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CB1 and CB2 gene expression in atopic and non-atopic asthma – expression levels before and after treatment with inhaled corticosteroids or leukotriene receptor antagonist

Carina Kozmus[1,2], Vojko Berce[3], Uros Potocnik[1,4]. [1]Centre for Human Molecular Genetics and Pharmacogenomics, Faculty of Medicine, University of Maribor, Maribor, Slovenia, [2]Institute for Molecular and Cell Biology, University of Porto, Porto, Portugal, [3]Department of Pediatrics, University Medical Centre Maribor, Maribor, Slovenia, [4]Laboratory for Biochemistry Molecular Biology and Genomics, Faculty for Chemistry and Chemical Engineering, University of Maribor, Maribor, Slovenia

Asthma is a chronic inflammatory disease of airways, characterized by hyper-responsiveness of the airways and reversible bronchial obstruction. Asthma pathogenesis is currently understood through the interaction of several genes and environmental influences. It is believed that endocannabinoids act as native modulators of the immune system, probably through activation of cannabinoid receptors. Our goal was to determine gene expression levels of CB2 (*CNR2*) and CB1 (*CNR1*) in untreated atopic and non-atopic asthmatics and after treatment with inhaled corticosteroids (ICS) or leukotriene receptor antagonist (LTRA). We analysed a case-control cohort of 229 children (5-18 years old) with newly detected mild/moderate persistent asthma (150 atopic, 79 non-atopic, and 13 undetermined atopy), and 271 controls. Blood samples were collected before treatment, and 72 matching samples obtained 4-6 weeks after ICS treatment and 105 after LTRA treatment. In ICS group fluticasone dry powder was prescribed (<12 years -200 μg/day, >12 years - 400 μg/day). In LTRA group patients were treated with montelukast (<12 years - 5 mg/day, > 12 years - 10 mg/day). Several clinical parameters [e.g. forced expiratory volume in 1 sec (FEV1), FEV1/ force vital capacity (FVC), and fraction of expired nitric oxide (FENO) were measured in asthmatics. RNA was extracted from blood leukocytes and gene expression was measured by qPCR. When comparing gene expression levels between asthmatics and controls we used Mann-Whitney U test. The influence of anti-asthmatic treatment on gene expression was analysed with Wilcoxon signed-rank test. Correlation between clinical parameters and gene expression was analysed by Spearman's correlation coefficient. Data are presented as median mRNA expression log2 (sample/control) ± interquartile range.

CNR2 and *CNR1* expression levels were higher in untreated asthmatics (*CNR2*, 0.60±1.28; *CNR1*, 0.44±2.97) ($p<0.0001$), in atopic (*CNR2*, 0.53±1.23; *CNR1*, 0.39±3.23) ($p<0.0001$) and non-atopic subgroups (*CNR2*, 0.61±1.35; *CNR1*, 0.86±3.31) ($p<0.0001$), compared to controls (*CNR2*, 0.02±1.00; *CNR1*, -0.14±1.16). *CNR2* expression was associated with eosinophilia in asthmatics ($r=0.31$, $p=0.046$), *CNR1* expression with FEV1/FVC in asthmatics ($r=-0.15$, $p=0.038$) and in atopic subgroup ($r=-0.25$, $p=0.005$), and with FENO in non-atopic subgroup ($r=0.33$, $p=0.008$). After ICS treatment, *CNR1* expression was lower in asthmatics (from -0.01±2.22 to -0.86±1.96, $p=0.0201$) and in atopic subgroup (from 0.38±2.56 to -0.87±1.66, $p=0.0300$). After LTRA treatment, *CNR1* expression was lower in asthmatics (from 1.20±3.06 to -0.45±1.80, $p<0.0001$), in atopic (from 0.83±3.33 to -0.44±1.78, $p<0.0001$) and non-atopic subgroup (from 1.98±3.03 to -0.47±1.91, $p<0.0001$). *CNR2* expression was lower in asthmatics (from 0.75±1.25 to 0.45±1.40, $p=0.0118$) and in atopic subgroup (from 0.71±1.28 to 0.42±1.43, $p=0.0480$). In atopic subgroup, the change in *CNR2* expression was associated with ICS treatment outcome (measured by ΔFEV1) ($r=-0.43$, $p=0.015$) while *CNR1* was associated with LTRA treatment outcome (measured by ΔFEV1/FVC) ($r=-0.42$, $p=0.004$).

Our results suggest the endocannabinoid system is up-regulated in asthma patients and correlated with severity of asthma and eosinophilic inflammation differently in atopic and non-atopic asthmatics. Following anti-asthmatic treatment, the improvement in asthma severity was associated with lower levels of *CNR2* (after ICS treatment) and *CNR1* (after LTRA treatment) in atopic asthma subgroup.