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Differential Expression of the *Tmem132* Family Genes in the Developing Mouse Nervous System

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Abstract

The family of novel transmembrane proteins (TMEM) 132 have been associated with multiple neurological disorders and cancers in humans, but have hardly been studied in vivo. Here we report the expression patterns of the five *Tmem132* genes (a, b, c, d and e) in developing mouse nervous system with RNA in situ hybridization in wholemount embryos and tissue sections. Our results reveal differential and partially overlapping expression of multiple *Tmem132* family members in both the central and peripheral nervous system, suggesting potential partial redundancy among them.

Keywords

Tmem132a ; *Tmem132b* ; *Tmem132c* ; *Tmem132d* ; *Tmem132e* ; Mouse; Central nervous system; Peripheral nervous system; embryos

1. Introduction

Despite decades of biochemical and genetic studies and recent high throughput mutagenesis efforts, a large number of the mammalian protein-coding genes still await investigation (e.g. (Pandey et al., 2014). Genome-wide association studies (GWAS) indicated that some of these genes are potentially important for human development and physiological function (Hirschhorn and Daly, 2005). The transmembrane protein family 132 (TMEM132) is a family of genes frequently associated with human genetic disorders, in particular neurological disorders, but has somehow evaded extensive molecular investigation (Gregersen et al., 2014; Sanchez-Pulido and Ponting, 2018).

Anxiety Disorder is a pediatric neurological disorder with a life-time occurrence of more than 20% (Meier and Deckert, 2019). A 2011 study found that *TMEM132D* was

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associated with panic disorder (PD), a major form of Anxiety Disorder, panic attacks and unipolar depression (Erhardt et al., 2011). Patients with PD exhibited higher expression of *TMEM132D* in frontal cortex. Higher expression of *Tmem132d* in cingulate cortex was also found to be associated with higher anxiety levels in a mouse model (Erhardt et al., 2011). The association between *TMEM132D* and PD was confirmed by subsequent independent studies in European (Erhardt et al., 2012; Haaker et al., 2014) and Japanese (Shimada-Sugimoto et al., 2016) populations with anxiety-related phenotype. Another *TMEM132* family member, *TMEM132E,* was also associated with PD (Gregersen et al., 2014).

TMEM132 family members were also implicated in other neurological disorders. Mis-sense mutations in *TMEM132E* were found in multiple patients with autosomal recessive non-syndromic hearing loss, and knockdown of *Tmem132e* in zebrafish disrupted mechanosensation (Li et al., 2015; Liaqat et al., 2019). GWAS also implicated *TMEM132E* in insomnia (Agbu et al., 2018). *TMEM132B* was implicated in Major Depressive Disorder (Hu et al., 2021). *TMEM132A* has not been associated with any human genetic diseases. However, *Tmem132a* expression was found in rat brain (Oh-hashi et al., 2003), and loss of *Tmem132a* led to neural tube closure defects (Dickinson et al., 2016).

Some indications of the potential function of the TMEM132 family members have emerged in recent structural, biochemical and genetics studies. TMEM132 proteins all contain extracellular Ig domains and intracellular WAVE complex binding domains, suggesting that they may mediate cell-cell interaction and intracellular actin network organization (Sanchez-Pulido and Ponting, 2018). Consistent with this notion, the loss of nematode *Tmem132* led to abnormal neurite projection (Wang et al., 2021). The *Drosophila Tmem132* homolog, *dtn*, was involved in reproduction and nociception, but the exact cellular mechanism was not revealed (Chon et al., 2021; Honjo et al., 2016). A recent biochemical study suggested that the TMEM132A protein interacted with WNTLESS and may be involved in WNT secretion (Li and Niswander, 2020).

The genetic studies in animal models and association with human diseases strongly indicate that the TMEM132 family of proteins is important for mammalian development and physiology, and deserves further investigation. In the current study, we compare the expression of *Tmem132* genes in mouse neural development and reveal differential and sometimes partially overlapping expression patterns of the family members. Our findings provide a basis for further functional studies of this important gene family in neural development and diseases.

2. Results and Discussion

2.1 Expression of the Tmem132 genes during early nervous system development

To explore the contribution of the *Tmem132* family genes to the early development of the nervous system, we examined their expression at embryonic day 8.5 (E8.5). Wholemount in situ hybridization (ISH) indicated that *Tmem132a* was expressed throughout the embryonic region, but not in the extraembryonic membranes (Fig. 1A). A sense probe for *Tmem132a* did not stain the embryos, indicating the specificity of the procedure (Fig. 1B). *Tmem132b* (Fig. 1C) and *Tmem132d* (Fig. 1E) did not exhibit any specific expression at this stage.

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Interestingly, *Tmem132c* was expressed in the dorsal midbrain, somites and the tail region (Fig. 1D). *Tmem132e* expression was present in the ventral neural tube (Fig. 1E and inset). At E9.5, *Tmem132a* continued to be expressed widely in the embryos (Fig. 2A), whereas *Tmem132b* and *Tmem132d* expression remained undetectable (Fig. 2B and D). *Tmem132c* continued to be expressed in somites and midbrain, and started to be expressed in head mesenchyme (Fig. 2C). Interestingly, *Tmem132e* expression was detected in the cranial and dorsal root ganglia (DRG) at E9.5 (Fig. 2E).

