, DeSibour. Neural control of ectopic filiform spines in adult tongue. Neuroscience. 1990; 36:831–838. [PubMed: 2234415]

29. Sollars SI. Chorda tympani nerve transection at different developmental ages produces differential effects on taste bud volume and papillae morphology in the rat. J Neurobiol. 2005; 64:310–320. [PubMed: 15898061]

30. Nosrat CA, Blomlof J, ElShamy WM, Ernfors P, Olson L. Lingual deficits in BDNF and NT3 mutant mice leading to gustatory and somatosensory disturbance, respectively. Development. 1997; 124:1333–1342. [PubMed: 9118804]

31. Plikus MV, Zeichner-David M, Mayer JA, Reyna J, Bringas P, Thewissen JGM, et al. Morphoregulation of teeth: modulating the number, size, shape and differentiation by tuning Bmp activity. Evol Dev. 2005; 7:440–457. [PubMed: 16174037]
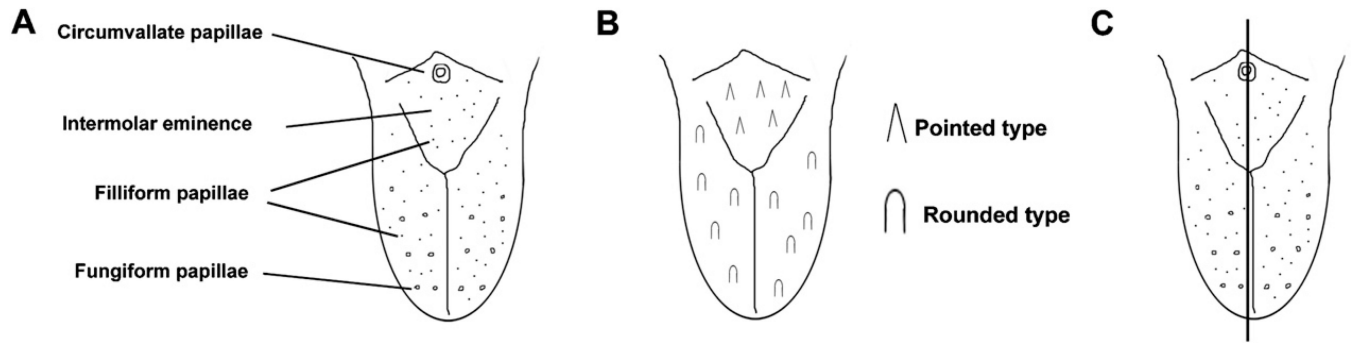
Fig. 1.
