G蛋白γ13亚单位在发育的嗅上皮和梨鼻中的表达模式研究

王小云[1#] 梁 洁[1#] 顾 复[1] 陈佳莹[1] 陈付学[1*] 李 峰[2*]

([1]上海大学生命学院, 上海 200469; [2]上海市(复旦大学附属)公共卫生临床中心, 上海 201508)

摘要 G蛋白亚单位以前被认为在味蕾中特异性的表达, 和味导素、苦味受体共表达于味蕾的II型细胞。目前的研究发现, Gγ13(G protein γ-subunit Gγ13)在小鼠不同发育时期嗅上皮和梨鼻均存在表达, 包括胚胎期15.5 d(E15.5)、生后期第0 d(P0)、生后期第5 d(P5)、生后期第10 d(P10)、生后期第21 d(P21)和成年期(>P40)。研究也表明, Gγ13可能是一个成熟嗅神经和梨鼻神经的分子标记物。mRNA原位杂交表明, Gγ13和Gα亚单位Gαolf(Gαolf在成熟嗅神经细胞中表达)的表达模式在嗅上皮是一致的, Gγ13和Gα亚单位Gαi2(Gαi2在成熟梨鼻嗅神经细胞中表达)在梨鼻上皮共定位。Gγ13的分布不同于标记细胞发育的标记物GAP43(growth associated protein 43)在嗅上皮的分布, 它的表达也不同于另外一个G蛋白亚单位Gγ8的表达分布。在P21的嗅觉系统, Gγ13蛋白在嗅上皮嗅毛中表达丰富, 在梨鼻的嗅毛表达也丰富。在主嗅球, 在颗粒细胞带、外网层、僧帽细胞带均发现Gγ13的阳性信号。而且, mRNA原位杂交也显示, Gγ13在僧帽细胞带表达, 表明Gγ13可能参与到僧帽细胞向大脑嗅皮质区的信号输送。在副嗅球, 在颗粒细胞层发现微弱的阳性信号。总之, 目前的研究表明, Gγ13可能参与嗅上皮和梨鼻的嗅分子信号传导过程。

关键词 G蛋白; 嗅神经; 梨鼻; 主嗅球; 副嗅球; 小鼠

The G Protein γ-subunit Gγ13 Is Expressed in the Developing Olfactory and Vomeronasal Neurons

Wang Xiaoyun[1#], Liang Jie[1#], Gu Fu[1], Chen Jiaying[1], Chen Fuxue[1*], Li Feng[2*]

([1]*School of Life Sciences, Shanghai University, Shanghai 200469, China*;
[2]*Shanghai Public Health Clinical Center, Fudan University, Shanghai 201508, China*)

Abstract The tissue localization of the G protein γ-subunit Gγ13 that has been believed to be specifically expressed in taste bud before, was studied in the olfactory and vomeronasal neurons at different ages: embryonic day 15.5, postnatal days 0, 5, 10, 21 and adult. Gγ13 appears to be a specific marker of the mature olfactory and vomeronasal neurons. *In situ* hybridization (ISH) reveals that Gγ13 distribution is identical to that of Gαolf, which is predominantly expressed in main olfactory epithelia (MOE), and that of Gαi2, which is predominantly expressed

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上海市科委项目(批准号: 15DZ2290200)资助的课题
#共同第一作者
*通讯作者。Tel: 021-37990333, E-mail: xlfeng006@163.com; Tel: 021-66136620, E-mail: chenfuxue@staff.shu.edu.cn
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#These authors contributed equally to this work
*Corresponding authors. Tel: +86-21-37990333, E-mail: xlfeng006@163.com; Tel: +86-21-66136620, E-mail: chenfuxue@staff.shu.edu.cn
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in mature vomeronasal organ (VNO). Gγ13 distribution differs from that of growth associated protein 43 (GAP43), and that of Gγ8. Gγ13 proteins are enriched in cilia of MOE and microvilli of VNO after P21 days. In main olfactory bulb (MOB), Gγ13-positive signals were present in the glomerular layer (GL), external plexiform layer (EPL) and mitral cell layer (MCL). Furthermore, the expression of Gγ13 was also detected in mitral cell layer with mRNA ISH, indicating that Gγ13 may be involved in the output of mitral cells to various parts of the olfactory cortex. In accessory olfactory bulb (AOB), the weak positive signals were also observed in the glomerular layer. In short, the current results collectively suggest that Gγ13 appear to be involved in signal transduction of MOE and VNO.

Keywords G protein; olfactory neuron; vomeronasal neuron; Gγ13; main olfactory bulb; accessory olfactory bulb; mice

It is believed that a huge varity of G protein coupled receptors (GPCRs) is specifically expressed in main olfactory epithelium (MOE). There are low different of olfactory receptors in rodents′ olfactory epithelium, whereas the number in human has been estimated to be ~350[1-2]. More and more evidences indicate that those olfactory GPCRs appear to employ the same G protein-mediated signal transduction pathway in olfactory sensory neurons. Gαolf, a G protein related to Gs[3], couples olfactory receptors in the cilia to adenylyl cyclase III, resulting in the increased formation of cAMP[4]. cAMP then activates a cyclic nucleotide-gated (CNG) cation channel consisting of three different subunits, CNGA2, CNGA4 and CNGB1. The increase in cellular Ca^{2+}-activates a Ca^{2+}-activated Cl^- channel that further depolarizes the cell membrane[5-7].

A second olfactory system called the accessory olfactory system or the vomeronasal system exists in most mammals[8]. The vomeronasal system responds to pheromones that mediate defined effects on individuals of the same species and modulate social, aggressive, reproductive, and sexual behaviors[9]. In the vomeronasal organ (VNO), two families of GPCRs, which in mice consist of 150 members each, have been identified. The V1 receptor family is expressed in vomeronasal sensory neurons together with the G protein Gαi2, whereas the V2 receptor family is expressed in a different population of neurons that co-expresses the G protein Go[10]. The transient receptor potential channel 2 (TRP2), which is expressed in vomeronasal sensory neurons, has been identified as a critical downstream mediator of the signal transduction pathway in vomeronasal sensory neurons[11].