To study the expression of *Tmem132a, Tmem132c* and *Tmem132e* in the nervous system in more detail, we performed ISH analyses on sections of E10.5 embryos. We found that *Tmem132a* was expressed throughout the neural tube at this stage (Fig. 3A, E and I), and the expression level increased in post-mitotic regions such as the motor neuron domain in the spinal cord (Fig. 3E). It was also highly expressed in the branchial arch (Fig. 3I). *Tmem132c* was expressed in the surface ectoderm (Fig. 3B, F and J). It was also expressed in dorsal telencephalon and posterior dorsal midbrain, but appeared to be absent from the rest of the neural tube (Fig. 3B, F and J). *Tmem132e* was expressed in the cranial (Fig. 3C) and spinal ganglia (Fig. 3G) labeled with *Isl1* expression (Fig. 3D and H). In addition, it was also expressed in the motor neuron regions labelled with *Isl1* and *Phox2a* (Fig. 3G, H, K and L; (Tiveron et al., 1996)). *Tmem132a* was also expressed in the cranial (Fig. 3I) and spinal (Fig. 3E) ganglia.

2.2 Expression of Tmem132a and Tmem132e in trigeminal ganglia at E14.5

At E14.5, *Tmem132a* (Fig. 4A) and *Tmem132e* (Fig. 4C) continued to be expressed in the trigeminal ganglia, which was labeled with strong *Isl1* expression (Fig. 4D). In addition, *Tmem132a* expression was enriched in whisker follicles and oral epithelium (Fig. 4A). *Tmem132c* was also expressed in the oral epithelium, but it was absent from the trigeminal ganglia (Fig. 4B).

2.3 Expression of Tmem132a, Tmem132c and Tmem132e in inner ear development

Point mutations in *TMEM132E* have been associated with hearing loss in humans, and knocking down *Tmem132e* expression in zebrafish affected inner ear development/function (Li et al., 2015; Liaqat et al., 2019). To better understand the contribution of Tmem132 family members to inner ear development, we examined the expression of *Tmem132a*, *Tmem132c* and *Tmem132e* in developing inner ears at E14.5 and E18.5 in the developing cochlea. At E14.5, both *Tmem132a* and *Tmem132c* were expressed in the cells lining the cochlear ducts (Fig. 5A, B), whereas *Tmem132e* was expressed in what appeared to be the vestibulocochlear ganglion, but not in the cochlear duct itself (Fig. 5C). At E18.5, both *Tmem132a* and *Tmem132e* were highly expressed in spiral ganglia (Fig. 5D and F). *Tmem132a* and *Tmem132c* were expressed in part of the sensory epithelium of the cochlear ducts at this stage (Fig. 5D and E). As point mutations in *TMEM132E* were associated with hearing impairment in humans despite overlapping expression of *Tmem132a* and *Tmem132e* in spiral ganglia (Li et al., 2015; Liaqat et al., 2019), it will be interesting to determine whether *Tmem132e* is required early, before *Tmem132a* expression in the vestibulocochlear nerve. Alternatively, the requirement for *TMEM132E* in human hearing may reflect a dosage effect.

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2.4 Expression of the Tmem132 family genes in caudal forebrain

A previous study detected *Tmem132a* in many regions of the adult rat brain with Northern hybridization (Oh-hashi et al., 2003). To reveal the dynamic expression patterns of all five *Tmem132* genes in brain development, we performed section ISH at E14.5, E18.5, P7 and P21. At E14.5, we found strong expression of *Tmem132a* in a specific domain of the caudal forebrain (Fig. 6A, F). By comparison with the known markers of the thalamus (*Ngn2, Gbx2;* Fig. 6G and H) and prethalamus (*Isl1;* Fig. 6I), we determined that this *Tmem132a* -enriched domain corresponded to the post-mitotic regions of the thalamus (Nakagawa and O'Leary, 2001). *Tmem132a* expression was also present in the hypothalamus, but at a much lower level (Fig. 6A, F). The other *Tmem132* genes were not expressed in the thalamus (Fig. 6B-E), but *Tmem132b* appears to be expressed in a small group of cells of the prethalamus (Fig. 6B), whereas *Tmem132d* and *Tmem132e* were expressed at low levels in the hypothalamus (Fig. 6D and E).

2.5 Expression of Tmem132 family members in the cerebral cortex and hippocampus

At E14.5, three *Tmem132* family members were expressed in the developing cerebral cortex. *Tmem132a* was expressed in postmitotic cells near the pia surface (Fig. 6A). *Tmem132b* expression was specific to the ventricular layer (Fig. 6B). *Tmem132c* was also expressed in the ventricular layer, but it was restricted to the cingulate cortex (Fig. 6C).

We analyzed the expression of the *Tmem132* family in more details at E18.5. To better distinguish different cell types, we labeled the neural progenitor cells with *Pax6* (Fig. 7A; (Englund et al., 2005; Stoykova and Gruss, 1994)), the intermediate precursors with *Tbr2* (Fig. 7B; (Englund et al., 2005)), the deeper layer projection neurons with *Tbr1* (layers V/VI, Fig. 7C; (Englund et al., 2005), intermediate layer projection neurons with *FoxP1* (layers III-V, Fig. 7D; (Ferland et al., 2003)), and upper layer projection neurons with *Cux1* (layers II-IV, Fig. 7E; (Nieto et al., 2004)). We found that *Tmem132a* was expressed at higher levels in the deeper layers and lower levels in the upper layers (Fig. 7F). *Tmem132b* was strongly expressed in the progenitors at the ventricular layer, and became weaker in the intermediate precursors (Fig. 7G). An additional domain of weak *Tmem132b* expression was also detected in deeper layers. A thin line of *Tmem132d* expressing cells were present at the bottom of the cortical plate (Fig. 7I). *Tmem132c* and *Tmem132e*, in contrast, did not show expression in the cerebral cortex at this stage (Fig. 7H and J).

