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Twisted gastrulation mutation suppresses skeletal defect phenotypes in *Crossveinless 2* mutant mice

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ABSTRACT

Bone morphogenetic protein (BMP) signaling controls various aspects of organogenesis, including skeletal development. We previously demonstrated that the pro-BMP function of Crossveinless 2 (Cv2) is required for axial and non-axial skeletal development in mice. Here, we showed that skeletal defects in the *Cv2*-null mutant were reversed by the additional deletion of *Twisted gastrulation* (*Tsg*). Whereas the $Cv2^{-/-}$ mutant lacks a substantial portion of the lumbar vertebral arches, $Cv2^{-/-}$;$Tsg^{-/-}$ mice have almost normal arches. Suppression of $Cv2^{-/-}$ phenotypes is also seen in the non-axial skeleton, including the ribs, humerus, skull, and laryngeal and tracheal cartilages. In contrast, the $Tsg^{-/-}$ phenotype in the head is not significantly affected by the *Cv2* mutation. These findings demonstrate that *Tsg* mutation is epistatic to *Cv2* mutation in the major skeletal phenotypes, suggesting that the pro-BMP activity of *Cv2* is, at least in part, dependent on *Tsg*. We also present genetic evidence for the context-dependent functional relationship between *Tsg* and *Cv2* during mouse development.

1. Introduction

BMP signaling plays important regulatory roles in a variety of aspects of vertebrate and invertebrate development, including skeletal development (reviewed in Wan and Cao, 2005). In addition, BMP signals regulate cell-type specification, maturation, cell growth, apoptosis, and dorsal–ventral axis determination (De Robertis and Sasai, 1996; Hogan, 1996; Massague and Chen, 2000; Hammerschmidt and Mullins, 2002; De Robertis and Kuroda, 2004; Yamamoto and Oelgeschläger, 2004; De Robertis, 2006). A number of factors that negatively regulate BMP signals in the extracellular space have been identified (anti-BMP factors). A typical example is a class of secreted antagonist proteins, including Noggin, Chordin, Follistatin, Cerberus, and Gremlin, that bind to, and inactivate, BMP proteins (Smith and Harland, 1992; Lamb et al., 1993; Sasai et al., 1994; Sasai et al., 1995; Hemmati-Brivanlou et al., 1994; Glinka et al., 1997; Hsu et al., 1998).

In contrast to these anti-BMP factors, several extracellular BMP-interacting proteins are reported to modulate BMP signaling in a positive manner. For instance, we previously reported in a study of knockout mice for Crossveinless 2 (Cv2),

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that Cv2 functions as an enhancer molecule of BMP signaling (pro-BMP factor) in mouse skeletal development (Ikeya et al., 2006). Cv2 is a BMP-binding protein that was first identified in a fly mutant study as being required for the formation of crossveins in the fly wing (Garcia-Bellido and de Celis, 1992). Genetic studies in flies have shown that the formation of these veins requires high BMP-signaling activity (involving Dpp and Gbb) and that Cv2 is essential for enhancing the local BMP signal near the receiving cells (Conley et al., 2000; O'Connor et al., 2006; Serpe et al., 2008). In mice, the *Cv2* mutant phenotype in the skeleton is enhanced in the *BMP4*$^{+/-}$ background, showing that Cv2 and BMP4 work together in the same direction for skeletal development (Ikeya et al., 2006). This idea is consistent with non-mammalian and in vitro studies that suggested pro-BMP activity of Cv2 (Conley et al., 2000; Coles et al., 2004; Kamimura et al., 2004; Ralston and Blair, 2005; Rentzsch et al., 2006; Moser et al., 2007; Serpe et al., 2008). A similar pro-BMP role has been reported for another Cv2-class molecule, kielin/chordin-like protein (KCP) in kidney repair (Lin et al., 2005). Interestingly, these factors (Cv2 and KCP) contain multiple cystein-rich repeats homologous to those in Chordin.

