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Expression of five *frizzleds* during zebrafish craniofacial development

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Abstract

Wnt/Planar Cell Polarity (PCP) signaling is critical for proper animal development. While initially identified in *Drosophila*, this pathway is also essential for the proper development of vertebrates. Zebrafish mutants, defective in the Wnt/PCP pathway, frequently display defects in convergence and extension gastrulation movements and additional later abnormalities including problems with craniofacial cartilage morphogenesis. Although multiple Frizzled (Fzd) homologues, Wnt receptors, were identified in zebrafish, it is unknown which Fzd plays a role in shaping the early larvae head skeleton. In an effort to determine which Frizzleds are involved in this process, we analyzed the expression of five zebrafish *frizzled* homologues *fzd2*, *6*, *7a*, *7b*, and *8a* from 2–4 days post fertilization (dpf). During the analyzed developmental time points *fzd2* and *fzd6* are broadly expressed throughout the head, while the expression of *fzd7a*, *7b* and *8a* is much more restricted. Closer examination revealed that *fzd7b* is expressed in the neural crest and the mesodermal core of the pharyngeal arches and in the chondrocytes of newly stacked craniofacial cartilage elements. However, *fzd7a* is only expressed in the neural crest of the pharyngeal arches and *fzd8a* is expressed in the pharyngeal endoderm.

Keywords

zebrafish; *frizzled 2*; *frizzled 6*; *frizzled 7a*; *frizzled 7b*; *frizzled 8a*; cartilage; craniofacial; Wnt; planar cell polarity

1. Results and discussion

Although initially discovered in *Drosophila* (Gubb and Garcia-Bellido, 1982), the Wnt/Planar Cell Polarity (PCP) pathway has since been proven to be critical in establishing and maintaining epithelial and mesenchymal cell polarity in multiple organisms. In the zebrafish, *Danio rerio*, the PCP pathway regulates convergence and extension movements and organ development (Solnica-Krezel et al., 1996; Rauch et al., 1997; Heisenberg et al., 2000; Topczewski et al., 2001; Dale et al., 2009). Several large screens in zebrafish have identified a number of mutations that lead to craniofacial defects (Neuhauss et al., 1996; Piotrowski et al., 1996; Schilling et al., 1996). One class of these mutations produces severely shortened jaws and is referred to as the “hammerhead” class (Piotrowski et al., 1996). While these mutants

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retain their ability to pattern the pharyngeal arch cartilages, the individual cartilage elements within the arches are shortened. When mutated in zebrafish, two genes that participate in the Wnt/PCP pathway, *glypican 4* (*knypek*) (Solnica-Krezel et al., 1996; Topczewski et al., 2001) and *wnt5b* (*pipe tail*) (Hammerschmidt et al., 1996; Piotrowski et al., 1996; Rauch et al., 1997), result in “hammerhead” phenotypes. In the chicken, members of the Wnt/PCP pathway are expressed within chondrogenic regions of the developing limbs (Hartmann and Tabin, 2000; Church et al., 2002) and regulate the cell polarity of the chondrocytes (Li and Dudley, 2009). Taken together, these data indicate the possibility that the Wnt/PCP pathway may control the behavior of craniofacial chondrocytes in zebrafish.

To further investigate how the Wnt/PCP pathway controls the morphogenesis of craniofacial cartilages in zebrafish, it is essential to identify potential Wnt/PCP receptors expressed during this process. Development of craniofacial cartilage takes place during several steps including migration of the cranial neural crest to pharyngeal arches, cartilage condensation, and the subsequent morphogenetic processes that shape the final cartilage elements (Schilling and Kimmel, 1997; Knight and Schilling, 2006). We sought to determine which of the twelve currently identified zebrafish Frizzled (*Fzd*) homologues may function as Wnt receptors to control this process. While the expression of several *fzd* genes has been previously shown (Kim et al., 1998; El-Messaoudi and Renucci, 2001; Sumanas et al., 2001; Thisse et al., 2001; Sumanas et al., 2002; Ungar and Calvey, 2002; Thisse and Thisse, 2004), the majority of this characterization was done in the early embryonic stages of development before the chondrocytes begin to condense. For our analysis we selected genes that have either been previously linked to Wnt/PCP signaling or have shown expression in the pharyngeal arches at the latest characterized stage. We therefore sought to characterize the expression of the *fzd* genes during this initial condensation and cartilage elongation and continue the analysis until 4 days post fertilization (dpf) when the formation of the larval craniofacial skeleton is mostly completed.

