Communication between cells is essential during embryonic development. The vertebrate limb bud provides us with a model in which to study signalling interactions between cells during patterning of embryonic tissues and organogenesis. In chapter 1, I give an introduction about limb bud development that is focused on the patterning of the anterior-posterior axis of the limb bud during outgrowth. Here I also introduce Sonic Hedgehog (SHH), which is a signalling molecule that is expressed by the cells of the polarising region. Shh mediates the organizing function of the polarising region and is required for patterning of the anterior-posterior axis of the distal limb. Mutual genetic antagonism between Gli3 and dHand prepatterns the limb prior to SHH signalling, resulting in the establishment of the polarizing region in posterior limb bud mesenchyme.

In chapter 2, I show that Gli3 and Alx4 genetically interact during limb and craniofacial development. Gli3 and Alx4 are both expressed in anterior limb bud mesenchyme and are required to keep the Shh expression domain posteriorly restricted. Disruption of either Gli3 or Alx4 results in the establishment of an ectopic Shh domain and preaxial polydactyly. However, analysis of limbs lacking both Gli3 and Alx4 reveal that these genes synergistically interact during patterning of all three groups of limb skeletal elements. In addition, skulls of embryos lacking both Gli3 and Alx4 exhibit craniofacial defects.

In Chapter 3, I have established that limb bud mesenchymal cells response differentially to SHH signalling. During limb bud patterning SHH differentially activates secondary signalling molecules, like BMP2 and Gremlin. Here I show that SHH also acts upstream of NOTCH signalling during limb bud development. SHH regulates the expression of Jagged-1 and Hey, which are involved in NOTCH signal transduction, in the limb bud mesenchyme. Furthermore, I provide evidence that SHH acts upstream of Jagged-1 during patterning of the limb bud vascular system.

The SHH signal is relayed to the AER by BMP antagonist Gremlin. In chapter 4, I describe the Gremlin mutant limb phenotype, which corresponds to the limb deformity limb phenotype. Gremlin mutant limbs exhibit fusions of zeugopodal elements and digit syndactyly, indicating that anterior-posterior patterning is affected. I show that the absence of Gremlin mediated BMP antagonism disrupts the feedback loop between SHH and the AER. In Gremlin deficient limb buds, activation of Fgfs and Bmps in the AER is disrupted and Shh expression is not propagated. Furthermore, mesenchymal limb buds cells undergo massive apoptosis in the absence of Gremlin.

In the final chapter, I discuss whether SHH acts as a morphogen during limb bud patterning. It has been proven that SHH patterns the neural tube directly and not through induction of secondary signals. However, it is still unclear whether SHH acts as a “true” morphogen during limb bud patterning. Furthermore, I discuss the role of NOTCH signalling during limb bud development. I propose future experiments that will allow us finding additional roles for NOTCH signalling during limb bud development.