Mouse tongue papillae. (A) Schematic diagram of mouse tongue papillae. (B) Schematic diagram of the two types of filiform tongue papillae; pointed type in the intermolar eminence and rounded type in the anterior region. (C) Schematic diagram of the direction of sections; sagittal sections including circumvallate papillae of the intermolar eminence.
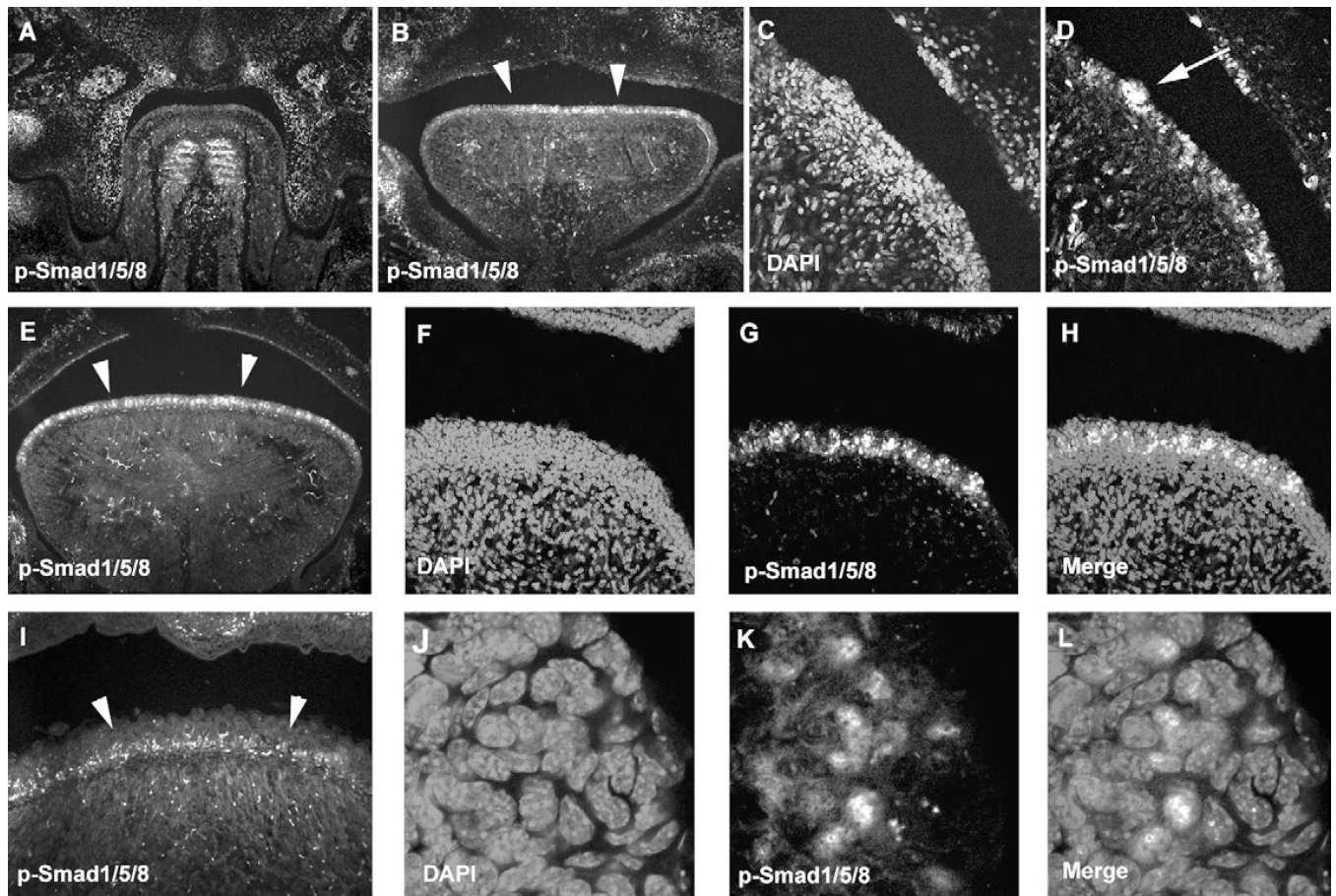
Fig. 2.
P-Smad1/5/8 in filiform papilla development. Immunohistochemistry of p-Smad1/5/8 in frontal head sections of the developing tongue at E13.5 (A), E14.5 (B–D), E16.5 (E–H, J–L) and birth (I). Arrowheads indicating p-Smad1/5/8 positive cells in tongue papillae (B, E, I). Arrow indicating p-Smad1/5/8 positive cells in fungiform papillae (D). DAPI (C, F, J), p-Smad1/5/8 (A, B, D, E, G, I, K), Merge images (H, L). (F–H, J–L) High magnification of E.
