Selected polymorphisms in UCP1 gene, study of its functional significance and association with the risk of obesity and metabolic syndrome

Metabolic syndrome (MetS) refers to the co-occurrence of several known metabolic risk factors, including obesity, impaired sugar or lipid metabolism, the presence of type 2 diabetes mellitus (T2DM), and high blood pressure. MetS is a serious problem which affects millions of people around the world. Despite advances that have been made in diagnosis, treatment and prevention, MetS is still the leading cause of death in developed countries. In the era of obesity epidemic, researchers have been trying for years to understand the mechanism of functioning and regulation of adipose tissue. In mammals, adipose tissue can be divided into white (WAT) and brown adipose tissue (BAT). The main role of WAT is fat storage, while BAT accumulates energy and dissipates it in a process called non-shivering thermogenesis. The best-characterized marker of BAT is uncoupling protein 1 (UCP1) which plays a key role in regulating thermogenesis, energy expenditure and oxidative stress protection.

There are many factors that modulate the thermogenic activity of BAT (e.g. cold exposure or lifestyle), subsequently affecting UCP1 expression. Stimulation of β3-adrenergic receptors (e.g. by norepinephrine) activates adenylate cyclase, causing an increase in cAMP levels that initiates lipolysis and the release of regulators of *UCP1* expression. It is postulated that the *UCP1* gene polymorphisms might be associated with altered transcription, translation and function of the UCP1 protein, leading to disturbances in fat metabolism, followed by increased risk of obesity and MetS.

The aim of this study was to investigate whether there is correlation between selected *UCP1* polymorphisms with the risk of obesity and MetS in the Polish population. Moreover, the functional significance of selected SNPs in terms of promoter activity was verified. A luciferase assay facilitated assessment of the functional significance of selected SNPs in the 5' *UCP1* region. Levels of *UCP1* and *UCP2* mRNA were also analysed in peripheral blood mononuclear cells (PBMC) from a group of healthy donors, as well as those with metabolic disorders (obesity or T2DM). In addition, NGS sequencing of the coding region and the 5' and 3' regions of the *UCP1* gene was performed to search for new, previously unknown genetic variants of *UCP1*.

The results presented in this study indicate that presence of the G allele at position A-3826G of the *UCP1* gene is associated with a lower risk of MetS. The remaining SNPs: A-

1766G, A-112/C, Ala64Thr, and Met229Leu have not been associated with a risk of developing MetS nor obesity in the Polish population. Functional studies using *UCP1* expressing cell lines: HepG2 and PAZ6, have shown that norepinephrine (NE) plays a key role in the mechanism of UCP1 activation in BAT. Presence of a mutant allele (G) in the -3826 polymorphism, located in close proximity to transcription factor binding sites, reduces the activity of UCP1 (PAZ6 cell line stimulated with NE). In this study, it was shown that PBMC do not express *UCP1* at a measurable level, while the level of *UCP2* mRNA in PBMC in the group of people with metabolic disorders is significantly lower than in the healthy control group. NGS sequencing confirmed earlier genotyped SNPs and allowed for identification of 39 new, possibly significant polymorphisms in term of susceptibility to MetS or T2DM.

Summarizing, the results of this study indicate a significant importance of the *UCP1* gene polymorphisms in the risk of obesity and metabolic syndrome.