FUNCTIONAL ANALYSIS OF NOVEL ABCC8 MUTATIONS FOUND IN CZECH PATIENTS WITH CONGENITAL HYPERINSULINISM

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Background
• Congenital Hyperinsulinism (CHI) is a heterogeneous genetically determined condition that is characterized by unregulated secretion of insulin from pancreatic β-cells. The most common and severe cases are caused by mutations in K<sub>ATP</sub> channel subunit SUR1 encoded by the gene ABCC8. To assess the pathogenic effect of novel ABCC8 mutations it is necessary to perform in-vitro functional study.

Objective and hypotheses
• The aim of our study was to identify Czech patients with CHI caused by ABCC8 mutations and to perform in-vitro functional study of novel ABCC8 mutations found in our cohort.

Methods
• The molecular genetic analysis of ABCC8 gene was performed on DNA samples of 42 Czech patients with CHI. Novel mutations were created by site directed mutagenesis and transfected into HEK293 cells for functional studies using radioactive Rubidium (⁸²Rb). Mutant and wild type (WT) channels were exposed to different drug conditions: control (DMSO), 100µM diazoxide, 100µM diazoxide and 10µM glibenclamide, 2.5mM NaCN and 20mM 2-deoxy-D-glucose and 2.5mM NaCN, 20mM 2-deoxy-D-glucose and 10µM glibenclamide. ⁸²Rb efflux was measured in a liquid scintillation counter using Cherenkov radiation.

Results

Conclusion
We report the biggest cohort of Czech patients with CHI published so far. The proportion of heterozygous mutations is much higher when compared to other published cohorts, most probably due to lack of consanguinity in the Czech population. Moreover, using in-vitro functional study, we have proved the pathogenic effect of 5 novel heterozygous ABCC8 mutations on the pancreatic K<sub>ATP</sub> channel function.

Supported by a grant from the Czech Ministry of Health (NT 11402), by the Grant Agency of Charles University (GAUK 248 213) and ESPE Short-term Research Fellowship for Klara Rozenkova.

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