In addition, heterotrimeric G proteins are believed to be central to wide variety of receptor-effector coupling pathway, and the alpha subunit of these proteins are the only critical determinant of G-protein receptor and G-protein effector interaction. However, it is becoming clear that the diverse βγ subunits also have distinct roles[12]. $G\gamma1^{-/-}$[13], $G\gamma3^{-/-}$[14], $G\gamma7^{-/-}$[15] mice support the notion that these three G protein subtypes contributes to the specificity of signaling pathways in the context of the organism *in vivo*. After released from the activated α subunit, the βγ subunit can regulate over 20 effectors including phospholipase C β2 (PLC-β), adenylyl cyclase, ion channels[16], PtdIns 3-kinase[17] and guanine nucleotide exchangers for small GTP binding proteins[18]. The free βγ dimer can also participate in regulatory events by binding to cytoplasmic proteins such as the β-adrenergic receptor kinase or phosducin[19].

Several G gamma subunits have been reported in olfactory system including MOE and VNO. On the other hand, loss of Bardet-Biedl syndrome (BBS) proteins leads to the missing of Gγ13 in olfactory cilia and defects in olfactory function in the mouse[20]. Hypomorphic CEP290/NPHP6 mutations results in the loss of Gaolf and Gng13 in cilia of olfactory neurons and causes anosmia[21]. Another study further

revealed that Ric-8B, besides interact with Gαolf, also interacts with Gγ13. Furthermore, guanine nucleotide exchange factor 8 B (Ric-8B) co-localizes with Gαolf, Gβ1 and Gγ13 in the cilia of olfactory sensory neurons[22]. Early researchers have already showed that Gγ8 is expressed during neurogenesis in the olfactory and vomeronasal neuroepithelia[23]. So far, it is still not known which Gγ subunit is involved in olfactory signal transduction in mature neuron. In this paper, we studied Gγ13 expression in the developing olfactory and vomeronasal neuroepithelia in detail. Our results showed that Gγ13 expression is gradually observed in cilia of olfactory neurons and VNO during the development of olfactory system, indicating the involvement in the signal transduction of MOE and VNO.

1 Experimental procedures

1.1 Tissue preparation

C57 mice of various postnatal ages (P0, P5, P10, P21, P40 and adult) were anesthetize with intraperitoneal injection of sodium pentobarbital (60 mg/kg), and perfused with ice-cold 4% paraformaldehyde in phosphate-buffered saline (pH7.4). The olfactory organs is dissected and postfixed in 4% PFA/PBS overnight at 4 ºC. Decalcification was carried out by incubation in 500 mmol/L EDTA pH8.0/PBS overnight (for young mice less than 3 weeks) or two nights (for older mice) at 4 ºC. For the embryos, embryonic head (E15.5) were dissected and fixed in 4% PFA/PBS overnight at 4 ºC. Then tissues were incubated in 10% sucrose/PBS for 2 h at 4 ºC, 20% sucrose/PBS for 2 h at 4 ºC, 30% sucrose/PBS for overnight at 4 ºC. Tissues were embedded in OCT compound and freeze in a mixture of dry ice and ethanol. Coronal section (10 μm thick) were cut and mounted on gelatin- and alum-subbed slides and stored at −20 ºC or −80 ºC.

1.2 *In situ* hybridization

In situ hybridization was performed as described in Ishii *et al*[46].

Gγ8 primer: 5′-AGA GTG TTC CAG CCC CCA GT-3′, 5′-ATA CTT CTG CGG GGG AGG AT-3′, 519 bp; *Gαolf* primer: 5′-CCT CAC TGC TGC CTC TTC TCC C-3′, 5′-GTG GCT GAA AAA GTT CCT CTT ATT CTG TTG-3′, 554 bp; *GAP43* primer: 5′-TCA TCA CAT TAT TGC CAT CCC-3′, 5′-TGG GAA GGA AAC ACA GAG ACA-3′, 825 bp; *Gγ13* primer: 5′ TTG CTG TCT CCT CCA AAA CCT-3′, 5′-TGT GGG TCA GGC TCA TAG GAT-3′, 325 bp.

1.3 Immunohistochemical staining

Standard double immunofluorescence was used to assist determining the cellular localization of Gγ13 protein in the olfactory and accessory system. The polyclonal primary antibodies used were specific for ACIII (rabbit sc-206), Gαolf (rabbit sc-385), Gγ13 (goat sc-26781), Gγ2 (rabbit sc-374), Gβ1 (rabbit sc-379), Gαi2 (rabbit sc-7276) (Santa Cruz Biotechnology, Santa Cruz, USA).

Standard double immunofluorescence protocols were used. Briefly, oven dried frozen sections were rehydrated with 0.1 mol/L PBS at pH7.0. For Gγ13 labeling, tissue were blocked in 3% BSA, 0.3% Triton X-100, 2% goat serum and 0.1% sodium azide in PBS for 1 h at room temperature and incubated for 2 days at 4 °C. For neuronal labeling, tissue were blocked in superblock (Pierce) and incubated in primary antibody overnight at 4 °C. All double immunolabeling were done sequentially with appropriate second primary antibodies (Alexa488 donkey anti-rabbit, Alexa555 donkey anti-goat, and Alexa568 donkey anti-mouse; Molecular Probes, Eugene, OR), and DAPI (dilution 1:1 000, Molecular Probes) was used to label cell nuclei. Nonspecific immunolabeling was tested by incubating with no primary antibody.

1.4 Imaging

Brightfield images were captured using a SPOT digital camera (Diagnostic Instruments, Inc) attached to a Nikon SA Microphot microscope and minimally processed using Image-Pro Plus image analysis

software (Media Cybernetics Inc., Silver Spring, MD). Fluorescent images were captured with the Leica TCS SP2 Spectral Confocal Microscope (Leica Microsystems Inc., Mannheim, Germany) using UV, Ar, GeNe and HeNe lasers and appropriate excitation spectrums. Leica Scanware software was used to acquire z-series stacks captured at a 0.3~0.4 m step size. Digital images were cropped, arranged and minimally adjusted for contrast and brightness for background standardization using Photoshop CS (Adobe Systems, Inc., San Jose, California).

2 Results

2.1 Gγ13 expression in the developing olfactory epithelium

GAP43 is a well characterized phosphor-protein that has been localized to the growth cones of most developing neurons including olfactory neurons[24]. The expression and distribution of Gγ8 vary considerably during the development of the olfactory epithelium, indicating that this protein has a specific function in olfactory neural development and regeneration[25]. Here, both GAP-43 and Gγ8 is used to label the immature olfactory neuron.

