All types of genetic variations contribute to the inter-individual genetic variability. However, at the same time they can cause variable congenital anomalies, such as congenital limb malformations (CLMs), the second most common group of birth defects. Despite a broad spectrum of diagnostic tools, the molecular causes of CLMs remain unknown in around 50% of cases worldwide. Since the discovery of the DNA structure and subsequent launching and completion of the Human Genome Project, scientists focused mainly on studying the genome through unravelling the mysteries of the DNA sequence. The recent advancement of the techniques investigating the 3D genome resulted in discovering the functional units of the genome, called the topologically associated domains (TADs). Functional studies of structural variants, i.e. submicroskopic aberrations (deletions, duplications, inversions), has shown that structural variants can disrupt TADs boundaries and lead to arising of abnormal gene expression. It is estimated, that at least 10% of the undiagnosed CLMs cases could be explained by such alterations of the regulatory landscape and modified TAD structure. Therefore the main objective of this project is to implement array comparative genomic hybridization (array-CGH) to identify structural variants, which disrupt the regulatory network of the undescribed genes and regions associated with the development of congenital limb malformations. In the ranks of this project a group of 24 patients presenting with CLMs, who have negative results of routine screening of all known causes of their anomalies will be subjected to array-CGH testing. The genome-wide approach will be utilized to detect undescribed, rare, potentially pathogenic structural variants, that disrupt the local regulatory landscape. All SVs will be analysed against the in-house data of structural variants detected in Polish patients with CLMs and publicly available databases. A novel approach, based on the most recent knowledge about TADs organization and their functionality will be implemented for the analysis of the structural variants of unknown significance (VUS). The most promising findings will be confirmed by means of Real-Time PCR. Family history, followed by the carrier state analysis will indicate to the structural variants, which are the most likely to be pathogenic in our cohort.

As a product of this project, it is expected that in some of the patients novel causes of congenital limb malformations will be detected. It is also expected that in some patients identification of yet undescribed genes responsible for limb development will be achieved. With the diagnostic yield of only around 50% of cases, which has been static in the last decade, there is a great need for discovering the remaining unknown causes of CLMs. We expect that as the result of the proposed research, some tests will be introduced to the routine testing, which will raise the quality and accessibility of the comprehensive genetic testing for patients presenting with congenital limb malformations. Through investigating the complexities of gene regulation and functionality of the genome organization the results of this project will also contribute greatly to the fields of medical and developmental genetics.