The *Tmem132* family genes were also differentially expressed in the hippocampus. *Tmem132a* was expressed in the hippocampus at P7 and P21 (Fig. 8A and F). *Tmem132b* was also expressed in hippocampus at both stages, but at a lower level (Fig. 8B and G). *Tmem132c* was not expressed in the hippocampus at either stage (Fig. 8C and H). *Tmem132d* expression was higher in the CA1 region than in CA3 region, and was absent in the dentate gyrus (Fig. 8D and I). On the contrary, weak *Tmem132e* expression was restricted to the dentate gyrus and CA3 region, but not in CA1 region (Fig. 8E and J).

2.6 Expression of Tmem132a in cerebellar granule layer

We next examined the expression of the *Tmem132a* in the cerebellum. At P21, *Tmem132a* was expressed strongly in the cerebellum (Fig. 9A). *Tmem132a* expression overlaps with

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that of *En2*, a marker of the cerebellar granule neurons (Fig. 9B; (Davis and Joyner, 1988)), suggesting that *Tmem132a* was expressed in the granule layer. *Calb1* labeled a thin layer of Purkinje cells that appeared to abut the *Tmem132a* expression domain (Fig. 9C; (Iacopino et al., 1990)). To investigate whether *Tmem132a* was also expressed in Purkinje cells, we compared the expression of *Tmem132a* and *Calb1* through double fluorescent in situ hybridization. We found that the *Tmem132a* expression domain abutted, but did not overlap with, that of *Calb1*, suggesting that *Tmem132a* was not expressed in Purkinje cells (Fig. 9D, D' and D").

3. Conclusions

Our gene expression analyses provide important information regarding the potential functions of the TMEM132 family members that may explain the previously observed association with human diseases and/or genetic data in model animals. Prior to neural tube closure, *Tmem132a* was widely expressed in the embryos, whereas other *Tmem132* family members were either absent or expressed at low levels. This observation is consistent with the frequent neural tube closure defects in a *Tmem132a* mutant mouse strain (Dickinson et al., 2016). Consistent with the association between *TMEM132E* and hearing loss in humans, we found specific expression of *Tmem132e* in spiral ganglia of the inner ear (Li et al., 2015; Liaqat et al., 2019). Interestingly, *Tmem132a* was also expressed in the spiral ganglia, suggesting possible redundant function between these two genes. The overlapping expression of *Tmem132a* and *Tmem132c* in the sensory epithelium of the cochlea suggests another potential role for this family of proteins in hearing that has yet to be elucidated.

Tmem132a, but not *Tmem132d*, was expressed at high levels in brain regions associated with emotional responses, such as the cortex, hippocampus and thalamus, which appears to contradict the GWAS association between *TMEM132D* and panic disorders (Erhardt et al., 2011; LeDoux, 2000). However, a gene expression analysis found elevated *TMEM132D* expression in the frontal cortex of panic disorder patients, suggesting that its expression may need to be kept at a lower level to ensure normal emotional circuit function (Erhardt et al., 2011). It would be interesting to see whether artificially increasing *Tmem132d* expression in lab animals, e.g. by expressing it under the control of a promoter highly active in frontal cortex, leads to panic disorder-like symptoms.

Human and mouse TMEM132 family members share similar domain structures including an extracellular Cohesin and three tandem ancient bacteria Ig domains, as well as intracellular protein phosphatase and WAVE complex binding motifs (Sanchez-Pulido and Ponting, 2018). Pair-wise BLAST analyses indicate that the overall peptide sequence similarities between TMEM132 family members range from 51% between TMEM132a and TMEM132e, to 72% between TMEM132c and TMEM132d. Therefore, it is likely that these proteins share redundant functions in cells where they are co-expressed, an interesting possibility awaiting direct functional testing.

Our results also suggest potential functions of *Tmem132* family genes in other aspects of nervous system function. For example, although *TMEM132D* was the only family member whose association with panic disorder has been well investigated (Erhardt et al., 2012;

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Erhardt et al., 2011; Erhardt and Spoormaker, 2013; Haaker et al., 2014; Hodgson et al., 2016; Howe et al., 2016; Inoue et al., 2015; Naik et al., 2018; Quast et al., 2012; Shimada-Sugimoto et al., 2016), the fact that all four other family members were expressed in developing cortex and/or hippocampus suggests that they may also play a role that has yet to be revealed. The strong expression of *Tmem132a* in the thalamus and cerebellar granule layer points to its potential roles in the functions of these brain regions. However, due to the preweaning lethality of the *Tmem132a* mutants, a tissue-specific mutagenesis approach may be needed to reveal such functions. Finally, the overlapping expression of *Tmem132a* and *Tmem132e* in other cranial and spinal ganglia suggests that they may have overlapping functions in other parts of the PNS in addition to hearing.

Online databases such as Genepaint (http://gp3.mpg.de) and Allen Brain Atlas (https://portal.brain-map.org/) provide valuable snapshots of gene expression patterns in development or adult. However, these databases are limited in terms of optimal stage selection (e.g., Only E14 expression was shown at Genepaint, and P56 for Brain Atlas), angle selection (e.g., only sagittal sections were shown at Genepaint and for many genes at Brain Atlas), lack of annotation and missing genes (e.g., *Tmem132b* was not examined at Genepaint or Brain Atlas). We carefully selected stages appropriate for each organ to compare the expression of the five *Tmem132* family genes, with known markers of various cell types and/or compartments as reference, providing deeper insights into potential connections between these genes and associated human diseases.