Agreed with previous study[26], GAP43 expression was detected since E15.5 (Fig.1A). Meanwhile, we also observed the expression of GAP43 in all points checked, including P0 (Fig.1E), P5 (Fig.1I) and P21 (Fig.1M). GAP43 expression was strictly limited to the basal part of olfactory epithelium since P5 (Fig.1I). Similar with previous study in rat[23], Gγ8 expression was found in neurons throughout the epithelium at E15.5 (Fig.1B). Newborn mice at P0 also expressed Gγ8 in the epithelium (Fig.1F). At P5, the expression of pattern was very similar to that seen at P0 (Fig.1J). A different pattern of Gγ8 expression was found at P21, when Gγ8-positive cells were located in the middle and basal part of the neuroepithelium (Fig.1N), which contains immature olfactory neurons and globose basal cells[27].

In situ hybridization (ISH) of Gγ13 was compared with that of Gαolf, Gγ8 and GAP43 in the serial sections of the developing olfactory epithelium. The distribution of Gγ13 was very similar with that of Gαolf, different from that of Gγ8 and GAP43. *Gαolf* (Fig.1C) and *Gγ13* (Fig.1D) mRNAs were detected at E15.5, at P0 both expression dramatically increase (Fig.1G [Gαolf] and Fig.1H [Gγ13]), at P5 keep stable (Fig.1K [Golf] and Fig.1L [Gγ13], then at P21 hybridization signal almost occupy the whole olfactory epithelium (Fig.1O [Gαolf] and Fig.1P [Gγ13]). In addition, *Gγ13* mRNA was also visualized by single-color RNA ISH in MOE at P0 (Fig.2A). Since P5, positive signals of both were most concentrated in the basal/apical part of the MOE (Fig.1K, Fig.1L, Fig.1O and Fig.1P).

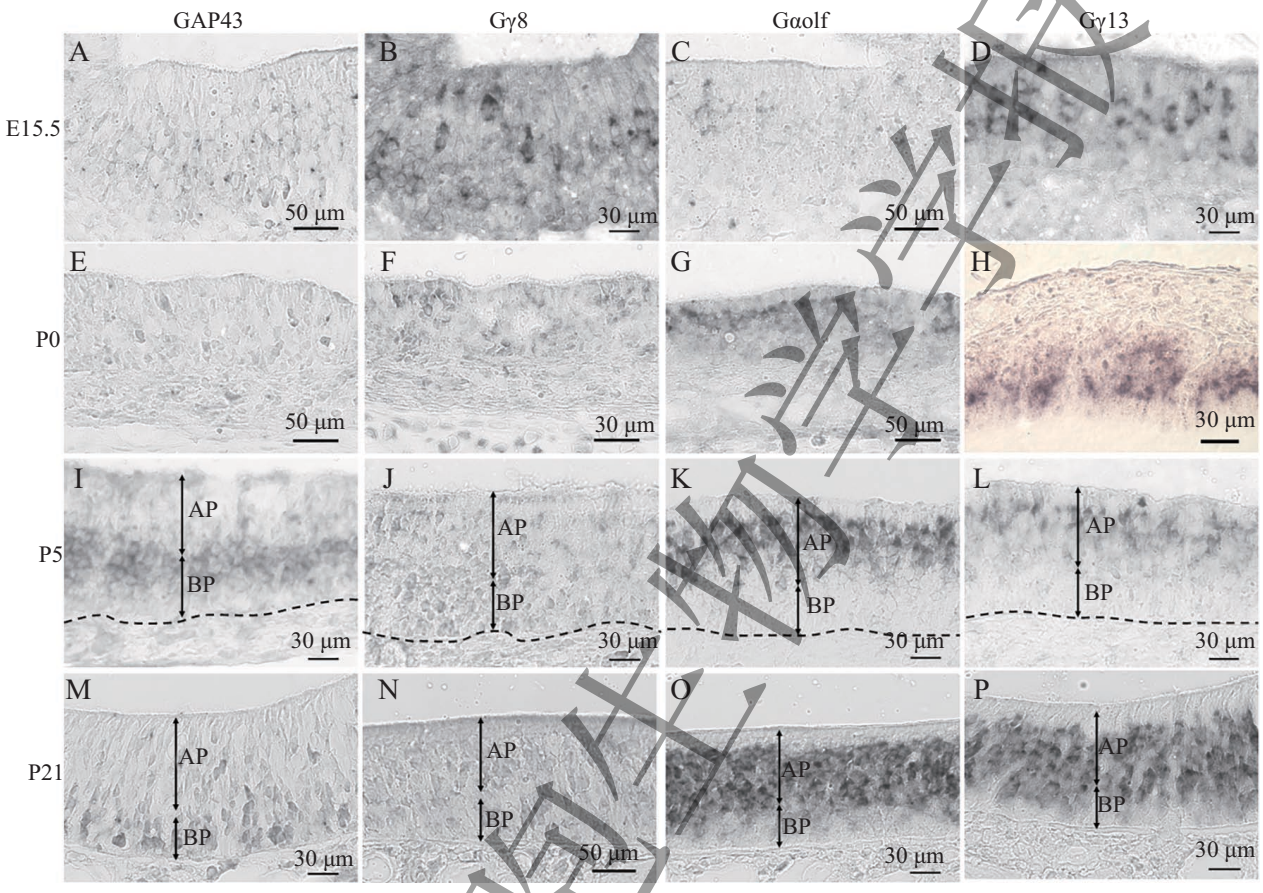
2.2 Gγ13 expression in the developing vomeronasal organ (VNO)

At E15.5, the weak signals of GAP43 were observed both in sensory parts (S-VNO) and non-sensory parts (NS-VNO) of epithelium (Fig.3A). At P0, the similar expression pattern was observed in VNO (Fig.3D). The expression increased significantly at P5 (Fig.3G) or P10 (Fig.3J). Positive signals almost became undetectable in the center of VNO by P21 (Fig.3M), few signals were found in the boundary of S-VNO and NS-VNO. Meanwhile, GAP43 was no longer detectable in the NS-VNO by P21 (Fig.3M).

Similar with the expression pattern of MOE, the Gγ8-positive signal were observed in NS-VNO and S-VNO at E15.5 (Fig.3B). After born (P0), the expression of Gγ8 dramatically increased (Fig.3E). The similar expression pattern was individually observed at P5 (Fig.3H), P10 (Fig.3K) and P21 (Fig.3N). In all development stages, Gγ8 expression was not found to be limited to specific region (basal or apical part), no regional variation was detected in the neuro-epithelium of VNO.