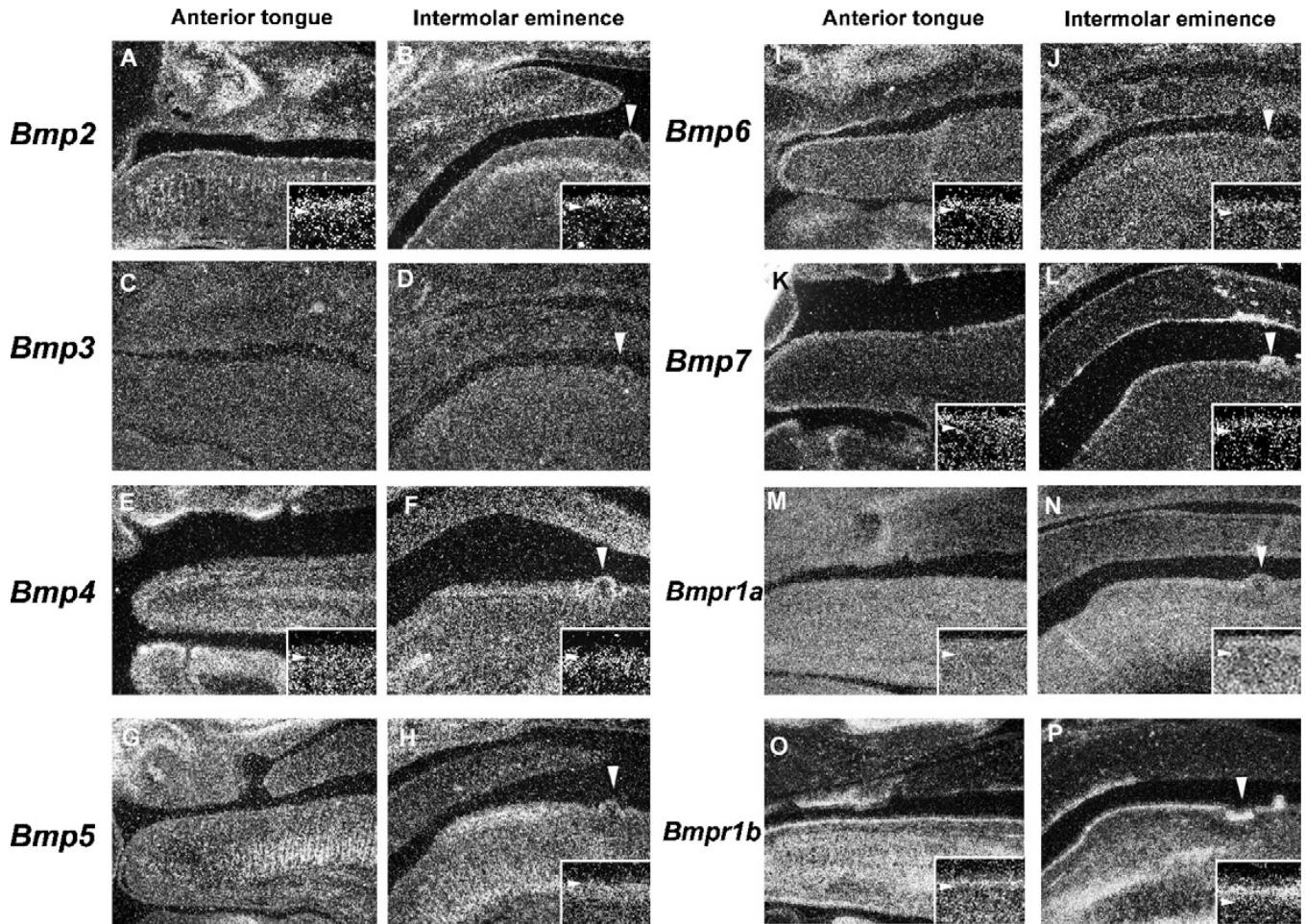
Fig. 3.
Bmp expression in filiform papilla development at E14.5. *In situ* hybridization of *Bmps* (A–L) and *Bmp receptors* (M–P) showing expression in sagittal head sections of the anterior tongue (A, C, E, G, I, K, M, O) and the intermolar eminence (B, D, F, H, J, L, N, P) at E14.5 *Bmp2* (A, B) *Bmp3* (C, D) *Bmp4* (E, F) *Bmp5* (G, H) *Bmp6* (I, J) *Bmp7* (K, L) *Bmpr1a* (M, N) and *Bmpr1a* (O, P). Arrowheads indicating the circumvallate papillae (B, D, F, H, J, L, N, P). (A, B, E, F, H, I–P) Inset is high magnification view of the filiform papillae. Arrowhead indicating the boundary between epithelium and mesenchyme in inset.
