Congenital malformations constitute a serious clinical, scientific as well as social and economic challenge. It is estimated that they concern 2-3% newborns. Multiple congenital anomalies are defined by the presence of at least two major birth defects from at least two distinct systems. A number of them are lethal leading to intrauterine death or infant death immediately after birth regardless of the treatment used. In a case of lethal defect there is no effective treatment in spite of intensive progress in medicine. Moreover, they account for rising costs of health care in every country and society. Despite the importance of many standard diagnostic methods, in most newborns (>50%) the causes of human malformations, which phenotypically do not correspond to specific and known genetic syndromes, remain unknown. Genetic factors play an important role in the etiology and pathogenesis of congenital malformations. About 1600 phenotype described in OMIM database still have an unknown molecular basis. Moreover, over 1500 of human genes have unknown function and have not been associated with any human disease.

The main objective of this study is: to expand the currently limited scientific knowledge about the genetic causes of severe and lethal congenital defects in human which lead to fetus death and stillbirth or infant death immediately after birth regardless of the treatment used, identification of new genetic causes in the etiology of severe and lethal congenital defects using contemporary high-throughput sequencing technologies including array CGH (Comparative Genomic Hybridization - aCGH) and trio-based whole exome sequencing – WES (fetus - proband and both parents) performed on the biological material derived from fetuses with multidefects in the second and third trimester; assessment of the prevalence of pathogenic submicroscopic chromosomal rearrangements and single nucleotide variants in the group of fetuses with at least two congenital malformations with severe phenotype and poor prognosis and; finally, an attempt at determining the relationship between a known chromosomal rearrangement or a single gene variant and the clinical outcome (genotype-phenotype correlation). Practical objectives of the project include: 1. Development of a diagnostic algorithm in a newborn with multiple malformations with the application of aCGH and WES; 2. Development of the rules for the interpretation of test results, especially of WES (but also aCGH) and assessment of their significance for genetic counselling in families with high risk for offspring with multiple birth defects; 3. Development of a next generation sequencing (NGS) based clinical diagnostic approach that will begin by testing for 23-chromosome copy number, to identify the most common known genetic causes of severe and lethal causes of multidefects, followed by genetic mutation panel analysis in non-aneuploid cases.

The project uses new genomic technologies and has 5 stages: I – recruitment of patients, collection of clinical data, II - aCGH study performed to assess the presence of the most common genomic defects known to cause fetal structural abnormalities, III - WES technique to DNA from fetal tissue, IV - trio-based WES study (proband – both parents), V - result summary and analysis, genotype-phenotype correlations, working up the diagnostic algorithm. For the implementation of a genetic studies fetal tissues (amniocytes, umbilical blood, other fetal tissues) will be collected, and if it is needed, peripheral blood from the parents as well. The study will include 100 fetuses with at least two congenital defects diagnosed during routine imaging studies, recruited among patients attending the Medical University in Wroclaw and other Dolny Slask obstetrics hospitals. After aCGH analysis in 100 fetuses, 70 proband's samples will be qualified to single WES study, and then in the remaining group of fetuses with the normal aCGH and proband's WES analysis without any causative sequence variant candidate, trio-based WES is planned in about 50 pair of parents.

Searching for the causes of congenital developmental defects, whose course is severe and unfortunately very often fatal, has very high impact in science allowing better understanding of human development and afterwards understanding of pathological mechanism of congenital defects. **Authors of project undertake this scientific subject** to elucidate and understand the causes and pathomechanism of congenital defects. Identification in the fetuses of genomic variants with aCGH and NGS methods will become the starting point for genotype-phenotype correlations and will increase the knowledge of genes that play a role in the pathogenesis of the congenital anomalies as well as the development of the diagnostic tests for such defects in fetuses and determination of recurrent risk in family counselling. It is also expected that applying new genomic methods in such a group of patients will lead to discovery of the new and interesting genetic causes of human disorders (novel monogenic syndromes). The meaning of these discovers goes beyond the problem of congenital defects, because it is suspected that some of identified genes associated with developmental defects in fetus, may be also important in pathogenesis of other non-lethal human disorders. The research methodology planned by us, i.e. sequential aCGH, WES and finally trio-based WES) will be conducted for the first time in Poland and one of the first worldwide. Their results, we believe, will be critically important for both cognitive and practical reasons.