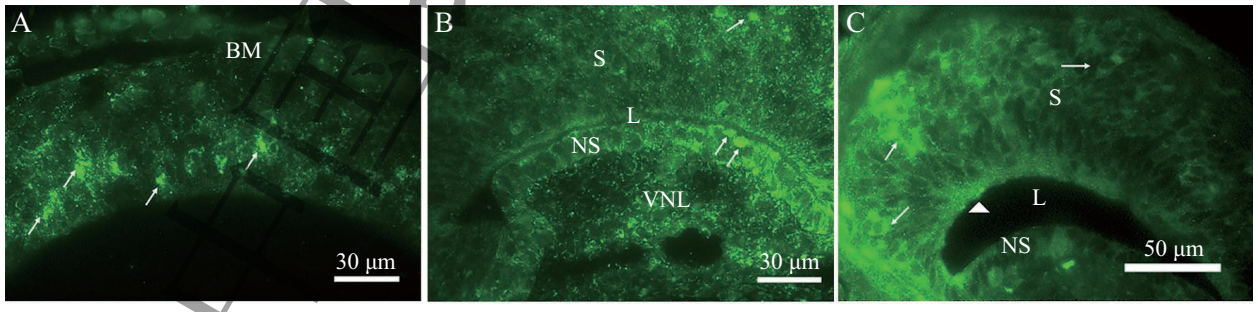
mRNA ISH of *Gγ13* was compare with that of *Gγ8* and *GAP43* in the developing VNO. The distribution of Gγ13 very differed from that of Gγ8 and GAP43. At E15.5, we observed a few signal of Gγ13

in both the S-VNO and NS-VNO (Fig.3C). At P0, positive signals were still detectable both in the S-VNO and NS-VNO (Fig.3F). In addition, *Gγ13* mRNAs were also visualized by single-color RNA ISH in VNO at P0 (Fig.2B). Immunostaining with anti-Gγ13 revealed the wide distribution of Gγ13 protein through the VNO at



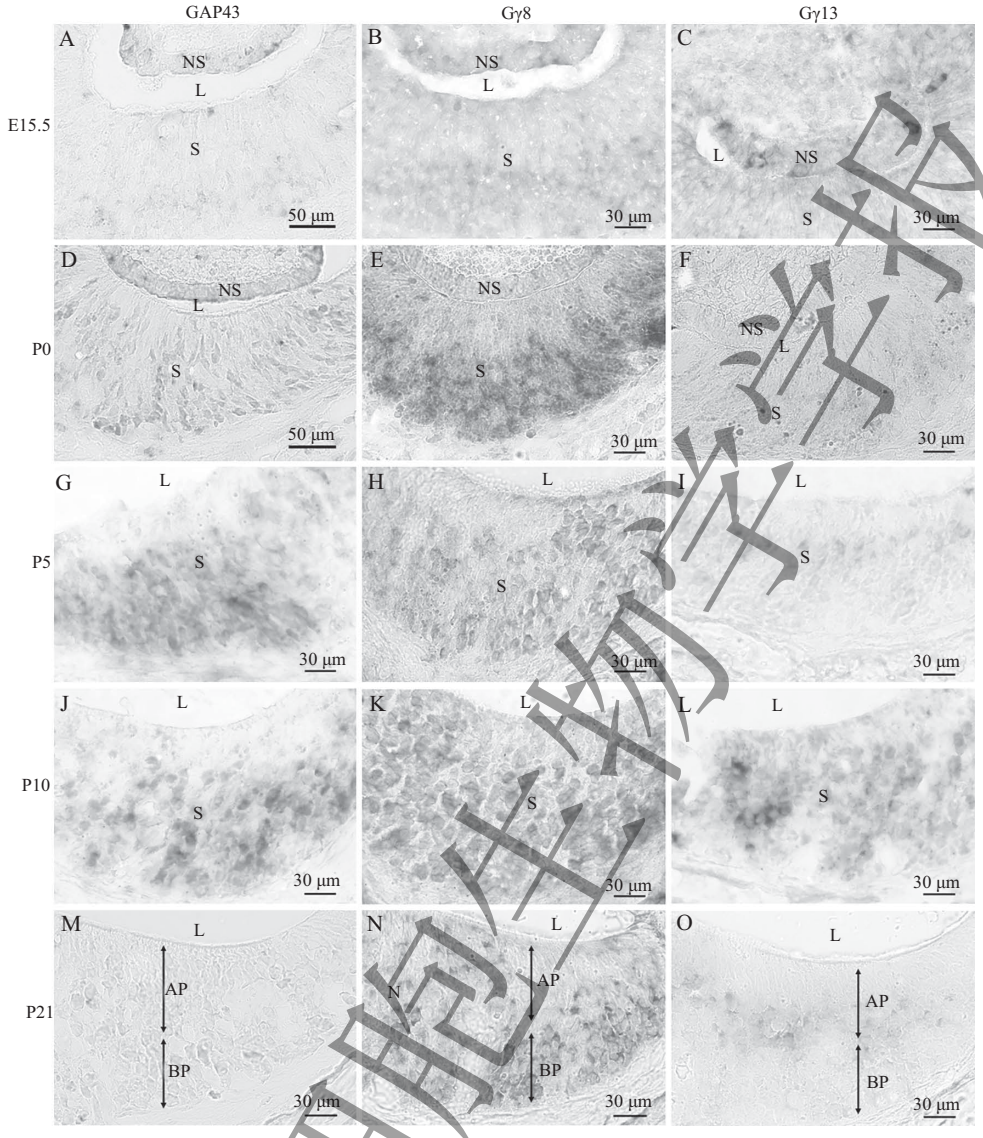
At E15.5, Gαolf (C), Gγ8 (B) and Gγ13 (D) expression is detected in MOE, except GAP43 expression (A). At P0, GAP43 (E), Gαolf (G), Gγ8 (F) and Gγ13 (H) expression is still detected in MOE. At P5, GAP43 expression is detected in the basal part of MOE (I). However, Gαolf (K) and Gγ13 (L) expression is detected in the apical part of MOE. Gγ8 expression evenly distribute into the basal and apical part of MOE (J). At P21, GAP43 (M) and Gγ8 (N) expression concentrate in the basal part of MOE, but Gαolf (O) and Gγ13 (P) expression mainly distribute into the apical part of MOE. MOE: main olfactory epithelium; AP: apical part of MOE; BP: basal part of MOE; dotted line: basal membrane of MOE.