Since a characteristic feature of BMP signals is their function as a morphogen (generating an activity gradient and evoking multiple-threshold responses) (Gurdon and Bourillot, 2001), fine spatial control of BMP signals by these pro- and anti-BMP factors is important for tissue formation to occur in the right place when the tissue is at the right size. In addition, these pro- and anti-BMP factors themselves are known to regulate one another in an intricate manner. The activity of the anti-BMP factor Chordin is itself regulated by multiple interacting factors, such as Twisted gastrulation (Tsg) (Harland, 2001), Xolloid-related protease (Ge and Greenspan, 2006) and Sizzled (Kimelman and Szeto, 2006). These interactions may be regulated in a context-dependent fashion, and their quantitative control remains elusive at the molecular level. For example, whether Tsg acts as a pro- or anti-BMP factor has been addressed through multiple approaches but has not yet been totally clarified (see Section 2.5). Similarly, it is still unclear whether Chordin in mice has any pro-BMP role, which has been suggested for Sog (fly Chordin homolog) and Chordino in *Drosophila* and zebrafish, in addition to its anti-BMP role (Zusman et al., 1988; Ashe and Levine, 1999; Decotto and Ferguson, 2001; Hammerschmidt and Mullins, 2002; Rentzsch et al., 2006). As for Cv2, a previous study in zebrafish and in vitro studies suggested that Cv2 could also function as an anti-BMP factor, at least under certain artificial conditions (Moser et al., 2003; Binnerts et al., 2004; Rentzsch et al., 2006; Serpe et al., 2008).

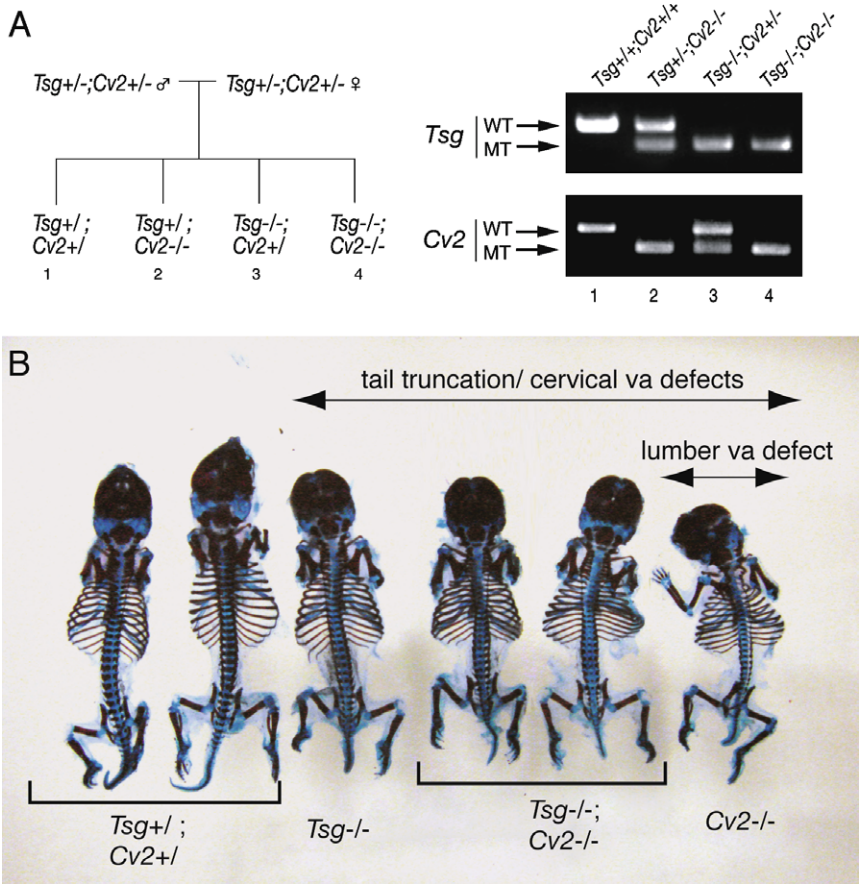

Fig. 1 – Genetic crossing of *Cv2* and *Tsg* mutants. (A) Analysis of genotypes by allele-specific genomic PCR. WT, wild type allele; MT, mutant allele. (B) Gross skeletal phenotypes of the compound mutants at E18.5. Skeletal preparations were stained with Alcian Blue and Alizarin Red. va, vertebral arch.