The *TMEM132* family members were also associated with non-neural traits, such as pulmonary function in human (Son et al., 2015), body weight in pig (Gong et al., 2019) and chicken (Tarsani et al., 2019), limb and kidney development in mice (Dickinson et al., 2016). Recent studies also implicated *TMEM132* genes in cancers of the mammary gland, colon, lung and ovary (de Almeida et al., 2019; Iwakawa et al., 2015; Karapetsas et al., 2015; Rudolph et al., 2013; Wan et al., 2022; Yu et al., 2015; Zhang et al., 2020; Zhang et al., 2022). Although it is beyond the scope of this study, which focuses on the expression of *Tmem132* genes in the developing neural tissues, it will be interesting to investigate their expression and function in other organs and various cancers to reach a more comprehensive understanding of the physiological roles of this important gene family.

4. Methods

4.1 Animals

C3H/HeNCrl male and female mice (Charles River Labs) were bred to generate embryos for the study. The noon of the day on which the vaginal plug was found was counted as embryonic day 0.5 (E0.5). Female pups/mice from the same strain were used for the study at P7 and P21. The use of the animals in this work was approved by the IACUC (#47340) at the Penn State University (PA, USA).

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4.2 RNA in situ hybridization in wholemount embryos

E8.5 and E9.5 embryos were dissected out of uteri in ice-cold PBS and fixed in 4% paraformaldehyde (PFA) for at least 4 hours. Subsequently, the embryos were subject to wholemount RNA in situ hybridization procedure described in detail in (Liu et al., 2012).

4.3 RNA in situ hybridization on cryosections

Whole embryos (E10.5, E14.5) or isolated tissues (whole heads or cochleae) of the embryos (E18.5) or young pups (P7) were fixed in 4% PFA for at least 4 hours. Older (P21) mice were anesthetized and perfused with 4% PFA according to (Dong et al., 2016), then brains were dissected, and further fixed for at least 4 hours. The tissues were then saturated with 30% sucrose, processed for cryosection and subject to a RNA in situ hybridization procedure described in detail in (Liu and Liu, 2014).

4.4 Double fluorescent RNA in situ hybridization on cryosections

Cryosections of P21 cerebellum were treated with H_2O_2 to inactivate endogenous peroxidase activity, acidified with HCl and acetylated before hybridized with Fluorescein-labeled *Tmem132a* probe and DIG-labeled *Calb1* probe overnight. On the following day, the sections were extensively washed and incubated with anti-Fluorescein POD (Sigma) overnight. On the third day, the sections were incubated with CF488-Tyramide (Biotium) for four hours before treated with H_2O_2 and incubated with anti-DIG POD (Sigma) overnight. On the final day, the sections were incubated with CF568-Tyramide for two hours and mounted with Vectashield with DAPI. Photos were taken with a Nikon E600 microscope and a QImaging micropublisher camera.

4.5 Synthesis of probes for RNA in situ hybridization.

The probe for *Tmem132a* was constructed by cloning the 700 base HindIII/KpnI cDNA fragment covering 20 bases of the 5' UTR and 680 bases of the coding region into pGEM3. All other probes were produced by PCR amplification of the 3' UTRs of the corresponding genes and cloned into pGEM3 or pGEM11. Fluorescein or DIG-labeled RNA probes were synthesized by in vitro transcription using Roche polymerases per manufacturer's instructions. DNA sequence information needed for designing RNA in situ hybridization probes were found at https://useast.ensembl.org/Mus_musculus/Info/Index. The following table lists the primers used for probe synthesis

Gene	Primers	Probe length
Tmem132b	forward primer: AACAAGAGCACAACCCCACA reverse primer: GGCCATTGGTCATGAGGTGA	976 bases
Tmem132c	forward primer: GCGGAGCTCTTGGAGAACAT reverse primer: TTCCACTCATTGGCCTGACC	1341 bases
Tmem132d	forward primer: CAGACCAAATGGACCTCCCC reverse primer: CCCACACATGCTCAACCTGA	1027 bases
Tmem132e	forward primer: CATCGTGTTTGTGCTGCGAT reverse primer: CCCAAGTCTAAGGAGCCGTG	1129 bases

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Gene	Primers	Probe length
Isl1	forward: CGACCCAGTCAATGGAAACT reverse: GCAATGCAAGAGCAAACAAA	854 bases
Tbr2	TCGGTACGGCGTTCAAAACT TTAATAGCACCGGGCACTCG	983 bases
Tbr1	TCCATCAAATCCATCGACTCC ACTCGACTCGCCTAGGAACA	858 bases
Cux1	AAGAAGGCTGCGAACTTGAA TGTGCCAACACAGACAGACA	1146 bases
FoxP1	GCCAACCACAGTCCAGATTT GTGGGGACCTTCAGGTGTAA	1077 bases
Calb1	CTGCTTCTATCTGGCGGAAG ACCCAAACCTGCATTAGGTG	866 bases