Fig.1 Gγ13 expression in the developmental MOE



RNA ISH with FLU-labeled Gγ13 probes shows the Gγ13 expression in MOE and VNO at P0. A: main olfactory epithelium. Arrow: positive signals in MOE. BM: basal membrane. B: Gγ13 expression is detected in sensory and non-sensory epithelium of VNO. Arrow: positive signals in VNO. C: immunostaining with anti-Gγ13 indicates the cell bodies expressing Gγ13. Arrow: Gγ13-positive cells. Triangle: one microvilli containing Gγ13 in VNO. S: sensory epithelium; NS: non-sensory epithelium; L: lumen; VNL: vermonasal nerve bundle.

Fig.2 Gγ13 expression in sensory and non-sensory epithelium of VNO at P0



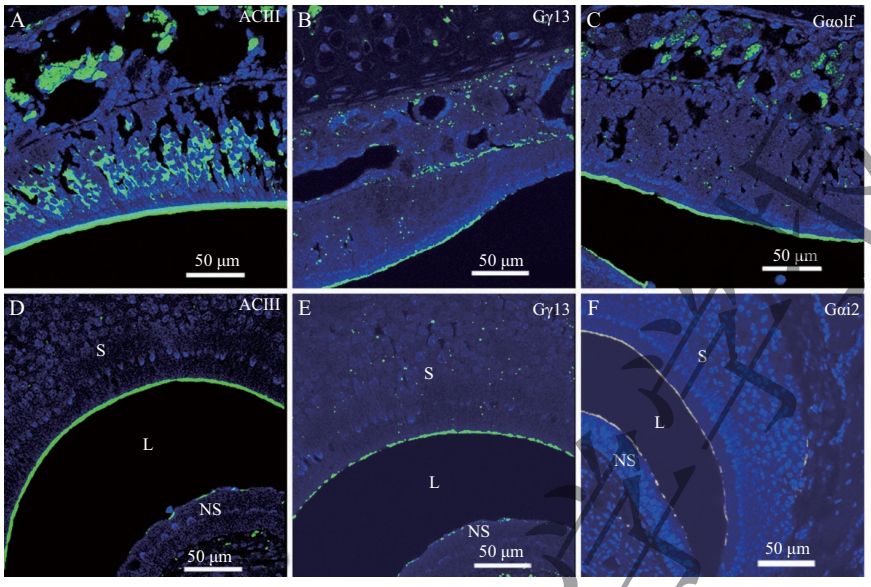
At E15.5, GAP43 (A), Gγ8 (B) and Gγ13 (C) expression is observed in VNO. GAP43 (A) and Gγ13 (C) expression is also detected in non-sensory epithelium in VNO. At P0, GAP43 (D), Gγ8 (E) and Gγ13 (F) expression in VNO. At P5, GAP43 (G), Gγ8 (H) and Gγ13 (I) expression in VNO. At P10, GAP43 (J), Gγ8 (K) and Gγ13 (L) expression in VNO. At P21, GAP43 (M), Gγ8 (N) and Gγ13 (O) expression in VNO. At P21, Gγ13 expression distribute into the apical part of VNO. S: sensory epithelium; NS: non-sensory epithelium; L: lumen; AP: apical part of sensory epithelium in VNO; BP: basal part of sensory epithelium in VNO.

Fig.3 Gγ13 expression in the developmental vomeronasal organ

P0 (Fig.2C). At P5 (Fig.3I) and P10 (Fig.3L), the high expression of Gγ13 was observed in neurons in the apical part of the neuro-epithelium that lies closer to the epithelial surface. By P21, we observed an adult-like labelling pattern of Gγ13, that Gγ13 expression was limited to apical part of VNO (Fig.3O).

2.3 Localization of Gγ13 in olfactory and vomeronasal neurons

It is believed that the distribution of Gγ8 is different from that of Gαolf[23]. In order to verify the distribution of Gγ13 in olfactory epithelium, we further compared the distribution of Gγ13 in MOE and VNO by confocal analysis. Immunostaining with anti-ACIII showed the expression of ACIII in MOE (Fig.4A). As expected, confocal analysis revealed the uniform expression pattern between Gγ13 (Fig.4B) and Gαolf (Fig.4C). Positive signals were most concentrated in the cilia of the olfactory neurons.



ACIII expression is observed in olfactory neuron and olfactory cilia (A). Gγ13 (B) and Gαolf (C) expression is observed in olfactory cilia. ACIII expression is also observed in VNO microvilli (D). Gγ13 (E) and Gαi2 (F) expressions are also observed in VNO microvilli. S: sensory epithelium; NS: non-sensory epithelium; L: lumen.

