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Examination of *Gbx2* function in zebrafish development

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Gbx2 is a member of the *Gbx* class of homeobox genes which encode DNA-binding transcription factors. The amino acid sequence of *Gbx2* is highly conserved across multiple species (e.g. mice, zebrafish, chicken, and frogs) with 100% sequence identity between the *Gbx2* homeodomains of the species examined. The function of *Gbx2* in mice and zebrafish has been studied with loss-of-function and hypomorphic (reduced expression) models in both species. The results of these studies have demonstrated a requirement for *Gbx2* in normal development of the mid/hindbrain organizer (isthmus) and anterior hindbrain. The hindbrain controls many basic life functions such as breathing and heartbeat. In early vertebrate development, the hindbrain is organized into eight distinct segments called rhombomeres. Rhombomeres give rise to hindbrain regions such as the cerebellum, pons, and medulla oblongata. In situ hybridization studies in *Gbx2* null and hypomorphic mice have shown that cranial nerve V, which is derived from rhombomeres 2 and 3, fails to develop normally without wild-type levels of *Gbx2*. Mice lacking wild-type levels of *Gbx2* die immediately after birth. To examine if *Gbx2* has a similar impact on cranial nerve V development in zebrafish, we injected a morpholino specific for zebrafish *Gbx2* into zebrafish embryos to silence the *Gbx2* gene early in development. Our findings have shown that zebrafish subjected to the morpholino have a similar phenotype in the hindbrain as *Gbx2* hypomorphic mice. Our present research will attempt to rescue the normal hindbrain phenotype in zebrafish embryos by simultaneously injecting the *Gbx2* morpholino with synthesized zebrafish *Gbx2* mRNA. We will also attempt to rescue the normal phenotype with synthesized mouse *Gbx1* mRNA and mouse *Gbx2* mRNA.