1.1 *fzd2* and *fzd6* are broadly expressed throughout the head

1.1.1 *fzd2* expression—The initial characterization of *fzd2* expression was focused on the first day of development (Sumanas et al., 2001). At 24 hours post fertilization (hpf) the expression was localized to the mesoderm within the tailbud. Thisse et al. (2001) observed expression in the tailbud mesoderm at 19–24 hpf, but also found additional expression domains within the cardiovascular system and the hypocord. In addition, 42–48 hpf embryos displayed expression in the head mesenchyme, otic vesicle, pectoral fin musculature and the pharyngeal arches 3 through 7 (Thisse et al., 2001). When *fzd2* morphants were observed, Sumanas et al. (2001) found that they produce shorter embryos with kinked or undulating notocords. This phenotype resembles those observed in *wnt5b* (*pipe tail*) mutants. These observations along with the fact that *Fzd2* transcripts can be found in the cultured chondrocytes of a two week-old mouse (Xu et al., 2001), led us to investigate the expression of this gene at later developmental stages.

Using *in situ* hybridization, we observed that *fzd2* is expressed throughout the head and pharyngeal arches at 2, 3 and 4 dpf (Fig. 1A–C). Similarly, *Fzd2* is expressed in an ubiquitous manner at a high level in the maxillary prominence, lateral nasal prominence and the mandibular prominence in stage-24 chicks (Geetha-Loganathan et al., 2009). In 2 and 3 dpf zebrafish, mRNA is detected within the pectoral fin bud (Fig. 1A–B) and the liver (Fig. 4I). Interestingly, although the level of expression within the pharyngeal arches is greater than other regions of the head in 3 dpf embryos (Fig. 1B), coronal sections revealed that the staining in this region was not within the cartilage but in fact was adjacent to the cartilage (Fig. 3N).

1.1.2 *fzd6* expression—While not previously characterized in zebrafish, mice with a targeted mutation in the *Fzd6* gene exhibit a whorled hair phenotype that is quite similar to the bristle and wing hair phenotypes observed in *Drosophila fz* mutants (Gubb and Garcia-Bellido, 1982; Vinson et al., 1989; Guo et al., 2004; Wang et al., 2006). Later studies have firmly established that *Fzd6* controls the polarity of these body hairs as part of the Wnt/PCP pathway (Devenport and Fuchs, 2008). Given that *Fzd6* plays a role in the mammalian Wnt/PCP pathway, we sought to determine the expression pattern of the zebrafish homologue. Our *in situ* results show that *fzd6* is expressed throughout the head in 2, 3, and 4 dpf embryos (Fig. 1D–F). Coronal sections of 3 dpf embryos revealed a uniform staining throughout the head including the brain (data not shown). Although *fzd6* expression is excluded from the craniofacial cartilage elements, it is expressed at a low level within the mesenchymal cells surrounding the cartilage (Fig. 3O). *fzd6* is also expressed within the pectoral fin bud (Fig. 1D, E) and the lateral line of 2, 3, and 4 dpf embryos (Fig. 1D–F).

