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# Detection of circulating miRNA levels in large cohort schizophrenia

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Schizophrenia is one of the most common severe mental disorders, with a lifetime risk of 1% in the population worldwide. Over the years, the diagnosis of schizophrenia has remained symptom-based, relying mainly on self-reports from patients, mental state examination, and clinical interviews, and lacking objective laboratory tests. Such a diagnostic strategy can sometimes lead to misdiagnosis and has been criticized widely. To remedy this embarrassing state of affairs, a set of biomarkers has been proposed based on physical and biological tests. In a currently finished study, global plasma miRNAs were profiled in a test cohort of 850 schizophrenia patients and 963 control subjects, using RNA sequencing, TaqMan Low-Density Array, and quantitative reverse transcription polymerase chain reaction (qRT-PCR) assays. The captured miRNAs were then validated by qRT-PCR assays in an independent cohort of 623 schizophrenia patients, 654 control subjects. The global plasma miRNA screening revealed eight miRNAs that were upregulated in schizophrenia, as revealed by both assay platforms. The qRT-PCR analysis showed the up-regulation of miR-17-5p and miR-193a-3p in schizophrenia but not in non-schizophrenia disorders.In this study, we designed a multistage case-control study with follow-up plan to investigate the plasma miRNA profile as noninvasive biomarkers for schizophrenia. We globally screened plasma miRNAs initially with both Solexa sequencing and TaqMan Low Density Array (TLDA) chips, followed by a stem-loop quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay.

A total of 726 patients with mental disorders were recruited in this study, of whom 564 were diagnosed as having schizophrenia according to DSM-IV criteria and 162 as having nonschizophrenia disorders including major depression, anxiety, bipolar disorder, and other atypical psychiatric diseases .All patients were diagnosed by at least two consultant psychiatrists using a structured interview and a strict assessment process. A total of 400 healthy subjects, matched on age, gender, smoking history, and education level, were recruited as control subjects.

TaqMan miRNA Assay (Applied Biosystems) was used to quantify mature miRNAs in plasma samples in accordance with the manufacturer's instructions. Briefly, 2 μL of total RNA was reverse-transcribed to cDNA using M-MLV reverse transcriptase (Promega, Madison, Wisc.) and stem-loop RT primers (Applied Biosystems). Real-time PCR was performed using TaqMan miRNA probes (Applied Biosystems) on the CFX-96 system (Bio-Rad, Hercules, Calif.). All reactions, including the no-template controls, were run in triplicate. After real-time PCR amplification, the Ct values were determined using the fixed threshold settings. Each miRNA was reverse transcribed and amplified separately.Diagnosis of schizophrenia is currently dependent on symptom-based criteria and lacks objective indicators. In this study, the authors investigated whether circulating miRNA can serve as a diagnostic biomarker for schizophrenia.

In this study, we designed a multistage case-control study with follow-up plan to investigate the plasma miRNA profile as noninvasive biomarkers for schizophrenia. We globally screened plasma miRNAs initially with both Solexa sequencing and TaqMan Low Density Array (TLDA) chips, followed by a stem-loop quantitative reverse transcription polymerase chain reaction (qRT-PCR) assay.

The schizophrenia patients in the validation cohort underwent a follow-up study under regular treatment with atypical antipsychotic drugs—risperidone and aripiprazole. The dosage for risperidone started at 1 mg/day and was gradually increased to 2–6 mg/day over 2–3 weeks, and that for aripiprazole started at 5 mg/day and was increased to 14–20 mg/day. Benzodiazepines were used temporarily in small dosages, if necessary, in patients with insomnia or anxiety, but combinations of other antipsychotics, antidepressants, or mood stabilizers were not allowed. A total of 107 patients reached the study endpoint, which was 12 months of treatment The definition of remission used in our study was that proposed by the Remission in Schizophrenia Working Group (16), in which both symptom and duration criteria must be met: participants had to maintain scores ≤3 on eight core items on the Positive and Negative Syndrome Scale (PANSS)—delusions (P1), concept disorganization (P2), hallucinatory behavior (P3), unusual thought content (G9), mannerisms/posturing (G5), blunted affect (N1), passive/apathetic social withdrawal (N4), and lack of spontaneity and flow of conversation (N6)—for at least 6 months.

To profile circulating miRNA levels, we used both Solexa sequencing and TLDA to detect miRNAs in plasma. The Solexa sequencing is a next-generation sequencing technology with the ability to read short fragments in a high-throughput pattern Because next-generation sequencing is not fully matured and can be influenced by sequencing errors, we used TLDA to filter out the false signal. To obtain a large amount of total RNA for genome-wide miRNA profiling (20 μg for Solexa sequencing and 2 μg for TLDA chips), we pooled the samples of low total RNA extraction yields (about 500 ng per 100 μL plasma), although this strategy's analysis accuracy has limitations. The pooled samples could mask variance and obscure heterogeneity, and some miRNAs of small effect may be missed. In addition, the pooled samples may generate a miRNA cumulative effect, leading to a false positive signal in

the genome-wide screening stage. This may be the reason why the other six candidate miRNAs failed to show a significant change in the test cohort of schizophrenia. To avoid generating a false positive outcome, however, we performed qRT-PCR validation individually in several independent sample groups to confirm an initial finding. Based on the assessment of the interassay deviations the reproducibility of the miRNA assay used was satisfactory, suggesting that our findings should be reliable. Moreover, there is no endogenous reference miRNA available for normalization of qRT-PCR data; this is the reason why we applied the miR-16 external standard curve to calibrate circulating levels of eight candidate miRNAs, as proposed in a previous study . The recovery rate of miR-130b and miR-193a-3p, which was calculated through the miR-16 calibration, was highly correlated with that through the miR-130b and miR-193a-3p calibrations suggesting that the results from the miR-16 external standard curve are reliable. However, the lower recovery rate of miR-16 calculation may affect the sensitivity of miRNA assay, which means that some useful signals may be missed.

The up-regulation of miR-17-5p and miR-193a-3p is a state-independent biomarker for schizophrenia, and these two miRNAs could be used to develop a diagnostic tool for schizophrenia.