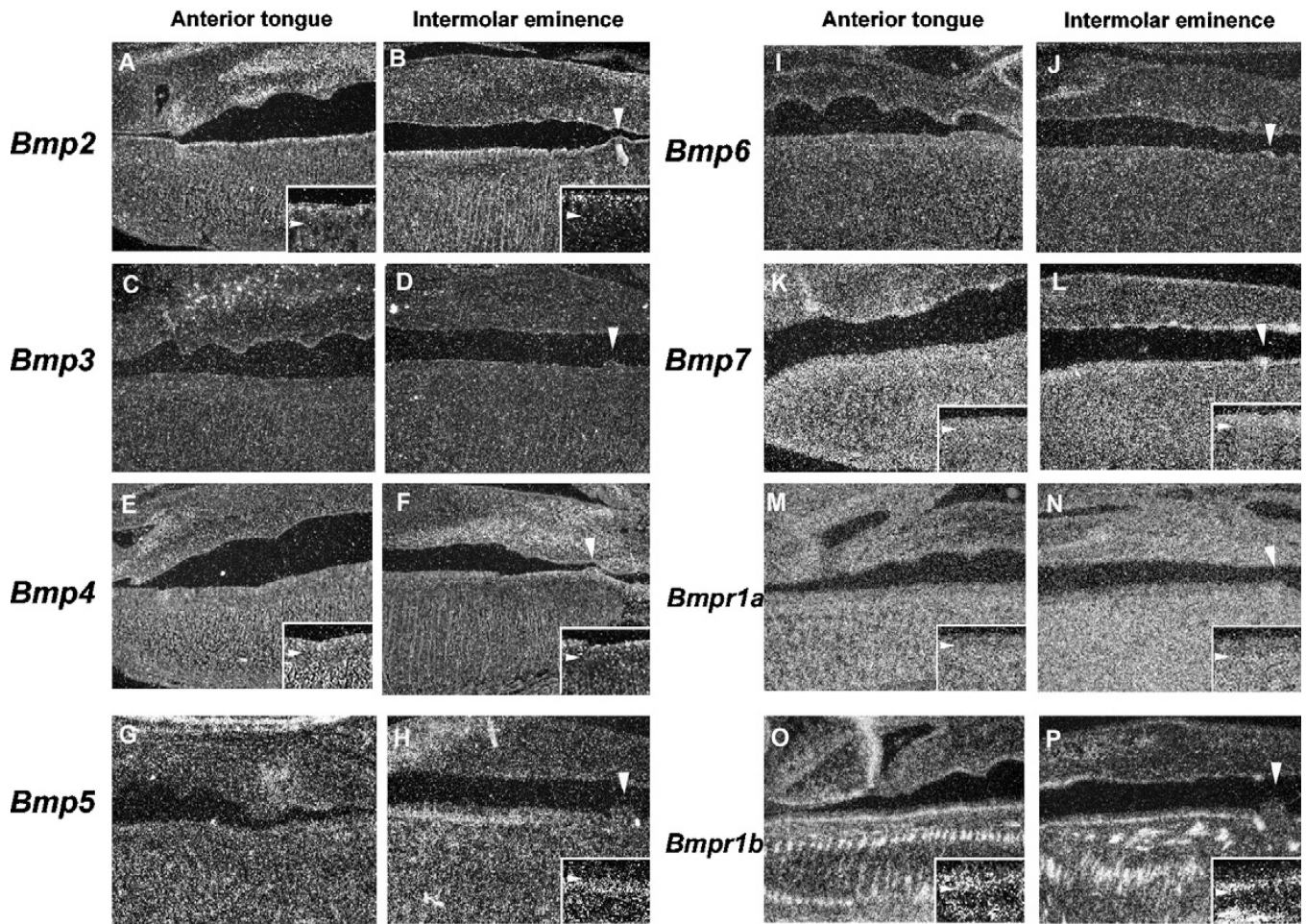
Fig. 4.
Bmp expression in filiform papilla development at E16.5. *In situ* hybridization of *Bmps* (A–L) and *Bmp receptors* (M–P) showing expression in sagittal head sections of the anterior tongue (A, C, E, G, I, K, M, O) and the intermolar eminence (B, D, F, H, J, L, N, P) at E16.5. Arrowheads indicate the circumvallate papillae *Bmp2* (A, B) *Bmp3* (C, D) *Bmp4* (E, F) *Bmp5* (G, H) *Bmp6* (I, J) *Bmp7* (K, L) *Bmpr1a* (M, N) and *Bmpr1a* (O, P) Arrowheads indicating the circumvallate papillae (B, D, F, H, J, L, N, P). (A, B, E, F, H, K–P) Insets are high magnification views of the filiform papillae. Arrowheads indicating the boundary between epithelium and mesenchyme in insets.
