we observed a reduction of *prdm12* expression which demonstrates the position of *prdm12* downstream of *zic1* in the gene regulatory cascade leading midbrain formation.

**Conclusions:** *prdm12* gene is a downstream target of *zic1* and likely to modulate signaling pathways that regulate the expression of genes in the midbrain domain. In addition, *prdm12* is also regulated by *wnt* signaling. We propose that *prdm12* has a crucial role in the midbrain formation regulated by *wnt* mediated *zic1*.

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## Zic1 targeted peripheral myelin protein (*pmp22*) regulates craniofacial morphogenesis


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**Introduction:** Peripheral myelin protein 22 (*Pmp22*), a member of the junction protein family Claudin/EMP/PMP22, contributes to the formation and maintenance of myelin sheaths in the peripheral nervous system. Deletions, duplications and mutations of *Pmp22* accounts for the majority of heritable demyelinating peripheral neuropathies, including Charcot-Marie-Tooth disease type 1A, hereditary neuropathy with liability to pressure palsies or a combination of both as a separate type called Charcot-Marie-Tooth disease type 1E. Apart from the establishment and maintenance of peripheral nerves, *Pmp22* and its family member have also been participated in a broad range of more general process including cell cycle regulation and apoptosis during development. We performed the functional analysis of *Pmp22* during *Xenopus* development. We found that *Pmp22* is a downstream of transcription factor Zic1 and it is responsible for craniofacial morphogenesis.

**Materials and Methods:** RNA encoding *Zic1*-GR and dnTCF-GR were synthesized in vitro with the Message Machine kit (Ambion, Austin, TX). The activity of the fusion proteins can be regulated by addition of dexamethasone to the culture medium of whole embryos. One blastomere of 2-cell stage embryos were injected in the animal pole region, with *Zic1*GR RNA (0.5 ng) and dnTCF-GR (1 ng). At early neurula stage (stage 13.5) the embryos were cultured in 0.1X NAM containing 10μm Dexamethasone (Dex; Sigma-Aldrich, St. Louis, MO) to activate both *Zic1*-GR and dnTCF-GR. Antisense morpholino oligonucleotides (*Zic1*-MO; 5'-AAGTCTTCCAACAATGGGCAGCGAA-3'; *pmp22*-MO; 5'-TCCCAGCAGTAAGAGGAGCATTTTC-3') were injected into the animal pole region of one blastomere at the 2-cell stage and embryos analyzed by *in situ* hybridization. To identify the injected side, β-galactosidase mRNA was co-injected as a lineage tracer.

**Results:** A *pmp22* morpholino (*pmp22*-MO) was designed to specifically interfere with translation of *pmp22* mRNA. Therefore, we observed the defective branch-arches formation during development after whole mount *in situ* hybridization. To make it confirm we performed *in situ* hybridization on sections of *pmp22*-MO injected embryos and the malformation of branch-arches was distinctly observed on the injected side of the sections. Two other branch-arches marker Sox9 and Sox11 was also down-regulated by the depletion of *pmp22*. In a large proportion of embryos injected with *Zic1*-MO we observed a reduction of *pmp22* expression in branch-arches. Using hormone inducible construct of *Zic1*, we analyzed the consequences of *pmp22*expression on the embryos. Embryos injected with 0.5 ng of *Zic1-GR* mRNA and treated with dexamethasone at the late neurula (stage 19) displayed an expansion of *pmp22 expression* in the branch-arches domain in more than 85% of injected embryos. These results further demonstrate the position of *pmp22* downstream of *Zic1* in the gene regulatory cascade leading branch-arches formation.

**Conclusions:** Our data suggests that pmp22 is a downstream target of transcription factor Zic1 mediated by Wnt signaling and *pmp22* has some crucial role in the maintenance of functional peripheral nerves as well as in the proliferation or differentiation of cells especially for the craniofacial morphogenesis.

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## Establishment of *hPer2* transfected HepG2 cell as hepatic circadian model


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**Introduction:** Circadian rhythms are biological,