# Abstract

**Background:** Metabolic syndrome (MS) with its growing worldwide prevalence and significant impact on the risk of cardiovascular disease and cancer development has emerged as a serious public health concern. Multiple mechanisms have been suggested to confer to its pathophysiology, however the exact contribution of each of MS risk factors still needs to be elucidated. The aim of the study was to evaluate molecular signatures in peripheral blood of individuals affected by MS and/or obesity through a whole-blood transcriptome analysis with the use of next generation sequencing (NGS) technology. Metabolically healthy obese phenotype was also investigated to determine the role of obesity in the development of metabolic abnormalities as well as to assess the extent of healthy obese exposure to carcinogenesis, metabolic and cardiovascular complications.

**Methods:** Out of more than 1000 individuals from the 1000PLUS cohort, 32 were recruited to 4 study groups: MS lean, MS obese, healthy obese and healthy lean, and matched for gender and age. MS was diagnosed based on the harmonized definition (Circulation 2009; 120:1640-5). Body mass index was used as a measure of obesity according to World Health Organization criteria. Eventually, each of the groups under investigation comprised 8 subjects. Whole-blood transcriptome analysis was performed with the use of Illumina NGS technology. Gene expression profiles’ comparisons were carried out and analyzed at pathway level (Ingenuity Pathway Analysis, Qiagen; DAVID Bioinformatics Resources 6.8).

**Results:** Principal component analysis of transcriptomic data revealed an efficient separation of groups in each comparison. Sets of enriched pathways and biological processes were identified for each of the phenotypes under study. The analyses revealed the activation of pro-inflammatory and pro-thrombotic pathways in subjects with MS when compared to their healthy counterparts, irrespective of their obesity status. Differences in the activation of pathways involved in hepatic abnormalities were also observed. Healthy obese presented the activation of mechanisms involved in fatty liver disease, while for MS obese activated processes were related to hepatic fibrosis. Moreover, obese study participants affected by MS demonstrated the upregulation of molecules involved in the process of carcinogenesis, when compared to the MS lean. Surprisingly, MS lean presented the downregulation of particular cancer pathways when compared to healthy lean subjects. Altered expression of genes involved in protein synthesis, including ribosome and mTOR signaling pathways, was also noted among obese and MS-affected subjects.

**Conclusions:** The evaluation of a whole blood transcriptome turned out to be a potentially useful tool for the assessment of metabolic health status as well as pathomechanisms involved in MS. The pathway enrichment analysis confirmed that MS is related to the state of inflammation and changes in the vascular system, independently of excess body weight. Our results also indicate that healthy obese, despite not fulfilling the criteria for MS diagnosis, could display an intermediate state with a lower degree of metabolic abnormalities, before they proceed to the full blown MS. Moreover, MS seems to enhance the progression of liver disease that starts already in the presence of obesity alone. Interestingly, obesity and not MS appears to be responsible for the increased risk of particular cancer pathways activation in MS patients.