1.2 Global expression patterns of *fzd7a*, *fzd7b*, and *fzd8a*

In *Xenopus*, *fzd7* regulates some aspects of gastrulation movements as part of the Wnt/PCP pathway (Djiane et al., 2000). Overexpression of zebrafish *fzd7a* and *fzd7b* disrupts proper gastrulation, however rescue experiments suggest that the *fzd7a* and *fzd7b* receptors signal through different noncanonical pathways (Knowlton and Kelly, 2004). The distinctive role of both *fzd7* genes is further emphasized by their different expression patterns. The expression of *fzd7a* was detected in the forebrain, hindbrain, midbrain, spinal cord, in the anterior part of the developing somites and within the lateral mesoderm (El-Messaoudi and Renucci, 2001) while *fzd7b* was expressed within the central nervous system, somatic and lateral mesoderm, lateral line, pectoral fin bud, ear and the pharyngeal arches (Sumanas et al., 2002; Ungar and Calvey, 2002).

During the studied stages, 2–4 dpf, *fzd7a* and *fzd7b* are expressed in the pharyngeal arches (Fig. 1G–L, Fig. 2A, B, D, E, G and H). Both genes are also expressed in the pectoral fin bud between 2–3 dpf (Fig. 1G–H, J–K, Fig. 4D and E). A few major differences between these two *frizzled* expression patterns are that *fzd7a* is strongly expressed in the brain in a specific pattern at 2 dpf, whereas *fzd7b* is not (Fig. 2A–B). In addition, *fzd7b*, unlike *fzd7a*, is expressed within the lateral line (Fig. 1J–L, Fig. 2B, E and Q). Furthermore, *fzd7a* is expressed at a low level within the heart (data not shown). When examined at a higher magnification (Fig. 2) it is clear that *fzd7a* is expressed at the dorsal part of the hindbrain from 2–4 dpf (Fig. 2A, D and G) and at 3 dpf strong staining of the tectum can be detected (Fig. 2D). A superficial dorsal view of an embryo at 2 dpf clearly shows the tectum, rhombic lip and dorsal rhombomere expression of *fzd7a* (Fig. 2J). As development proceeds, this expression becomes localized more laterally as seen in (Fig. 2M). This magnification also revealed that *fzd7a* is expressed in the retina adjacent to the lens (Fig. 2P). Sectioning also revealed that *fzd7a* is expressed in the sensory hair cells of the ear at 55 hpf and 3 dpf (data not shown).

In *Xenopus* *fzd8* is involved in the regulation of convergent extension movements (Wallingford et al., 2001). Guided by the observations that zebrafish *fzd8a* is present in the pharyngeal arches before the condensation of the chondrocytes (Kim et al., 1998; Thisse and Thisse, 2004), mouse *Fzd8* transcripts are present in cultured chondrocytes from two week-old animals (Xu et al., 2001), and that the noncanonical Wnt pathway is responsible for convergence and extension in zebrafish, we sought to determine if *fzd8a* is expressed at the critical stages of craniofacial cartilage morphogenesis.

Whole mount *in situ* revealed that *fzd8a* is specifically expressed within the pharyngeal arches from 2–4 dpf (Fig. 1M–O, Fig. 2C, F, and I). During this period of development *fzd8a* is also expressed within the anterior and posterior lateral line (Fig. 1M–O, Fig. 2C, F, O, R, and Fig. 4K). At 2 dpf *fzd8a* is also expressed in the otic vesicle (Fig. 2L), in the sensory hair cells of

the ear at 55 hpf and 3 dpf (data not shown), and the olfactory bulbs (Fig. 2R) at 3 dpf. In addition, *fzd8a* is expressed within the chondrocytes of the pectoral fin bud at 55 hpf (Fig. 4F) and 3 dpf (data not shown) and has a low level of expression in the heart at 3 dpf (data not shown).

1.3 *fzd7a*, *fzd7b*, and *fzd8a* are expressed in different tissues within the pharyngeal arches