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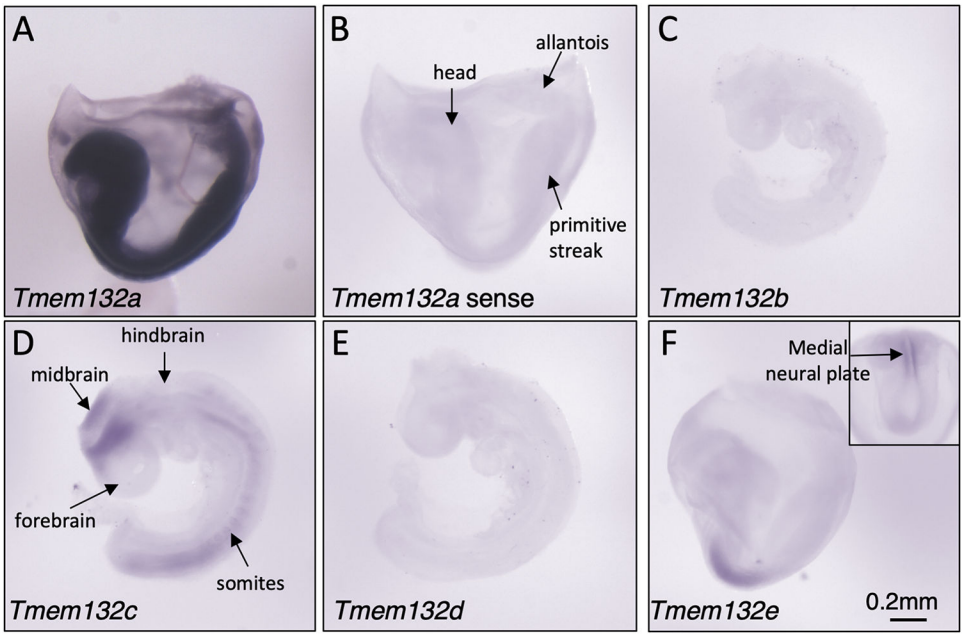

Figure 1. The expression of Tmem132 family genes at embryonic day 8.5

Lateral views of E8.5 embryos after wholemount in situ hybridization. Purple staining shows gene expression of *Tmem132a* (A), *Tmem132b* (C), *Tmem132c* (D), *Tmem132d* (E) and *Tmem132e* (F). (B) shows an embryo treated with *Tmem132a* sense probe as a negative control. The inset shows the ventral view of the same embryo in F with the neural tube expression of *Tmem132e*.

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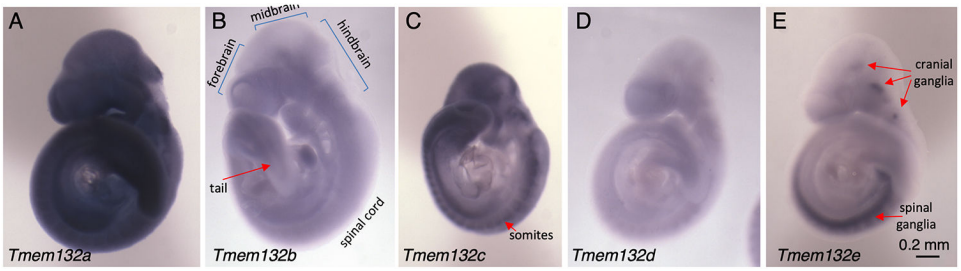

Figure 2. The expression of Tmem132 family genes at embryonic day 9.5.

Lateral views of E9.5 embryos after wholemount in situ hybridization. Purple staining shows expression of *Tmem132a* (A), *Tmem132b* (B), *Tmem132c* (C), *Tmem132d* (D) and *Tmem132e* (E).

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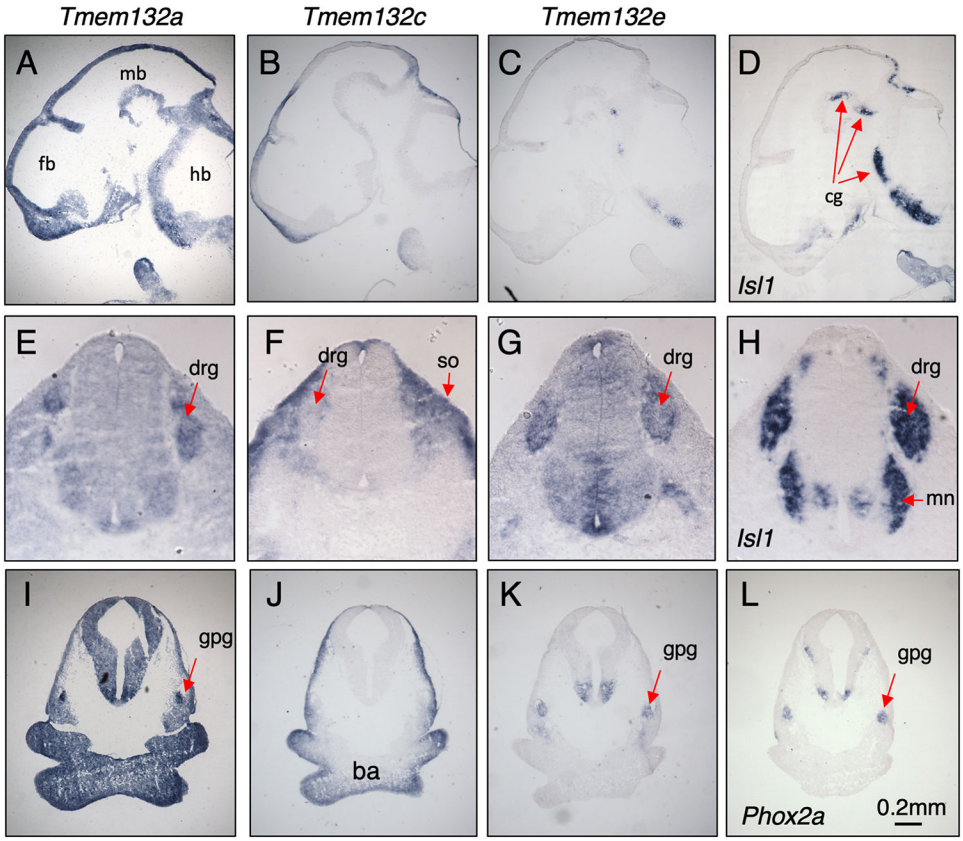