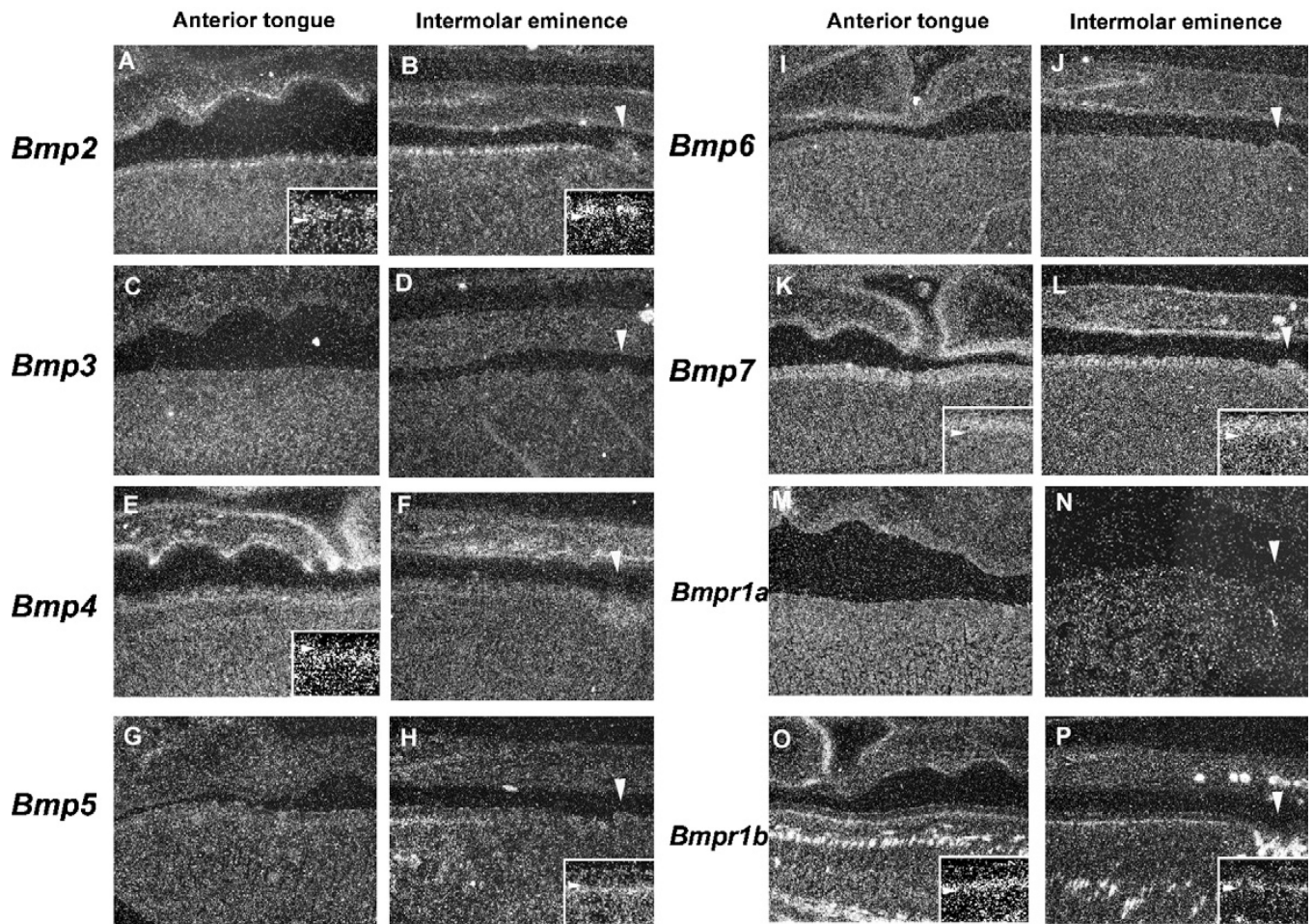
Fig. 5.