Although the whole mount *in situ* analysis of *fzd7a*, *fzd7b* and *fzd8a* revealed that all three genes are expressed in specific patterns in the pharyngeal arches from 2 – 4 dpf, it was not clear where this staining was with respect to the cartilage elements themselves. To determine the location of this expression within the arches we first looked at the posterior pharyngeal arches at 55 hpf. At this stage the cells within the anterior craniofacial cartilage elements have stacked and the most posterior craniofacial cartilage elements are beginning to condense (Schilling and Kimmel, 1997). To label the neural crest within the pharyngeal arches, we cut sagittal sections of 55 hpf *Tg(fli1a:egfp)^{y1/+}* embryos (Fig. 3B). In this transgenic line, in addition to endothelial cells, GFP labels cranial neural crest derivatives (Lawson and Weinstein, 2002) and provides a great reference to compare our other sections with. Sagittal sections revealed that although *fzd7a* is expressed in the neural crest at this stage, it is excluded from the mesodermal core and the pharyngeal endoderm (Fig. 3D). This expression is similar to *Fzd7* expression in the mandibular prominence of stage-24 chicks where it is ubiquitously expressed in most of the tissue but is absent from the mesodermal core (Geetha-Loganathan et al., 2009). Next, we found that *fzd7b* is expressed within the neural crest and the mesodermal core of the pharyngeal arches. In contrast to *fzd7a* and *fzd7b*, zebrafish *fzd8a* is clearly excluded from the mesodermal core and neural crest and is only present in the pharyngeal endoderm (Fig. 3E and F). The expression of *fzd7a* is highly similar to that of *fli1a:egfp* (Fig. 3B and D). Interestingly, close examination of transverse sections revealed that stacking chondrocytes at 55 hpf within the symplectic cartilage express *fzd7b*, but not *fzd7a* or *fzd8a* (Fig. 3G–I).

Given that each of these genes had a different expression at the time of cartilage condensation, we wanted to look later in development to see if this pattern of expression was maintained when the chondrocytes had finished their initial morphogenesis movements within each cartilage element at 3 dpf. When sections of the *fzd7a*, *fzd7b* and *fzd8a* 3 dpf *in situ* hybridizations were examined, it was clear that none of the craniofacial cartilages expressed any of those genes (Fig. 3J–L). The expression that was observed within the pharyngeal and pharyngeal arches in the lateral, dorsal and ventral views of these whole mount *in situ* hybridizations at 3 dpf was due to the expression within the surrounding tissue of the cartilage elements (Figs. 1 and 2). The *col2a1a* probe strongly stained the palatoquadrate and Meckel's cartilages at 3 dpf, indicating that the *in situ* hybridization conditions used for the experiments in this study may be suitable (Fig. 3M). Nevertheless, probe penetration of cartilage at 3 dpf is difficult and it is possible that the *fzd* genes may be expressed by the chondrocytes but are not detected in the sections of the whole mount *in situ* hybridizations. To increase probe penetration we attempted to over digest the embryos with proteinase K and collagenase type 1 prior to the whole mount *in situ* hybridization and performed hybridization on sections, neither of which demonstrated that *fzd7a*, *7b* or *8a* are expressed in the cartilage (data not shown).

1.4 Neuronal and gut expression patterns of *fzd7a*, *fzd7b*, and *fzd8a*

While the lateral views of the whole mounts provided us with a general view of the different *fzd* expression patterns, we obtained a more complete picture of *fzd7a*, *7b*, and *8a* expression within the zebrafish brain by observing sagittal sections at 55 hpf. These sections illustrated that *fzd7a* is expressed within the forebrain, midbrain tegmentum, dorsal hindbrain and hypothalamus (Fig. 4A). At this stage *fzd7b* is also expressed within the tectum, tegmentum, thalamus and the rhombic lip (Fig. 4B). The most complex expression pattern in the brain was

observed with *fzd8a*, and includes strong expression domains in the telencephalon, along the border between the hypothalamus and ventral thalamus as well as in the more rostral hypothalamus, hypophysis (pituitary) and in the midbrain tegmentum (Fig 4C). The expression of *fzd8a* within the midbrain tegmentum is particularly intensive laterally (compare to Fig. 2O). In addition to the brain, *fzd7a* and *fzd8a* are also expressed within the neural tube (Fig. 4G, J and L). Coronal sections at 55 hpf demonstrate that *fzd7b* is expressed in the swim bladder as well as some mesenchymal tissue surrounding the gut (Fig. 4H). More posteriorly, we found that *fzd8a* is expressed in the liver, gut, perivascularity and along the ventral borders of the myotomes (Fig. 4J–L).