Figure 3. The expression of Tmem132a, Tmem132c and Tmem132e in the CNS and PNS at E10.5.

(A-D) Sagittal sections showing gene expression in the brains and cranial ganglia (E-H) Transverse sections through the neural tube at the thoracic level. (I-L) Coronal sections through the hindbrain and branchial arches. (A, E, I) *Tmem132a*, (B, F, J) *Tmem132c*, (C, G, K) *Tmem132e*, (D) *Isl1* to show cranial ganglia. *(H) Isl1* to show motor neurons and dorsal root ganglia. *(L) Phox2a* to show motor neurons and cranial ganglia. ba: branchial arch; drg: dorsal root ganglia; gpg: glossopharyngeal ganglia; mn: motor neurons; so: somites.

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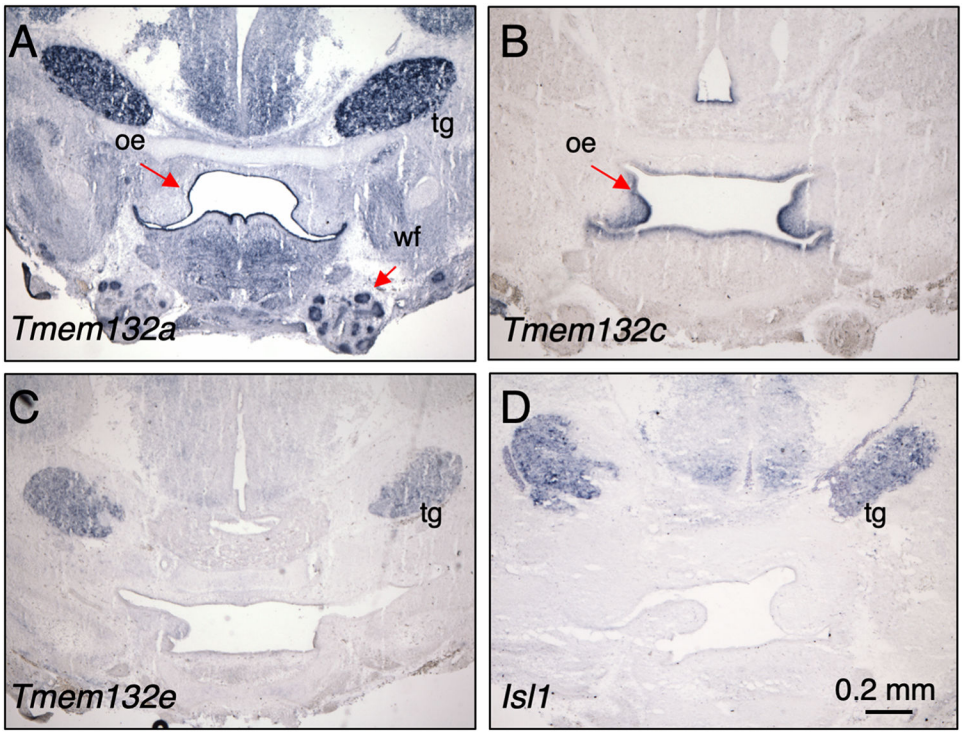

Figure 4. The expression of Tmem132a, Tmem132c and Tmem132e in the craniofacial region at E14.5.

Coronal sections to show the craniofacial regions after RNA in situ hybridization. (A) *Tmem132a* expression in the trigeminal ganglia (tg), oral epithelium (oe) and whisker follicles (wf). (B) *Tmem132c* expression in the oral epithelium. (C) *Tmem132e* expression in the trigeminal ganglia. (D) *Isl1* serves as a marker for the trigeminal ganglia.

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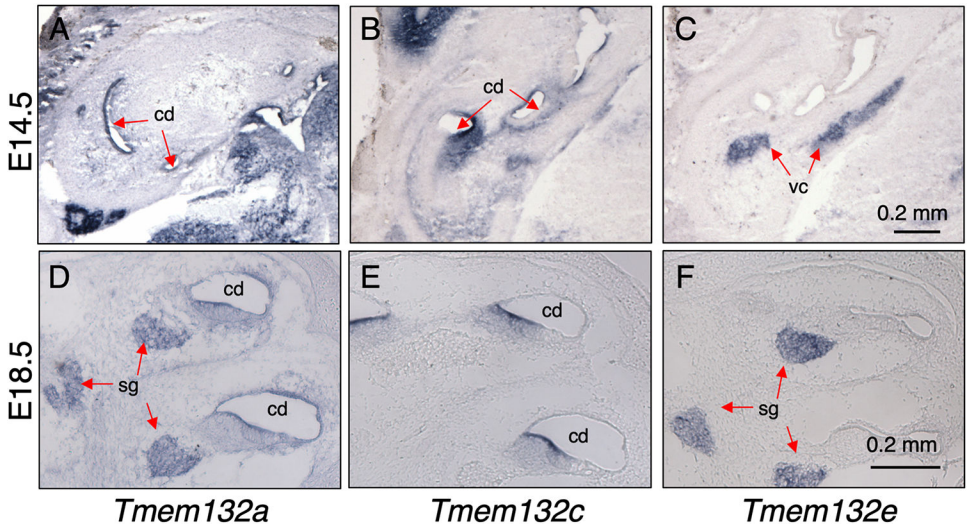

Figure 5. The expression of Tmem132a, Tmem132c and Tmem132e in inner ear development.