Bmp expression in filiform papilla development at birth. *In situ* hybridization of *Bmps* (A–L) and *Bmp receptors* (M–P) showing expression in sagittal head sections of the anterior tongue (A, C, E, G, I, K, M, O) and the intermolar eminence (B, D, F, H, J, L, N, P) at birth. Arrowheads indicate the circumvallate papillae *Bmp2* (A, B) *Bmp3* (C, D) *Bmp4* (E, F) *Bmp5* (G, H) *Bmp6* (I, J) *Bmp7* (K, L) *Bmpr1a* (M, N) and *Bmpr1a* (O, P). Arrowheads indicating the circumvallate papillae (B, D, F, H, J, L, N, P). (A, B, E, H, K, L, O, P) Insets are high magnification views of the filiform papillae. Arrowheads indicating the boundary between epithelium and mesenchyme in insets.
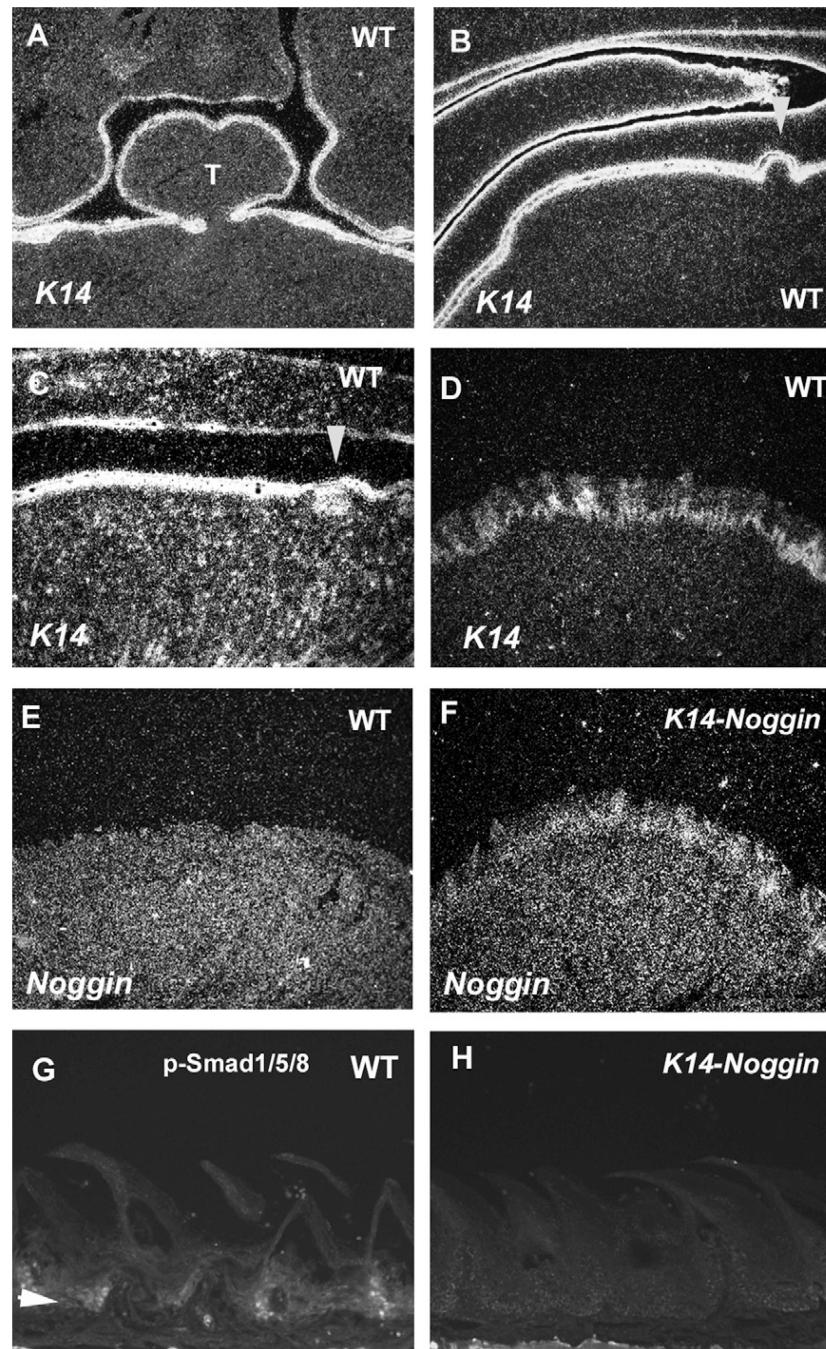
Fig. 6.