1.5 The expression of *fzd7a*, *fzd7b*, and *fzd8a* is normal in Wnt/PCP mutant embryos

Our analysis suggests that *fzd7a*, *fzd7b*, and *fzd8a* are good candidates to play the role as receptors of the Wnt/PCP pathway during craniofacial development. To test whether Wnt/PCP directly controls the expression of these receptors we conducted *in situ* hybridization with *fzd7a*, *fzd7b*, and *fzd8a* probes in *gpc4* (*knypek*), *wnt5b* (*pipe tail*), and *wnt11* (*silberblick*) (Piotrowski et al., 1996; Heisenberg et al., 2000) mutants and their wild-type siblings at 3 dpf (Fig. 5). Although the overall length of the mutant embryos was reduced due to abnormal convergence and extension movements, the expression patterns of the three *frizzled* genes appeared to be normal. This is consistent with the observation that mutations affecting Wnt/PCP impair cell movements but not tissue patterning (Topczewski et al., 2001).

Taken together, the data presented here demonstrate that *fzd7a*, *7b*, and *8a* are expressed in a tissue specific manner in the pharyngeal arches during craniofacial cartilage morphogenesis and that these patterns are not disrupted by mutations in *gpc4* (*knypek*), *wnt5b* (*pipe tail*) or *wnt11* (*silberblick*). Further experiments will determine what role these *frizzled* receptor homologues may have during craniofacial development.

2. Experimental Procedures

2.1 Zebrafish maintenance, embryo production and staging

AB (ZDB-GENO-960809-7), *wnt5b*^{ta89} (ZDB-GENE-980526-87), *gpc4*^{m818} (ZDB-GENE-011119-1), *wnt11*^{tz216} (ZDB-GENE-990603-12) and *Tg(fli1a:EGFP)*^{y1/+} (ZDB-GENO-070209-102) (Lawson and Weinstein, 2002) zebrafish lines were maintained as described in Topczewski et al. (2001). Embryos were collected from natural spawning and staged according to morphology described in Kimmel et al. (1995).

2.2 *fzd7* nomenclature

fzd7a was originally referred to as *fz7* (El-Messaoudi and Renucci, 2001). *fzd7b* was originally referred to as either *fzd7a* (Ungar and Calvey, 2002) or *fzd7b* (Sumanas et al., 2002). To eliminate confusion, we follow the nomenclature standards set forth on ZFIN (<http://zfin.org>) and refer to these genes as *fzd7a* (ZDB-GENE-990415-223) and *fzd7b* (ZDB-GENE-990415-229), respectively.

2.3 In situ hybridization

Anti-sense RNA probes were synthesized using the following cDNA clones: *fzd7a* (Witzel et al., 2006), *fzd7b* (accession number **BC049397**), *fzd8a* (accession number **BC056273**, ZDB-GENE-00032803), *fzd2* (accession number **BC056524**, ZDB-GENE-990415-224), and *col2a1a* (Yan et al., 1995) (ZDB-GENE-980526-192). The zebrafish *fzd6* homolog was identified using bioinformatics methods and the antisense RNA probe was synthesized from a cDNA clone (accession number **BC065361**, ZDB-GENE-040426-1451). Whole mount *in situ* hybridization was performed as described in (Topczewski et al., 2001). All of the probes

were hybridized in 50% formamide hybridization buffer except for *col2a1a*, which was hybridized in 65% formamide hybridization buffer.

