The horse is a species domesticated about 5500 years ago. Excavations indicated that one of the oldest types of horses usage was mares milking. In the later time period, due to expansion of the highperformanced cattle dairy utility, the mares milk has not played any important role in nutrition of people living in majority of world parts. In the last few years we can observe an increasing interest in mare's milk utility again, mainly because of the current knowledge about its composition and health-promoting features. Despite the interest in the mare's milk as a food product is relatively new phenomenon in the European culture, in Asian countries (eg. Mongolia, Kazakhstan and Uzbekistan) mare's milk is consumed for many centuries, without interruption, due to the traditions of kumiss (low-alcohol drink obtaining from fermented horses milk) production. In the European countries and USA the growing attention is paid on using of mares milk in the supporting treatment of numerous diseases (e.g. liver, stomach, gall bladder and pancreas function disorders; respiratory diseases - tuberculosis, chronic bronchitis and asthma). Therefore, we can observe the dynamically developing farms specializing in mares milk production. An example of such activity is German horse stud "Haflinger Gestüt Meura", where the horses are milked automatically and the sale of offered milk and milk related products (cosmetics, liqueurs, etc.) is very high. Mare's milk in terms of basic chemical composition is similar to human's milk and different to cow's milk. For this reason, the milk of horses is considered as valuable substitute of brest milk (especially for children with allergies to cow's milk protein). Mare's milk, has a high lactose content and low fat concentration. The mare's milk protein fraction is characterized by the low amount of caseins (considered as one of the major milk allergens) and by the high, in relation to other species, the level of bioactive protein - lysozyme and lactoferrin. Studies on ruminants indicated the existence of relationship between milk protein polymorphisms (including βlactoglobulin and  $\alpha$ -lactalbumin) and different milk composition traits as well as milk allergenicity. Till date, in the literature, there are no data regarding the association of polymoporphic variants of genes encoding horses milk proteins and milk composition traits.

This project assumes the association of polymorphisms in genes encoding major whey proteins (LGB1, LGB2 and LALBA) and their expression level, which can be the reason of milk composition traits variability, observed in previous studies. The aim of this project is molecular analysis of genes encoding the most abundant mare's milk whey proteins including searching for polymorphisms in their structural parts (exons and introns). After bioinformatical ana lysis of detected polymorphic variants (to find polymorphisms affecting sequences potentialy related to genes expression regulation, e.g. within the tagret sites for microRNAs), the most promising polymorphisms will be genotyped in larger animal gruop to asses their interbreed distrinution. Moreover, an association study will be performed to check whether investigated polymorphisms are related to genes expression variability (application of the results of mRNA and proteins levels obtained in NCN SONATA grant, which is conducted in Poznan University of Life Sciences). The horse is a species domesticated about 3,500 years BC and is characterized by the unique phenotypic diversity. This variation is mainly an effect of a long artificial selection, which is conducted by human since the moment of domestication, to improve economically important traits. Besides, the nucleotide sequence of the domestic horse is known for a long time, the above mentioned variation of the Equus Caballus phenotypes is still not fully explained at DNA level. The broad aim of this project is to extend the knowledge about molecular genetics of the domestic horse in relation to the genes encoding the major whey proteins In the future, results obtained in this study may be helpfull in mares milk composition genetic markers describing, which can be usefull in animals selection (to obtain milk with the most needed chemical properties as an valuable component for application in human nutrition as well as in cosmetic and pharmaceutical industry and well known correlation between mare's milk quality and correct development of newborn foal.) However, the main objective of this project is to contribute in better understanding of the mechanisms regulating genes expression in the mammary gland of mare and to explain the molecular background of significant intra- and interbreed differences observed in the horses milk composition.

Ongoing own investigations regarding 5'-flanking variants of genes encoding whey proteins and their expression level (during PhD thesis conducting) showed the existence of significant differences of mRNA and milk protein levels between analyzed breeds, as well as between individual horses (among each breed). Preliminary results have indicated the significant effect of breed and lactation stage on genes expression level. Screening for 5'-flanking polymorphic variants revealed the existence of 14 SNPs (Single Nucleotide Polymorphisms). If all animals were analyzed together, no statistically significant associations (genotype  $\rightarrow$  gene expression) were noticed. When horses were divided into particular breeds, the meaningful influence of c.-718G>A polymorphism in LGB1 gene on milk  $\beta$ -laktoglobulin level was recorded for Polish Cold-blooded Horse mares. The lack of spectacular results of associations between 5'-flanking variants and analyzed genes expression can suggest, that observed intra- and interbreed differences in mRNA and milk proteins level are related to the polymorphisms located in other regions of investigated genes. Therefore, it is justified to expand the previously conducted analyses by the structural parts of LGB1, LGB2 and LALBA genes.