Fig.4 Gγ13 proteins are observed in cilia of MOE and microvilli of VNO

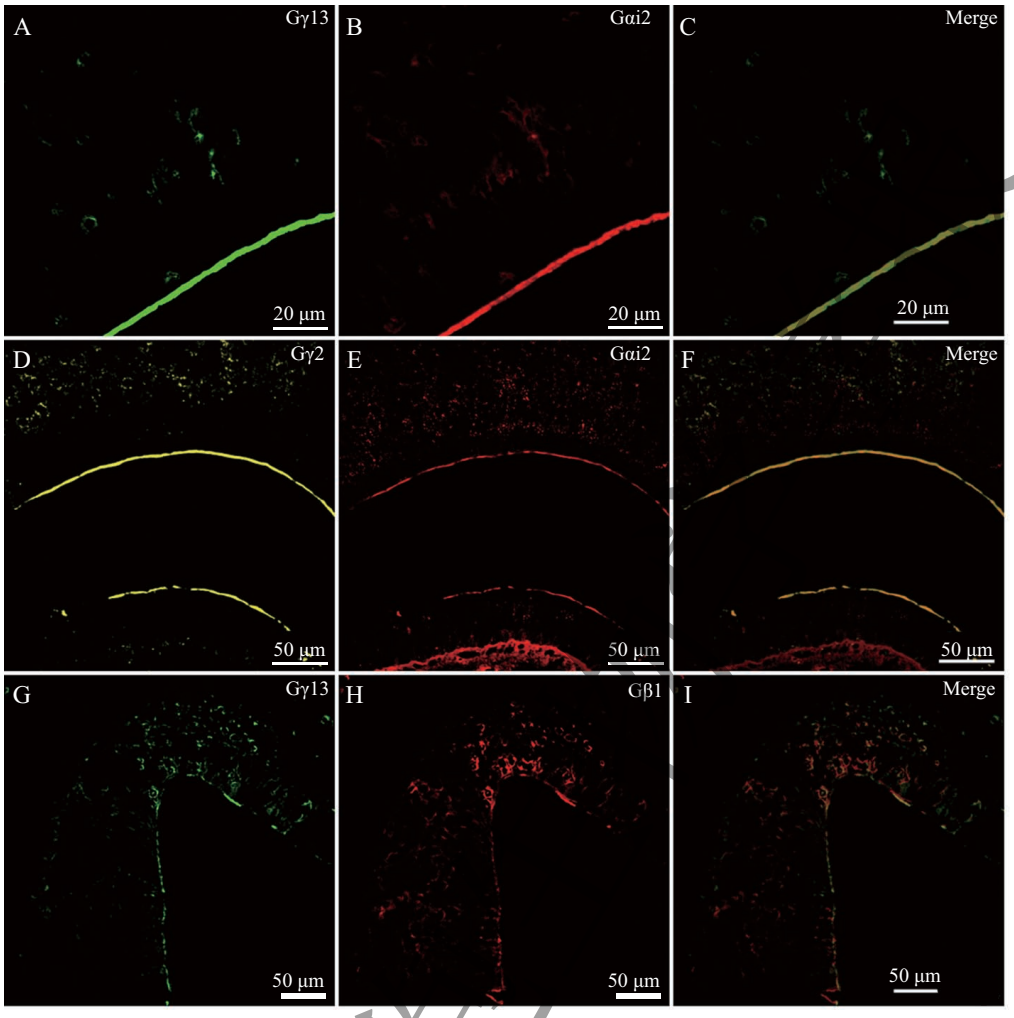
The intensive staining was also detected in the axon bundle under the basal lamina (Fig.4B and Fig.4C). In VNO, immunostaining with anti-ACIII showed the microvilli of VNO (Fig.4D), where positive signals were also observed after immunostaining with anti-Gγ13 (Fig.4E). Meanwhile, the microvilli was also showed to be Gαi2-positive (Fig.4F). Furthermore, Gγ13 completely co-localized with Gαi2 (Fig.5A-Fig.5C), partially with Gβ1 (Fig.5G-Fig.5I), after confocal analysis with double staining. On the other hand, Gγ2 also co-localized with Gαi2 in microvilli of VNO (Fig.5D-Fig.5F).

In addition, the distribution of Gγ13 protein was also investigated in P0. Many positive cells were found throughout the epithelium of VNO. In this stage, the Gγ13 proteins were kept in cell bodies (Fig.2C). In the followed stages (P21), the positive signals were concentrated in the lumen surface of VNO epithelium (Fig.4E).

2.4 The distribution of Gγ13 in the main olfactory bulb and accessory olfactory bulb

In mice, olfactory neurons in MOE project to glomeruli in MOB, and in VNO to that in AOB[2]. Gγ13 expression in MOE and VNO indicate the possible distribution of Gγ13 proteins in MOB and AOB. As expected, Gγ13 proteins were indeed observed in GL (including olfactory nerve and glomeruli) (Fig.6A and Fig.6B), and EPL as well (Fig.6C and Fig.6D). On the other hand, ISH also revealed the expression of Gγ13 in mitral cell layer (Fig.6F), where GAP43 expression was also detected in P21 MOB (Fig.6E). In short, Gγ13 proteins were widely observed in main olfactory system at P21, including cilla, olfactory nerve bundles, glomeruli layer and mitral cell layer (Fig.6G). However, Gγ13 proteins were only detected in mitral cell layer in MOB at P0 (Fig.6H).

The similar specific distribution of Gγ13 with Gαi2 in VNO indicated the possible regional distribution of Gγ13-positive neurons in AOB. Furthermore, the distribution of several G proteins in AOB were investigated by confocal analysis. Compared to the special distribution of Gαi2 protein in the anterior part of AOB (Fig.7A and Fig.7B), the weak staining was observed for Gγ13 protein in AOB (Fig.7C and Fig.7D). However, the strong positive signals of Gγ13 were also found in EPL (Fig.7C). Interesting,



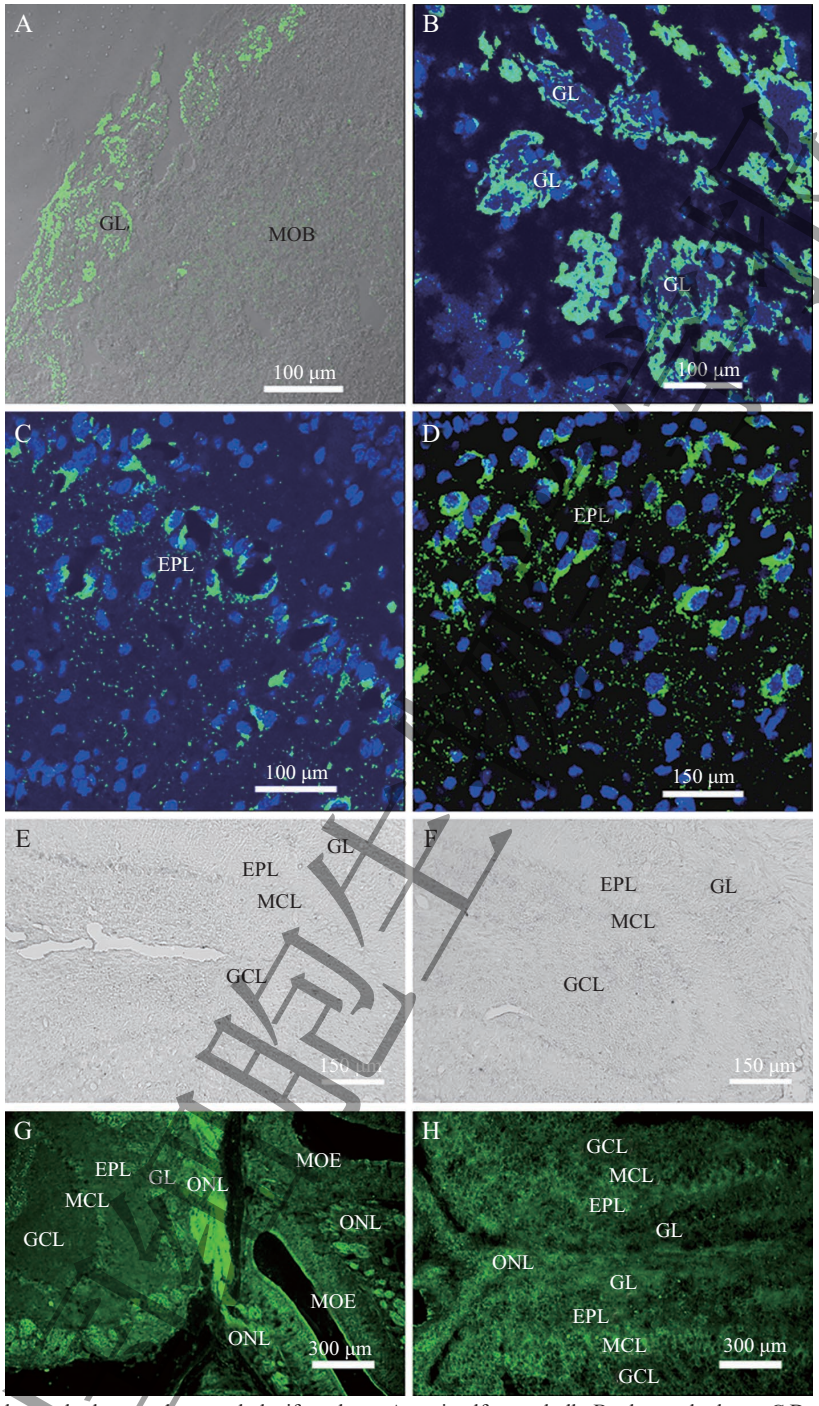
A: Gγ13 expression in the microvilli of VNO; B: Gαi2 expression in the microvilli of VNO; C: Gγ13 co-expresses with Gαi2; D: Gγ2 expression in the microvilli of VNO; E: Gαi2 expression in the microvilli of VNO; F: Gγ2 co-expresses with Gαi2; G: Gγ13 expression in the microvilli of VNO; H: Gβ1 expression in the microvilli of VNO; I: Gγ13 co-expresses with Gβ1 in microvilli of VNO.