K14 and *Noggin* expression and p-Smad1/5/8 localization in tongue papilla of wild-type and *K14-Noggin* mice. *In situ* hybridization showing the expression of *K14* (A–D) and *Noggin* (E, F) in frontal head sections (A, D–F) and in sagittal head sections (B, C) of wild-type (A–E) and *K14-Noggin* (.F) at E12.5 (A), E14.5 (B), E16.5 (C) and five months of age (D–H). Arrowheads indicating the circumvallate papillae (B, C). (G, H) Immunohistochemistry of p-Smad1/5/8 in sagittal head sections of wild-type (G) and *K14-Noggin* mice (H) at five months of age. Arrowhead indicating p-Smad1/5/8 positive cells (G). T = Tongue.
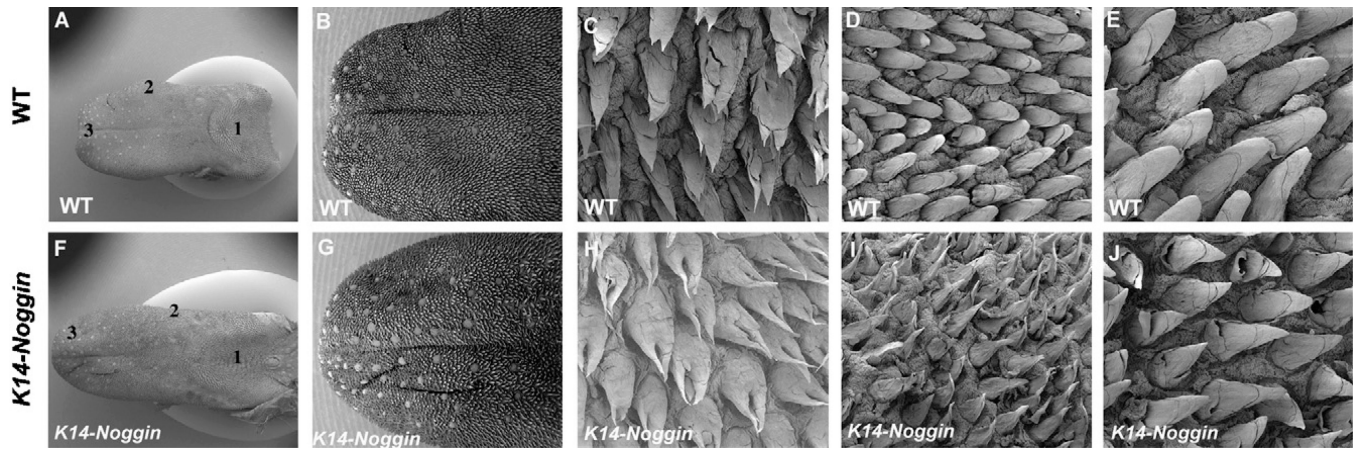
Fig. 7.
SEMs of Filiform papillae in *K14-Noggin* (A, F) Oral view of the entire tongue. (B, G) Oral view of the anterior part of the tongue. (C, H) High magnification of the intermolar eminence (1 in A, F). (D, I) High magnification of the side edge of the tongue (2 in A, F). (E, J) High magnification of the anterior part of the tongue (3 in A, F). Wild-type (A–E) and *K14-Noggin* mice (F–J) at five months of age.

Table 1

Summary of the expression of *Bmps* and *Bmprs*.

	E14.5		E16.5		NB	
	Anterior	**Intermolar**	**Anterior**	**Intermolar**	**Anterior**	**Intermolar**
Bmp2	++	++	++	++	++	++
Bmp3	–	–	–	–	–	–
Bmp4	+	++	+	+	+	–
Bmp5	–	+++	–	+++	–	+
Bmp6	+	+	–	–	–	–
Bmp7	+	+	++	++	++	++
Bmpr1a	++	++	++	++	–	–
Bmpr1b	+++	+++	+++	+++	++	++

(−) no, (+) low, (++) medium, and (+++) high level of expression, Anterior, Anterior tongue; Intermolar, Intermolar eminence.