

Human embryonic development is an extremely complex process that depends on the interplay between a large number of genes and their regulatory elements. The expression pattern of most developmentally important genes is controlled spatially and temporally and undergoes complex regulatory mechanisms. The interaction between regulatory sequences, such as enhancers or silencers and gene promoters requires their close physical proximity. This includes long range regulation in which the regulatory modules are, in linear organization of genome, located up to 1 million base pairs from the target gene. However, as chromosomes are organised in three-dimensional structures, even two distal DNA fragments could physically come close together. Regulatory elements are often located in non-coding DNA, that until recently remained untargeted in molecular diagnostic approaches mostly due to difficulties in data interpretation and limited knowledge on its biological function. However, a significant number of disease-causing alterations in limb malformations are related to mutations in non-coding part of the genome affecting gene regulatory landscape.

The main objective of this study is to demonstrate that genomic structural mutations (deletions, duplications, inversions, translocations), identified in patients with congenital limb malformations, could interfere with gene regulation by disrupting the proper enhancer-promoter interactions. We aim to develop relevant functional assays to study the disruption of gene regulatory landscape, thereby contributing to better understanding of molecular mechanisms behind the disease. To achieve this goal, we are going to apply the 4C-seq (Circularized Chromosome Conformation Capture sequencing) approach that enables to screen for lost and/or gained interactions between gene and regulatory elements. We expect to find novel regulatory elements involved in skeletal morphogenesis and define regulatory architecture of a certain region of the genome, thus supplementing the cis-regulatory maps of human tissues that should be introduced as a tool for understanding the mechanisms of gene regulation in health and disease.

We assume that within the ranks of this project, we will collect representative data to highlight the importance of non-coding part of the human genome in the development of specific limb phenotypes. The result of our study will complement the basic science: developmental genetics and biology, but also contribute to the clinical genetics by improving genetic counselling for patients and their families. We aim to introduce the non-coding part of the genome into molecular diagnostics by developing new genetic tests for certain limb phenotypes. The upgrading of genetic counselling for patients with congenital limb disorders seems to be particularly important, regarding that the molecular cause of the disease in approximately 50% of cases remains unsolved. This approach will also allow for the informed family planning, because it helps to estimate the risk of reoccurrence of a certain malformation in next child or in next generation. Noteworthy, it adds value to prenatal and preimplantation tests. Only the comprehensive molecular diagnostics enables to consciously decide on family planning, thereby contributing to prevention of birth defects. The experimental strategy, that we intend to establish in our group will supplement the ongoing research on genetic background of a variety of congenital birth defects with special focus on congenital skeletal malformations.