Fig.5 Gγ13 co-localizes with Gαi2 and Gβ1 in the microvilli of VNO

Gγ2 expression was also detected in anterior part of AOB and EPL (Fig.7E and Fig.7F).

3 Discussion

Firstly, Gγ13 is found in taste bud and it may play an important role in signal transduction of taste[28]. Meanwhile, Gγ13 is also co-expressed with Gαo, Gβ3 and Gβ4 in retinal ON bipolar cells[29]. Recently, more and more data suggested that Gγ13 also played an important role in signal transduction of olfactory. Loss of cilia–centrosomal protein CEP290/NPHP6[21] and Bardet-Biedl syndrome protein[20] both result in anosmia caused by the selective loss of Gαolf or/and Gγ13 proteins in cilia of olfactory sensory neurons. Furthermore, Ric-8B interacts with Gαolf and Gγ13 and co-localizes with Gαolf, Gβ1 and Gγ13 in the cilia of olfactory sensory neurons[22]. Interestingly, the expression of Gγ13 is extended to testis in our previous study, except taste, retinal and neuronal tissues. Gγ13 is a very special G gama unit, shared a less homology with other G gama unit. In most Gγ subunits, the X in the CAAX sequence is a leucine, however in some (Gγ1, Gγ8, Gγ11 and Gγ13) X is a serine, which permits the addition of a farnesyl group[30]. Previous research have already suggested that Gγ8 played a very specific role in the development and turnover of

