Duchenne muscular dystrophy (DMD) is the most common inherited and incurable human muscle disorder caused by lack of dystrophin due to mutations in the gene encoding this protein. As dystrophin gene is located on the chromosome X the DMD sufferers are boys with the estimated prevalence of 1 per 5000 live birth. Dystrophin deficiency is a cause of progressive muscle wasting, which appears at the onset and exacerbates with increased physical activity of a child and is eventually leading to severe disability and premature death. Dystrophin is present in different tissues, however, life-threating symptoms of full-length dystrophin deficiency (tissue-specific shorter forms of dystrophin cannot replace the full-size protein) come from a severe muscular dysfunction, thus investigating of muscle-debilitating mechanism is on the focus of both scientist who are interested in understanding molecular background of this pathology and physicians who are searching for an effective DMD therapy. Dystrophin localizes to the cytoplasmic face of the sarcolemma and anchors a group of proteins known as the dystrophin-associated protein (DAP) complex with actin filaments and microtubules. These interactions provide a link between the cytoskeleton, DAP in the membrane of the muscle fibre and the extracellular matrix components bound by DAP and has been attributed with a role in mechanical strengthening of the sarcolemma during the contractile activity. While disruption of this link contributes to the DMD pathology, additional pathogenic mechanisms have also been implicated. These include an abnormal calcium homeostasis that cannot be explained by membrane ruptures alone and is considered as a major cause of dystrophic muscle impairment. One of commonly described features of dystrophic muscles, which is observed in *mdx* mice (animal model of DMD) as well as in humans suffering from Duchenne muscular dystrophy is a gradual fibrosis and calcification. These processes lead to a replacing of dying necrotic muscle fibers with fibroblasts, adipocytes and calcium minerals. Finally, total muscle mass is seemingly unaffected (or even increased) but in fact the real muscle volume and efficiency are dramatically reduced. Moreover, muscle regenerative potential is severely compromised. Despite intensive studies the origin of calcium minerals (myositis ossificans) within dystrophic muscle is still elusive and it seems to be a very complex phenomenon. In fact, our preliminary experiments concerning this issue led us to unexpected but highly replicable and inspiring results. Therefore, the general purpose of this project is to identify the cellular origin of muscle calcification in *mdx* mice and ultimately define biochemical processes involved. On a basis of literature data and our preliminary results we have formulated a few working hypothesis explaining this phenomenon. First of all we focus our attention on abnormalities concerning endoplasmic reticulum (ER-stress) and aberrant calcium homeostasis particularly due to an excessive activity of P2X7 receptor (one of nucleotide-activated ionotropic receptors). Previously we found that muscle calcification in *mdx* mice depleted of P2X7 is much lower than in *mdx* mice expressing this receptor. In our experiments we will use immortalised myoblasts and myotubes derived from dystrophin-positive control and dystrophin-negative (*mdx*) mice. Moreover we will also work on primary myoblasts isolated from control, *mdx* and P2X7-deficent *mdx* mice. We will use various modern biochemical and molecular approaches to identify the cellular origin of calcium deposits in skeletal muscles of dystrophic mice (a role of myoblasts, myotubes and may by others if necessary will be considered), explain the biochemical basis of muscle ossification considering metabolic diversity of skeletal muscles and understand signaling pathways leading to the ectopic muscle calcification.

We believe that an accomplishing these goals will allow explaining an interesting biological phenomenon which is additionally closely linked with the real medical problem, that increases its importance. While a role of the ER-stress in tissue calcification seems to be well documented, a potential functional relationship between ER-stress and deregulation of calcium homeostasis particularly due to an aberrant susceptibility of muscle cells to purinergic (nucleotide) stimulation is of high novelty. This project is a continuation of our long-standing study on Duchenne muscular dystrophy, which are performed in a close collaboration with Professor Dariusz C. Górecki (University of Portsmouth UK).