MicroRNAs serve as prediction and treatment-response biomarkers of attention-deficit/hyperactivity disorder and promote the differentiation of neuronal cells

Liang-Jen Wang[1], Ho-Chang Kuo[2,3], Sheng-Yu Lee[4,5], Lien-Hung Huang[6], Yuyu Lin[6], Pei-Hsien Lin[6] and Sung-Chou Li[6]

[1]Department of Child and Adolescent Psychiatry, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan; [2] Department of Pediatrics, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan; [3] Kawasaki Disease Center, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; [4]Department of Psychiatry, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [5] Department of Psychiatry, College of Medicine, Graduate Institute of Medicine, School of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan; [6] Center for Mitochondrial Research and Medicine and Genomics and Proteomics Core Laboratory, Department of Medical Research, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan

Background Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable neurodevelopmental disorder. Our research team previously also applied next-generation sequencing (NGS) to identify WBC miRNAs as high-performance biomarkers for identifying ADHD. Our previous study using structural magnetic resonance imaging (MRI) indicated that the gray matter (GM) volume was negatively correlated with the ΔCt values of miR-126-5p, miR-140-3p, and miR-30e-5p.

Aims & Objectives This study aimed to examine whether miRNA expression abundance in total white blood cells (WBCs) facilitated the identification of ADHD and reflected its response to treatment. Furthermore, whether miRNA markers facilitated the growth of the human cortical neuronal (HCN-2) cells was also investigated.

Methods Total WBC samples were collected from 145 patients and 83 controls, followed by RNA extraction and qPCR assays. Subsequently, WBC samples were also collected at the endpoint from ADHD patients who had undergone 12 months of methylphenidate treatment. The determined ΔCt values of 12 miRNAs were applied to develop an ADHD prediction model and to estimate the correlation with treatment response.

Results The prediction model applying the ΔCt values of 12 examined miRNAs (using machine learning algorithm) demonstrated good validity in discriminating ADHD patients from controls (sensitivity: 96%; specificity: 94.2%). Among the 92 ADHD patients completing the 12-month follow-up, miR-140-3p, miR-27a-3p, miR-486-5p, and miR-151-5p showed differential trends of ΔCt values between treatment responders and non-responders. In addition, the *in vitro* cell model revealed that miR-140-3p and miR-126-5p promoted the differentiation of HCN-2 cells by enhancing the length of neurons and the number of junctions. Microarray and flow cytometry assays confirmed that this promotion was achieved by repressing apoptosis and/or necrosis.

Discussion & Conclusion The findings of this study suggest that the expression levels of miRNAs have the potential to serve as both diagnostic and therapeutic biomarkers for ADHD. The possible biological mechanisms of these biomarker miRNAs in ADHD pathophysiology were also clarified.