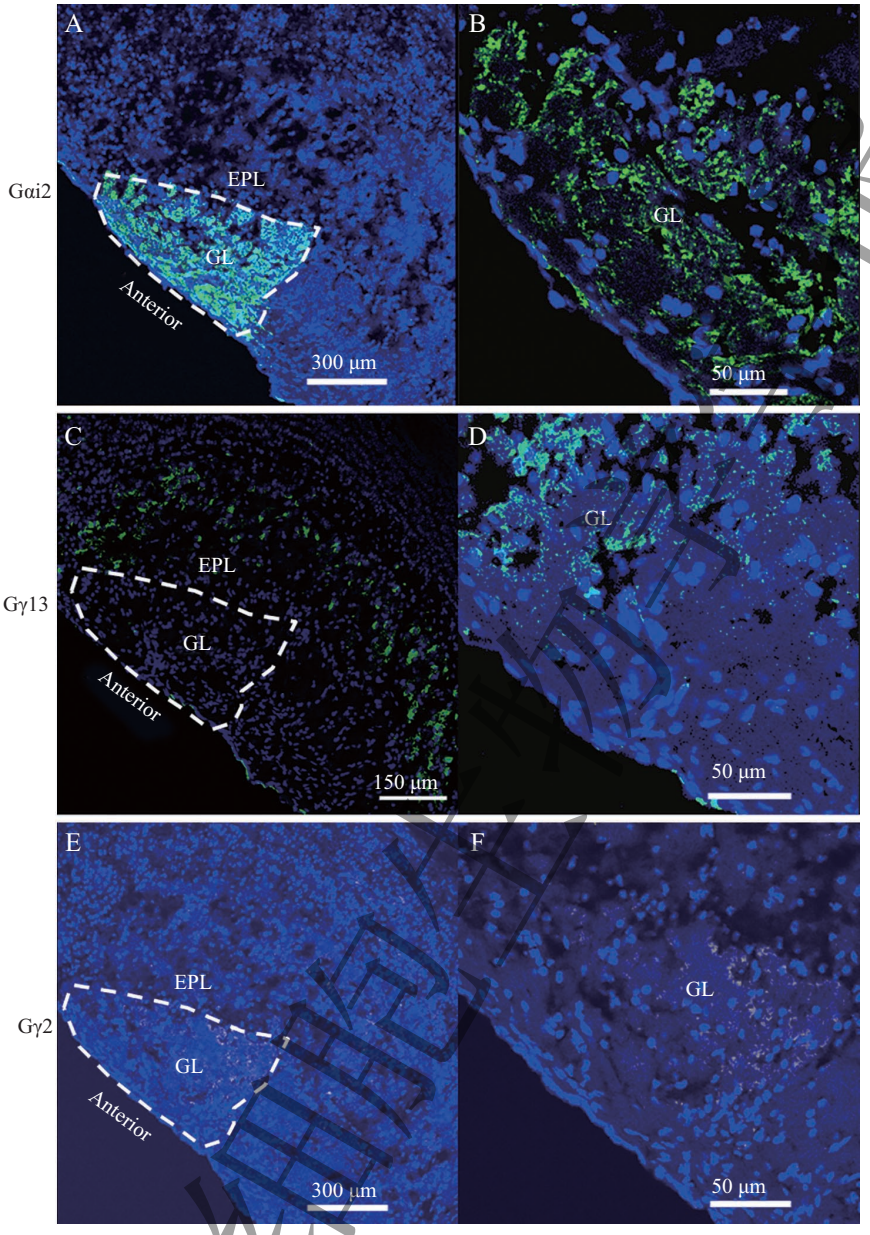


Gγ13 expression is found in glomerular layer and external plexiform layer. A: main olfactory bulb; B: glomerular layer; C,D: external plexiform layer; E: mRNA ISH reveals the expression of GAP43 in MCL; F: mRNA ISH reveals the expression of Gγ13 in EPL and MCL; G: at P21, immunostaining with anti-Gγ13 reveals the distribution of Gγ13 protein in the main olfactory system, including cilia of the olfactory neurons, olfactory nerve bundle, GL and MCL; H: at P0, the intensive staining with anti-Gγ13 is observed in MCL. MOB: main olfactory bulb; AOB: accessory olfactory bulb; GL: glomerular layer; EPL: external plexiform layer; MCL: mitral cell layer; GCL: granule cell layer; ONL: olfactory nerve layer; VNL: vomeronasal nerve layer.

Fig.6 Gγ13 expression observed in main olfactory bulb

olfactory and VNO neurons[23]. Thus, the current data urge us to think about the pattern of Gγ13 expression during the development of olfactory and VNO neurons.

The current results shows that the expression pattern of Gγ13 is similar with that of Gαolf in MOE, different from that of GAP43 and Gγ8. GAP43, also known as B50 or F1, is a marker for immature neurons, expressed during axonal growth until synaptic



A,B: Gαi2 expression in the anterior part of AOB; C,D: Gγ13 expression in the anterior part of AOB and EPL; E,F: Gγ2 expression in the anterior part of AOB. GL: glomerular layer; EPL: external plexiform layer.

Fig.7 Gγ13 expression in accessory olfactory bulb

connections are made[26]. Gγ8 is also a specific marker of the immature olfactory neurons[23]. GAP43 and Gγ8 both tend to be expressed in basal region of MOE after birth. In contrast, Gγ13 and Gαolf expression is concentrated in the upper part of MOE after birth, indicating that Gγ13 is expressed in mature neuron. Indeed, Gγ13 conditional knockout showed the absence of Gγ13 and Gαolf in olfactory epithelium. Furthermore, behavioral tests indicated that the mutant mice had a remarkably reduced ability to perform an odor-guided search task[31].

The current results also show more *Gγ13* mRNA than Gαolf in MOE by *in situ* hybrizi at E15.5. Several proteins, related to olfactory neurons, have been found in MOE at embryo stage. For example, Gαolf was found expression at E15.5[32], Gβ at E14[33], OMP at E13.5[34]. In addition, olfactory receptor (*OR*) gene expression was first evident in the mouse olfactory

epithelium at E11.5-E12.5[33]. Gαs was founded wide expression throughout the OE[32]. Thus, it is possible that Gγ13, interacting with Gαs in early OE, may play an important role in gene regulation and axon guidance in early olfactory sensory neurons.

It is controversial for Gγ13 expression in VNO. Runnenburger[35] failed to find Gγ13 expression in VNO, but Kerr[22] did. Our results further suggested that Gγ13-positive cell was found only in neurons located in the apical region of the epithelium after birth, where Gαi2 and V1R pheromone receptors were normally expressed[36], and not in neurons located in the basal region, where Gαo and V2R pheromone receptors are expressed[37]. The high expression of Gγ13 in the apical region of mouse VNO and the enrichment of these proteins in microvilli, together support the idea that Gγ13 is the major γ-subunit of G proteins involved in the sensory transduction of apical region in VNO.

Furthermore, Gγ13-positive cells were found at E15.5, both in sensory-VNO and nonsensory-VNO. This was not the first report of ectopic neural marker in the fetal VNO. In the mouse VN sensory epithelium, OMP expression is also observed both in sensory-VNO and nonsensory-VNO at E13.5[38], PGP 9.5 at E13-E14 in mouse[38-39], in rat at E16[40]. Interestingly, V1R and V2R expressions are further detected at E13.5[41]. Gγ8 and Gγ13 expression at E15.5 further suggest that two G gamma subunits appears to play an important role in signal transduction during prenatal VNO neural development. It was known that Gαo and Gαs, not Gαi2 were expressed in the fetal VNO[36], Gγ8 and Gγ13 may interact with those G proteins, involve in the development of VNO.

One issue concerning development in rodents is when, during ontogeny, the VNO becomes functional. In mice fluorescent beads injected into the amniotic fluid surrounding E18 fetuses, do not enter the VNO, although they are found in all other regions of the nasal cavity[42]. Histological studies indicated that the VN duct, was not open in E19 mice, opening only at P5. Before born, mice AOB may not be functional[36]. Thus, it is still unknown why pheromone receptors and Gγ13 is expressed in the fetal VNO.

Among the 12 Gγ subunits, Gγ8 and Gγ13 is both expressed in olfactory system and eyes[23]. Two Gγ subunits both have a same X in the CAAX sequence, both interact with Ric-8B[22], both begin to express in early embryo (this paper). A difference between Gγ8 and Gγ13 is presented at expression pattern in MOE and VNO: Gγ13 expression in mature, Gγ8 in immature cells. It seems that two Gγ subunits carry out a different physiological function in sense neuron. Except a common X in the CAAX sequence with Gγ8, via which PDZ domain proteins interact with specific subunits[43], Gγ13 is still unique in possessing an asparagine-proline-tryptophan (NPW) tripeptide prior to the C-terminal isoprenylation signal sequence; this conserved tripeptide ends in phenylalanine (i.e. NPF) in all other mammalian Gγ polypeptides[44]. The NPW motif is also found within the Gγ-like (GGL) domains of the R7 subfamily of mammalian RGS proteins[45]. Kerr *et al*[22] has further showed that a GEF protein, Ric-8B can interact with Gαolf and Gγ13, may regulate the odorant signal transduction. Thus, the current study collectively indicate that Gγ13 play an important role in regulating the odorant signal transduction.

In a word, the current study reveals that Gγ13 proteins are enriched in cilia of MOE and microvilli of VNO after P21 days, Gγ13 appears to be a specific marker of the mature olfactory and vomeronasal neurons. In main olfactory bulb, Gγ13-positive signals were present in the glomerular layer. In accessory olfactory bulb, the weak positive signals were also observed in the glomerular layer. The current results collectively suggest that Gγ13 appear to be involved in signal transduction of MOE and VNO.

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