7 Summary

In this study the mouse mutant *„short digits“ (Dsh)* was analysed. *Dsh* is a radiation induced mouse mutant with autosomal semidominant mode of inheritence. The *Dsh* phenotype was initially described in 1993 by Selby et al. Aim of the study was the morphological and molecular characterisation of the Dsh mutant and to unravel the genetic basis , which is causitive for the phenotypes.

Homozygous *Dsh/Dsh* mice are characterised by a multitude of internal and skeletal defects, including severe midline defects. The *Dsh/Dsh* and *Shh-/-* phenotypes are very similar. The shared phenotype is characterized by holoprosencephaly. Using a genetic complementation test we were able to demonstrate that *Dsh* and *Shh* are allelic. However, sequencing of a genomic 14 kb *Shh* locus and *Shh* cDNA showed no mutation in the canditate gene *Shh*. Using a positional cloning approach we were able to demonstrate that Dsh is caused by a large chromosomal inversion: Despite analysing a thousand meioses in the initial fine mapping we could not substantially reduce the interval of the *Dsh* Locus on chromosome 5 as expected. The observed suppression of recombination in proximity of the breakpoints is due to the chromosomal rearrangement. Identification of the inversion breakpoints was done with a classic screen using southern blot hybridisations, the inversion was confirmed by FISH analyses on chromosomes. The sequence based analyses of the breakpoint region shows that no gene is affected through the *Dsh* inversion, which compromises 11,7 Mb. Using quantitative Realtime PCR we could show, that the expression of the flanking genes on both sides of the breakpoints are not affected by the *Dsh* inversion, and therefore can not be causative for the mechanisms which lead to the *Dsh/Dsh* or *Dsh/+* phenotypes.

The *Dsh* inversion, which telomer breakpoint is located 13 kb upstream of the *Shh* gene, leaves the exons, the promoter and two enhancers intact. The inversion leads to a position effect, which affects the regulation of the *Shh* gene. In humans, translocations 15 to 265 kb 5’ of the *Shh* promoter lead to holoprosencephaly phenotypes; these findings and the *Dsh* mouse mutant point to the existance of cis-regulatory elements. These elements are needed especially for genes associated with development, like *Shh*, to ensure correct spatio-temporal gene expression. Using a bioinformatic *in silico* analysis, we were able to show that the 1 Mb *Shh* “gene desert” harbours 5 putative cis-regulatory DNA elements. This result is a good explanation for the observed dysregulation of Shh gene expression caused by the *Dsh* inversion.

In early development homozygous *Dsh/Dsh* embryos are lacking *Shh* transcript, which explains the severe midline defect, which is manifesting to the same extent in *Shh-/-* embryos. In later stages of development (E13.5) we were able to show reexpression of Shh in the proboscis using in situ hybridisation. In the hereozygote *Dsh/+* embryos the inversion leads to an expected 50% reduction of *Shh* transcript in comparison to the wildtype. Beginning with E 13.5 *Shh* is strongly upregulated and ectopicially expressed in the limb compared to the wildtype, which leads to the activation of hedgehog target genes , like e.g. *Pthlh*. The *Ihh-Pthlh* feedback loop, which is essentiell for the development of digits and joints is disturbed, which results in a delayed chondrocyte differentation and the consequent development of the *Dsh/+* phenotype: The short digits, hence the name of the mutant, are caused by a fusion of the first and second phalanx in digit 2-5 and a shortened proximal phalanx in digit 1. The described heterozygous phenotype is very similar to the human congenital disease brachydactyly type A1 , which is caused by mutations in *IHH* leading to shortening or loss of the middle phalanges. In addition we could show structural changes in the morphology of the cerebellum of Dsh/+ mice. In this study the functional analysis of the in silico identified putativ cis-regulatory DNA elements was initiated. The generated transgene reporter mice and the analysis of the resulting embryos were mentioned in brief. In summary the genetic and molecular analysis of the *Dsh* mouse mutant shows new insights in the complex regulation of *Shh* gene expression and the involvement in the processes of development and disease.