(A-C) Coronal sections through the inner ear at embryonic day 14.5 (E14.5) showing the expression of *Tmem132a* (A), *Tmem132c* (B) and *Tmem132e* (C). (D-F) Sections of the E18.5 cochlea showing the expression of *Tmem132a* (D), *Tmem132c* (E) and *Tmem132e* (F). (G-I) Sections of the postnatal day 7 (P7) cochlea showing the expression of *Tmem132a* (D), *Tmem132c* (E) and *Tmem132e* (F). cd: cochlear duct; vc: vestibulocochlear nerve; sg: spiral ganglia.

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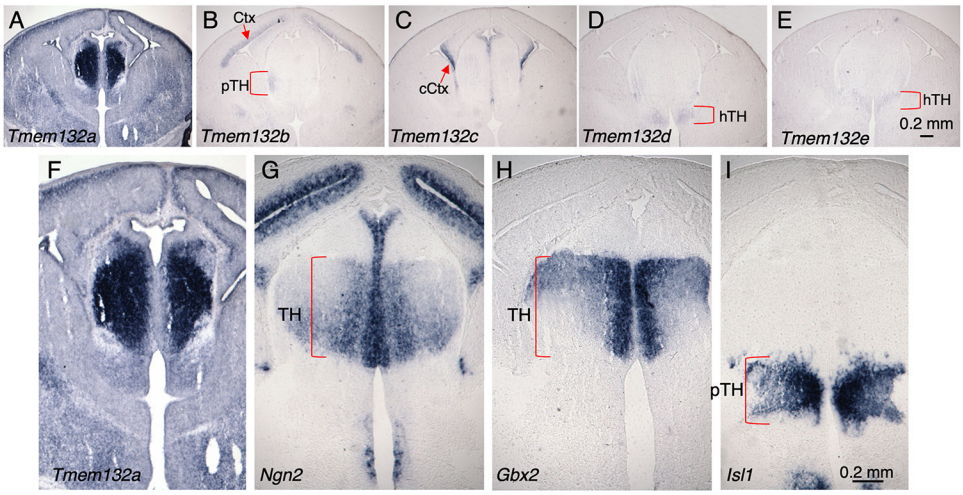

Figure 6. The expression of the Tmem132 family genes in the cerebral cortex and caudal forebrain at embryonic day 14.5.

Transverse sections of the E14.5 embryos showing the expression of *Tmem132a* (A, F), *Tmem132b* (B), *Tmem132c* (C), *Tmem132d* (D) and *Tmem132e* (E). The expression of *Ngn2* (G) and *Gbx2* (H) mark the thalamus (TH), whereas the expression of *Isl1* (I) marks the prethalamus (pTH). cCtx: cingulate cortex; Ctx: Cortex; hTH: hypothalamus

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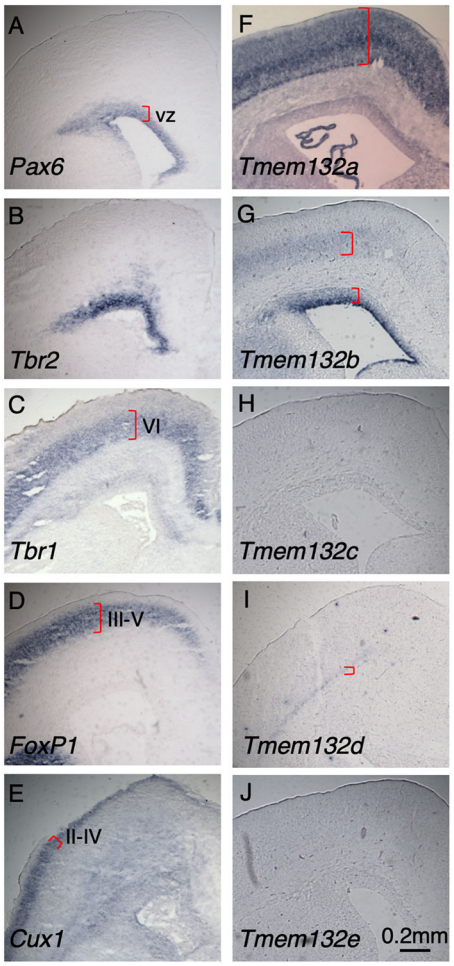

Figure 7. The expression of Tmem132 family genes in embryonic day 18.5 cerebral cortex

Cross sections of E18.5 brains processed for RNA in situ hybridization. (A) *Pax6* marks the progenitors in the ventricular layer. (B) *Tbr2* marks the intermediate precursors. (C) *Tbr1* marks the deeper layers of projection neurons. (D) *FoxP1* marks the intermediate layers of projection neurons. (E) *Cux1* marks the superficial layers of projection neurons. (F) *Tmem132a* expression in all layers of the cortical plate. (G) Strong *Tmem132b* expression in the ventricular layer and weak expression in the deeper neuronal layers. (H) No *Tmem132c* expression in the cortex. (I) Weak *Tmem132d* expression in a thin layer of deep neuronal layers. (J) No *Tmem132e* expression in the cortex. Brackets outline the domains of gene expression.

