# ABSTRACT


## Molecular Pathology of Acute Intermittent Porphyria

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Acute intermittent porphyria (AIP) is autosomal dominant disorder caused by the partial deficiency of porphobilinogen deaminase (PBGD), the third enzyme in the heme biosynthetic pathway. AIP is manifested by life-threatening acute neurological attacks that can be provoked by various factors such as drugs or alcohol.

Objective of this study was to identify and characterize the molecular lesions in Czech and Slovak AIP patients. To identify disease-causing mutations screening was performed by PCR, denaturing gradient gel electrophoresis (DGGE), automated DNA sequencing and restriction fragment lenght polymorphism (RFLP) assays. Total of 35 individuals from 8 families were analyzed to detect asymptomatic carriers. Seven mutations were identified, including 3 novel mutations. Of particular interest, one patient had two mutations R173Q and Q204K, both located in exon 10 on the same allele. To further characterize these mutations, human PBGD was cloned into the pGEX-4T-1 vector and mutations were generated by site-directed mutagenesis. The wild-type and mutated enzyme species were expressed in *E. coli* as GST fusions and purified by affinity chromatography. Conditions of the PBGD enzyme assay were determined and specific activities, thermostability and pH optima of mutated enzymes were measured and compared to the wild-type protein. R173Q was the causative mutation with major effect on the PBGD function. The Q204K mutated PBGD had high residual activity but thermostability was decreased by 75%. pH optimum for Q204K mutated protein was the same as for the wild-type.

To conclude, three novel mutations were identified in AIP patients. These studies provide accurate detection of asymptomatic carriers and emphasize the molecular heterogeneity of AIP. An unusual case of AIP with two mutations both located in 10. exon of the PBGD gene was characterized at molecular and enzymatic level.



**Keywords:** acute intermittent porphyria, porphobilinogen deaminase, mutations

**Klíčová slova:** akutní intermitentní porfyrie, porfobilinogen deamináza, mutace