2.4 Cyrosections

Following the whole mount *in situ* hybridizations, the embryos were postfixed with 4% paraformaldehyde, mounted in sucrose agarose blocks, and cut into 10 µm sections. The *Tg (fli1a:EGFP)^{y1/+}* fish were cut into 16 µm sections and fluorescent images were collected with a 510 LSM META confocal microscope.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Lateral views of zebrafish *fzd2*, *6*, *7a*, *7b*, and *8a* mRNA expression at 2, 3, and 4 days post fertilization (dpf). (A–C) *fzd2* is expressed within the brachial arches (arrowheads), throughout the head and within the pectoral fin bud (asterisks in A–B). (D–F) *fzd6* is expressed within the brachial arches (arrowheads), throughout the head, within the posterior lateral line (arrows in D–F) and within the pectoral fin bud (asterisks in D–E). (G–I) *fzd7a* is expressed within the brachial arches (arrowheads), in the brain and within the pectoral fin bud (asterisks in G–H). (J–L) *fzd7b* is expressed within the brachial arches (arrowheads), in the anterior and posterior lateral line (arrows in J–L) and within the pectoral fin bud (asterisks in J–K). (M–O) *fzd8a* is expressed within the brachial arches (arrowheads) and in the anterior and posterior lateral line (arrows in M–O). (M) At 2 dpf *fzd8a* is also expressed within the midbrain.

Fig. 2.

Higher magnification of the gene expression of zebrafish *fzd7a*, *7b* and *8a* reveals specific patterning within the head. (A–I) Lateral views of the embryo show all three genes expressed within the pharyngeal arches (arrowheads). (A–C) Gene expression of *fzd7a*, *7b* and *8a* at 2 days post fertilization (dpf). (A) *fzd7a* is expressed in the dorsal part of the hindbrain (arrow) and within the forebrain (asterisk). (B–C) *fzd7b* and *8a* are expressed within the anterior lateral line (asterisks). (D–F) Gene expression of *fzd7a*, *7b* and *8a* at 3 dpf. (D) *fzd7a* is clearly expressed in the tectum (arrow). (E–F) *fzd7b* and *8a* are expressed within the anterior lateral line (asterisks). (G–I) Gene expression of *fzd7a*, *7b* and *8a* at 4 dpf. (G) The gene expression of *fzd7a* in the dorsal hindbrain is still visible. (J–L) Dorsal views of *fzd7a*, *7b* and *8a* gene

expression at 2 dpf. (J) A superficial dorsal view of 2 dpf clearly shows the tectum (arrow), rhombic lip (asterisk) and dorsal rhombomere expression (arrowhead) of *fzd7a*. *fzd7b* and *fzd8a* are expressed at the midline (arrowhead) and in the otic vesicle (arrow) (K and L respectively). (M–O) Dorsal views of the gene expression of *fzd7a*, *7b* and *8a* at 3 dpf. (M) The expression in the rhombic lip and dorsal rhombomeres (arrowhead) has moved laterally. (O) The dorsal view of *fzd8a* expression clearly shows the anterior lateral line expression (asterisks). (P–R) Ventral views of *fzd7a*, *7b* and *8a* gene expression at 3 dpf. (P) *fzd7a* is expressed in the retina adjacent to the lens (arrow). (Q–R) Ventral views of *fzd7a* and *7b* expression reveal expression in the anterior lateral line (asterisk). (R) At 3 dpf *fzd8a* is expressed within the olfactory bulbs (arrows). Bar equals 100 μ m.

Fig. 3.

Sections of zebrafish craniofacial cartilage reveal tissue specific gene expression of *fzd7a*, *7b*, and *8a*. (A) Schematic of a lateral view of a zebrafish at 55 hours post fertilization (hpf) highlighting the location of pharyngeal arches. Anterior is to the left. (B) Sagittal section at 55 hpf of *Tg(fli1a:EGFP)^{Y1/+}* that expresses GFP within the neural crest tissue within the pharyngeal arches. Overlay of Nomarski image and *fli1a:EGFP* expression. (C) Schematic drawing of two pharyngeal arches at 55 hpf illustrating the different gene expression patterns of *fzd7a*, *7b* and *8a*. (D-F) Sagittal sections of the pharyngeal arches at 55 hpf. (D) *fzd7a* is expressed within the neural crest tissue of the pharyngeal arches. (E) *fzd7b* is expressed within the mesodermal core and the neural crest tissue. (F) *fzd8a* is expressed within the pharyngeal endoderm of the pharyngeal arches and is excluded from the neural crest and the mesodermal core. Note the similarity between the expression pattern of *fzd7a* at 55 hpf (D) and the *fli1a:EGFP* (B). (G-I) At 55 hpf *fzd7b*, but not *fzd7a* or *8a*, are expressed within the

