A role of unconventional myosin VI in cardiac muscle and its involvement in the development of cardiomyopathy.

Cardiomyopathy is a disease affecting the cardiac muscle itself, in which the heart is enlarged and weakened by impairment of its pump function. A prevalence of cardiomyopathy is about 0.02% worldwide. Cardiomyopathies are classified according to their predominant pathophysiological features, the most prevalent ones are dilated cardiomyopathy and hypertrophic cardiomyopathy. Dilated cardiomyopathy is characterized by ventricular enlargement (dilation), contractile dysfunction of the left (the heart's main pumping chamber) or both heart ventricles, and congestive heart failure symptoms. Hypertrophic cardiomyopathy is characterized by the abnormal growth and arrangement of muscle fibers, leading to thickened heart walls usually occurring in the left ventricle. Hypertrophic cardiomyopathy is the most frequent cause of sudden death in young adults but the nature of this severe disease has yet not been fully understood on the molecular level.
In most cases, hypertrophic cardiomyopathy is caused by a single mutation within the genes encoding proteins of the sarcomere (a basic subunit of the cardiomyocytes containing the contractile apparatus. Despite numerous studies not all mutation have been characterized, the knowledge on new genes possibly involved in the cardiomyopathy development is continuously expanding. One of such new genes is a gene encoding unconventional myosin VI, a motor proteins that unlike muscle myosins cannot form filaments. Instead, it fulfills its transporting functions by tissue-specific interaction with proteins involved in various functions crucial for the given tissue, most probably incliding also striated muscles. In 2004 it was shown that a point mutation within the myosin VI gene was associated with hypertrophic cardiomyopathy thus implying that myosin VI could be involved in the disease development. But despite that there is decade since this observation there no reports addressing this important association. Studies performed in the group of Prof. Maria Jolanta Rędowicz from the Nencki Institute of Experimental Biology has revealed that this motor protein could play important role in striated muscle and possibly in the development of hypertrophic cardiomyopathy.
Therefore I would like to expand theses studies on understanding the role of unconventional myosin VI (MVI) in cardiac muscle, and to evaluate its possible involvement in the development of cardiomyopathy.

To address the objective of this project I am going to perform the following research tasks:

1. Analysis of myosin VI expression levels in cardiac muscle and neonatal cardiomyocytes.
During cardiac muscle development a change the level of several protein expression was observed. Often, proteins that are present in the embryonic heart are downregulated (i.e. their synthesis is diminished) their around birth and upregulated (i.e. their synthesis is enhanced) again in cardiomyopathy. To address whether MVI level is also changing during cardiomyocyte development, I am going to assess MVI expression level during subsequent stages of heart development.

2. Identification of potential MVI partners in cardiac muscle and neonatal cardiomyocytes.
Comparison between the MVI partners identified in hearts of animals of different developmental stages and in isolated cardiomyocytes will allow to identify interactions that exist during heart development and in the adult heart, and to assess whether they are altered in isolated cardiomyocytes. This will indicate pathways in which MVI is involved in these hearts.

3. Analysis of the morphology of cardiac muscle of the MVI knockout mice.
Most of the information on MVI functions in vivo comes from studies on Snell's waltzer (SV) mice that do not synthesize the MVI and therefore serve as a model for MVI knock out animals. Several defects were observed in these mutant mice, including hearing, brain, kidney and brush border. No studies so far were performed on cardiac muscle despite the fact that these animals exhibited elevated blood pressure indicating that in addition to kidney defects there could be also some changes in the heart function. I am going to assess the Snell's waltzer mice heart to body mass index to test whether their hearts are enlarged (which is a symptom of hypertrophy) in respect to control animals. Detailed analysis of the mice hearts functions (such as for example blood pressure, ECG or echocardiography) is also planned.

4. Analysis of the effect of MVI knockdown on neonatal cardiomyocytes.
I would like to test whether and how MVI knockdown (i.e. inhibition of its synthesis possible by genetic manipulations on a cell level) affects neonatal cardiomyocytes. I expect that these cells will reveal defects in cell structure as well as in the time-course of cardiomyocyte maturation.

5. Analysis of the effect of overexpression of the MVI mutant associated with human cardiomyopathy on neonatal cardiomyocytes.
I am going to test how overexpression of the MVI mutant (achieved by genetic manipulation on a cell level) associated with cardiomyopathy affects the cardiomyocyte morphology and function.

Implementation of this project and the obtained results will contribute to the better understanding the role of MVI in the myocardium in physiology and pathology, as well as will shed new insights into the molecular mechanisms leading to the development of cardiomyopathy.