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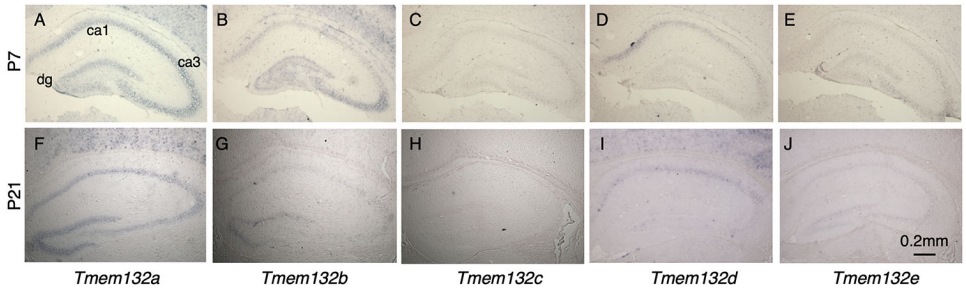

Figure 8. The expression of Tmem132 family genes in the hippocampus

Transverse sections of the brains processed for RNA in situ hybridization. (A-E) The expression of *Tmem132a* (A), *Tmem132b* (B), *Tmem132c* (C), *Tmem132d* (D) and *Tmem132e* (E) at postnatal day 7 (P7). (F-J) The expression of *Tmem132a* (F), *Tmem132b* (G), *Tmem132c* (H), *Tmem132d* (I) and *Tmem132e* (J) at P21.

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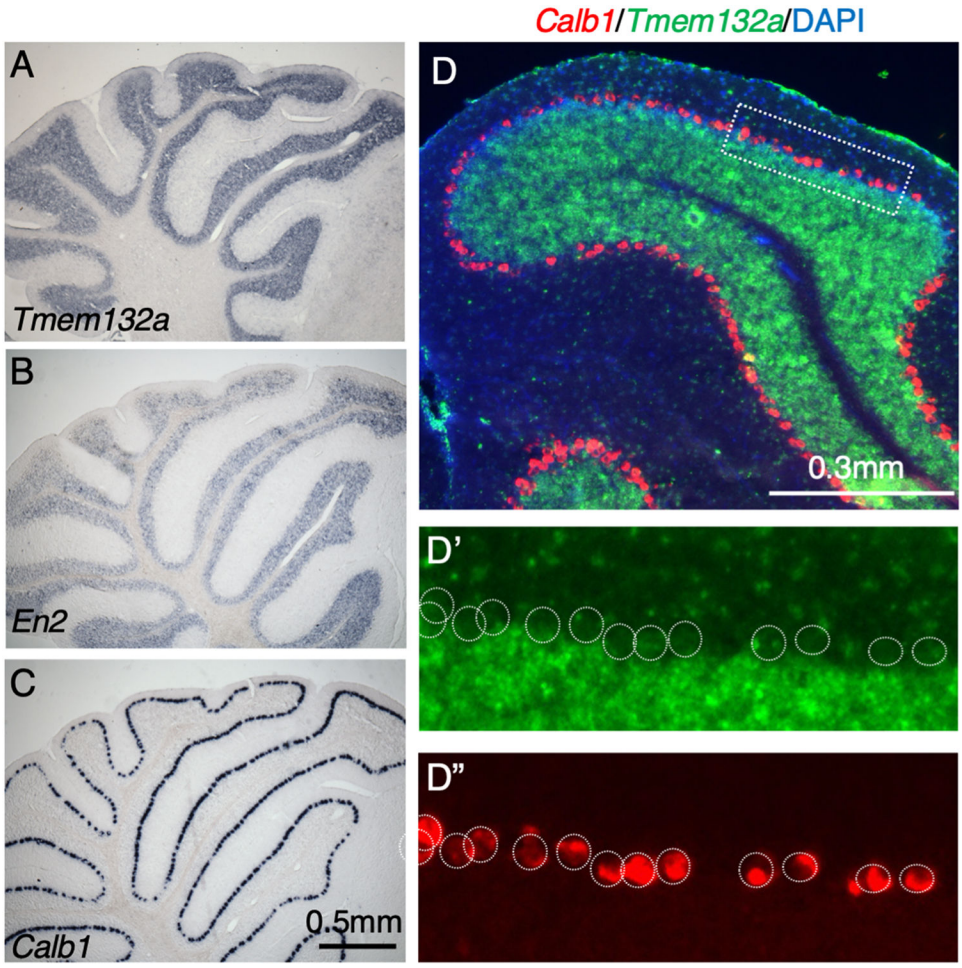

Figure 9. The expression of Tmem132 family genes in the cerebellum

Sagittal sections of the cerebellum processed for RNA in situ hybridization. All panels show expression at postnatal day 21 (P21). *Tmem132a* (A) was expressed in the granule layer marked with *En2* expression (B). *Calb1* expression marked Purkinje cells (C). (D) Double fluorescence in situ hybridization showing the absence of *Tmem132a* expression in Purkinje cells. D' and D" show the close-up view of the highlighted area in D. The positions of *Calb1*$^{+}$ cells were indicated by circles in D' and D".