symplectic cartilage elements (arrows). (J–L) At 3 days post fertilization (dpf) *fzd7a*, *7b* and *8a* are no longer expressed within the craniofacial cartilage, but are expressed in the tissue surrounding the cartilage elements. (M) At 3 dpf it is clear that *col2a1a* is not expressed in the trabeculae but is expressed in the more posterior cartilage elements of the palaquadrates and Meckel's cartilage. (N–O) Much like *fzd7a*, *7b* and *8a*, *fzd2* (N) and *fzd6* (O) are expressed in the tissue surrounding the cartilage elements and not directly in the cartilage at 3 dpf. Abbreviations: (e) eye, (mc) Meckel's cartilage, (pq) palaquadrates, (t) trabeculae, (ep) ethmoid plate, (nc) neural crest, (pe) pharyngeal endoderm, (mes) mesodermal core, (ov) otic vesicle, and (b) pharyngeal arches. Bar equals 50 μ m.

Fig. 4. Sagittal sections of 55 hours post fertilization (hpf) reveal specific expression of *fzd7a*, *7b*, and *8a* within the brain. (A) *fzd7a* is expressed within the dorsal hindbrain (arrowhead), forebrain (asterisk), midbrain tegmentum (arrow) and the hypothalamus. (B) *fzd7b* is expressed within the tectum (arrowhead), rhombic lip (asterisk), thalamus (arrow) and the tegmentum. (C) *fzd8a* is expressed in the telencephalon, rostral hypothalamus, along the border between the hypothalamus and the ventral thalamus (asterisk), the midbrain tegmentum (arrowhead), and the hypophysis (pituitary) (arrow). (D–F) Transverse sections of the pectoral fin bud at 55 hpf. (D) *fzd7a* is expressed throughout the pectoral fin bud at a low level (arrow). (E) *fzd7b* is expressed throughout the pectoral fin bud including the cartilage (arrow). (F) *fzd8a* is expressed within the cartilage of the pectoral fin bud (arrow). (G) A coronal section of a 55 hpf embryo reveals that *fzd7a* is expressed within the neural tube (arrow). (H–J) Coronal sections at 55 hpf (H) and 3 dpf (I–J) reveal staining within the gut. (H) *fzd7b* is expressed in the swim bladder and some mesenchymal tissue surrounding the gut. (I) *fzd2* is expressed in the liver. (J) A more posterior coronal section demonstrates that *fzd8a* is expressed in the liver and along the ventral borders of the myotomes (arrow). (K) A lateral view at 3 dpf illustrates that the expression of *fzd8a* along the ventral borders of the myotomes is also within the anterior side of each segment of the trunk (arrow). This view also shows the expression of *fzd8a* within the lateral line (arrowhead) and within the gut. (L) Coronal section at 3 dpf shows expression of *fzd8a* in the

neural tube, gut and in the vasculature (arrow). Abbreviations: (nt) neural tube, (g) gut, (nc) notochord, (liv) liver, (sb) swim bladder, (h) hypothalamus, (tm) tegmentum, (t) telencephalon, and (rh) rostral hypothalamus. Bar equals 50 μm .

Fig. 5.

Expression of *fzd7a*, *7b* and *8a* within *wnt5b*, *gpc4*, and *wnt11* mutants is normal at 3 days post fertilization. (A, E, and I) wild type siblings. (B, F, and J) *wnt5b* (*pipe tail*) mutants. (C, G, and K) *gpc4* (*knypek*) mutants (D, H, and L) *wnt11* (*silberblick*) mutants. The different shapes of the *wnt11* (*silberblick*) mutant heads are due to the synophthalmia phenotype. Bar equals 100 μ m.