



## Data in Brief

## miRNA profiling in autism spectrum disorder in China



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## ABSTRACT

Autism spectrum disorder (ASD) is a clinically complex and heterogeneous disorder. It is characterized by impaired social abilities, disordered language, isolated areas of interest, and repetitive behaviors. Evidence suggested that the neuropathology of ASD is widely distributed, involving epigenetic regulation in the brain. MiRNAs are a group of endogenous non-coding RNAs that play a critical role in neurodevelopment, neuroplasticity, and other fundamental neurobiological processes. To study miRNA profiling in Autism spectrum disorder in China, we performed miRNA microarray followed quantitative reverse transcription-polymerase chain reaction (qRT-PCR). Here, we describe detailed methods and analysis on these microarray data which has been deposited in Gene Expression Omnibus (GEO): [GSE67979](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE67979).

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## 1. Direct link to deposited data

The deposited data can be found at: <http://www.ncbi.nlm.nih.gov/pubmed/?term=GSE67979>.

## 2. Experimental design, materials and methods

## 2.1. Sample collection and RNA isolation

We totally recruited 20 patients and 20 controls. Peripheral blood samples were obtained from patients and controls at Xiangya Hospital [1]. Total RNA was harvested by the TRIzol (Life Technologies) according to the manufacturer's instructions [2]. The quality and quantity of RNA were examined using an Agilent Bioanalyzer (Santa Clara) and K5500 micro-spectrophotometer (Kaiao). The total RNA was saved at  $-80^{\circ}\text{C}$  until use.

## 2.2. miRNA expression profiling by microarray

RiboArray™ miDETECT™ Human Array microarrays (RIBOBIO), which contained 2578 assay probes corresponding to the entire set of primate miRNAs, were used to screen the miRNA expression. Microarray was incubated 10 min in  $65^{\circ}\text{C}$  followed by 1 h in  $37^{\circ}\text{C}$  for

prehybridization. 2.5  $\mu\text{g}$  total RNA of each sample was labeled by Cy5. The Cy5-labeled RNA samples could be readily visualized with comparable intensity under UV. Labeling efficiency (1.0 to 3.6 was accepted for good microarray result) can be calculated by the concentration of CyDye and RNA measured by K5500 micro-spectrophotometer. Cy5-labeled RNA was denaturated 3 min in hybridization solution, incubated 20 s on ice, and hybridized to microarray 16 h in  $37^{\circ}\text{C}$ . All microarrays were successively washed by  $6\times$  SSPET,  $3\times$  SSPET,  $0.5\times$  SSPET, and  $0.5\times$  SSPET. Then, developer and coverslip were plus for scan (GenePix 4000B laser scanner), followed by bioinformatic analysis (digitized using the R software). Fig. 1 showed part of the signal collection. Fig. 2 showed the log<sub>2</sub> scale of the expression signal values that were plotted, including control probes.

## 2.3. miRNA qRT-PCR analysis

Reverse transcription of total RNA to cDNA was carried out in a 20  $\mu\text{L}$  reaction volume using RevertAid First Strand cDNA Synthesis Kit (Fermentas) according to the manufacturer's instructions. Quantitative PCR assays (CFX96, Bio-Rad) was carried out in a 20  $\mu\text{L}$  reaction volume containing 1  $\mu\text{L}$  cDNA in the 15 autism patients and 15 health controls. It was programmed for an initial denaturation step ( $95^{\circ}\text{C}$ , 3 min) followed by 40 amplification cycles ( $95^{\circ}\text{C}$ , 10 s;  $60^{\circ}\text{C}$ , 20 s). The miRNA qRT-PCR primers were provided by RiboBio (Guangzhou, China) [3]. Has-miR-16-5p was used as an internal reference in the qRT-PCR experiments [4,5]. Differences between control and experimental samples were calculated using the  $2^{-\Delta\Delta\text{Ct}}$  method.

