Abstract

Purines are organic compounds with miscellaneous functions that are found in all living organisms in complex molecules such as nucleotides, nucleosides or as purine bases. The natural balance of purine levels is maintained by their synthesis, recycling and degradation. Excess purines are excreted in the urine as uric acid. Purine nucleotides may be recycled by salvage pathways catalysing the reaction of purine base with phosphoribosyl pyrophosphate. A completely new central molecule of purine metabolism, inosine monophosphate, can be synthesized from precursors during the *de novo* purine synthesis (DNPS). DNPS involves ten steps catalysed by six enzymes that form a multienzymatic complex, the purinosome, enabling substrate channelling through the pathway. DNPS is activated under conditions involving a high purine demand such as organism development.

Currently, three DNPS-disrupting disorders have been described: ADSL deficiency, AICA-ribosiduria and PAICS deficiency. All three disorders are caused by genetic mutations leading to the impaired function of particular enzyme causing insufficient activity of respective DNPS step, manifested biochemically by accumulation of substrate of deficient enzyme, biologically by disruption of purinosome formation and clinically by unspecific neurological features, which contributes to difficulties in DNPS disorders diagnosis. We assumed, that defects in other DNPS enzymes remain unseen due to the rarity of DNPS patients and the lack of diagnostic methods caused by the commercial unavailability of most of the DNPS substrates.

Our hypothesis was supported by the data from gnomAD database revealing the possibility of mutations in five of six genes coding enzymes of the pathway. Therefore, we prepared biochemical and inorganic procedures for synthesis of DNPS substrates and their multiple isotopically labelled analogues. All prepared compounds were utilized as standards for the development of LC-MS/MS diagnostic methods. We also produced human cell models of known and putative DNPS disorders. Cell lines were characterized by genetic sequencing, protein activity assays and determination of DNPS substrates accumulation in cell medium and lysates.

Our results initiated an international collaboration leading into description of a new DNPS disorder the PAICS deficiency and encouraged us to screen the urine and dry blood spot (DBS) samples of patients with nonspecific neurological impairment lacking a diagnose. We determined physiological values of DNPS substrates detectable in urine and DBS. DBS samples did not reveal any significantly altered values. However, we identified three modestly and one extremely elevated value within urine samples resulting in further investigation with the aim to prove a presence of DNPS disorder.

Keywords: *De novo* purine synthesis, Adenylosuccinate lyase deficiency, AICA-ribosiduria, PAICS deficiency, Human cellular model, Purinosome, Dried blood spots, DBS, Screening, Tandem mass spectrometry, HPLC–MS/MS, Unspecific neurological symptoms.