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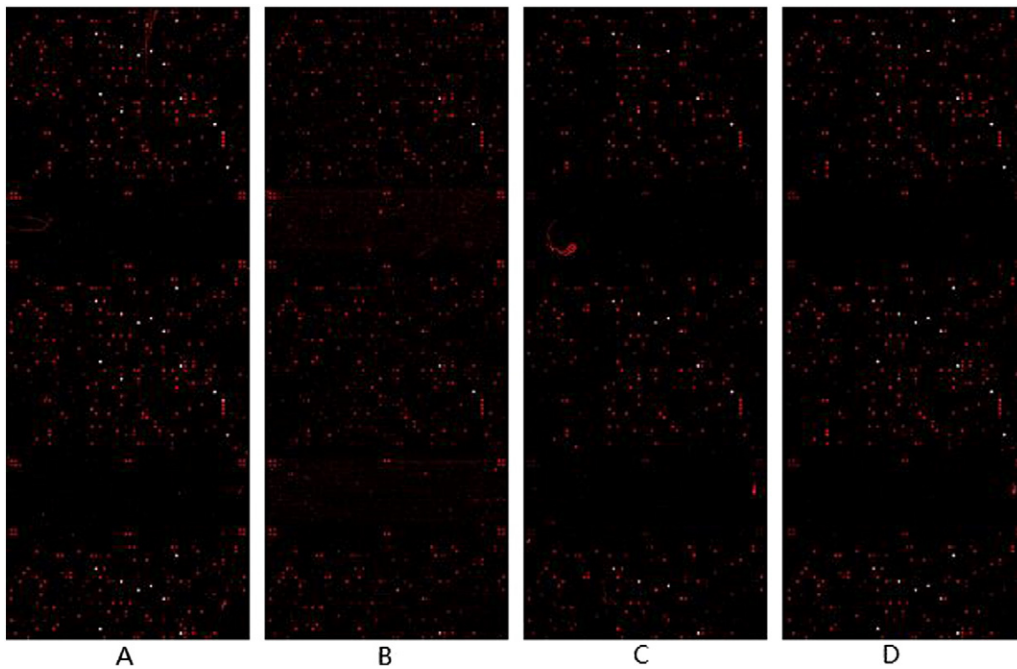


Fig. 1. Part of the signal collection.

### 3. Discussion

We have studied miRNA profiling of ASD by using RiboArray™ miDETECT™ Human Array (A10101-1-12-19, 1×12K). Here, we describe the detailed steps of the microarray analysis. Part of the signal collection and box plot of raw data were provided. As a result, several aberrantly expressed miRNAs related to ASD were discovered. This study confirmed that using miRNA array to study the easily accessible peripheral blood is valid and repeatable [6–8].

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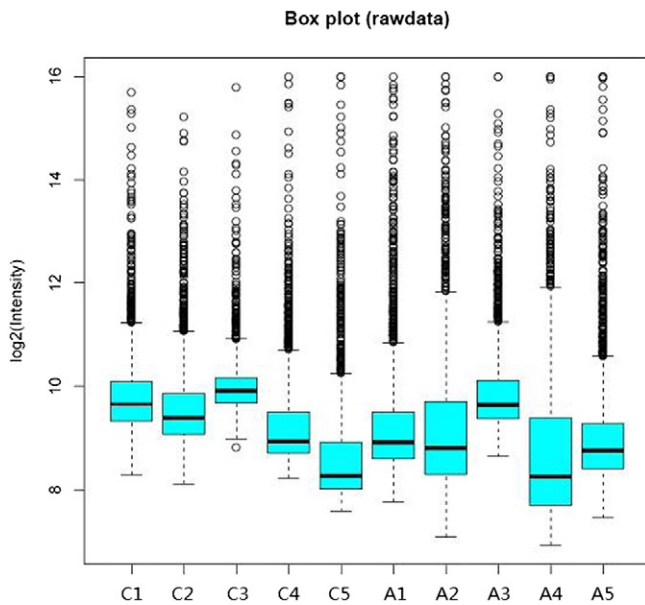


Fig 2. Box plot of raw data. Note: C1-5 means controls, A1-5 means patients.

### Conflict of interest

The authors declare no conflict of interests.