# Neuronal miRNA from circulating vesicles are altered in individuals with schizophrenia

Michelle M. Barnett [1,2], William R. Reay [3], Michael P. Geaghan [4], Dylan J. Kiltschewskij [1,2], Melissa J. Green [5,6], Judith Weidenhofer [1], Stephen J. Glatt [7], Murray J. Cairns [1,2]*

[1] School of Biomedical Sciences and Pharmacy, Faculty of Health and Medicine, The University of Newcastle, Callaghan, NSW 2308, Australia [2] Precision Medicine Research Program, Hunter Medical Research Institute, Newcastle, NSW 2305, Australia [3] Menzies Institute for Medical Research, University of Tasmania, Hobart, TAS 7000, Australia [4] Kinghorn Centre for Clinical Genomics, Garvan Medical Research Institute, Darlinghurst, NSW 2010, Australia [5] Discipline of Psychiatry and Mental Health, School of Clinical Medicine, University of New South Wales, Sydney, NSW 2052, Australia [6] Neuroscience Research Australia, Sydney, NSW, Australia [7] Psychiatric Genetic Epidemiology and Neurobiology Laboratory (PsychGENe lab), Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, Syracuse, New York, USA

## Introduction

- The molecular composition of neurons is specific to the functional state and is altered during disease.
- While these alterations may be detected in postmortem analyses, the ethical constraints, small sample sizes and other confounders are insurmountable for well-powered population studies and biomarker development.
- Given that neurons release extracellular vesicles (EVs) which reflect their origin, we hypothesized that the fractionation of neuron derived EVs provides an opportunity to specifically profile their encapsulated contents non-invasively from blood.
- To explore this possibility, we determined miRNA expression in MAP1B-enriched serum EVs derived from neurons from a large cohort of individuals with schizophrenia or no history of psychiatric illness.

## Methods

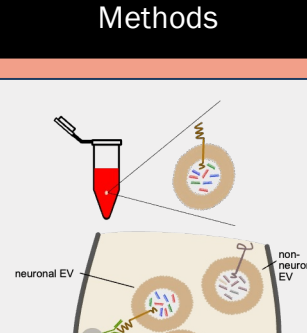


**Figure 1. Fractionation of human serum to enrich for neural EVs.** Individual serum aliquots (100µL) were incubated with anti-MAP1B coupled magnetic beads overnight at 4°C with end-on-end rotation. On the magnetic stand, supernatant was removed and beads washed (x3), supernatant removed following each wash. Vesicles remaining attached to magnetic beads represent neuronal origin EVs and were eluted in glycine buffer (pH3.0). Total RNA was extracted using phenol/chloroform extraction and isopropyl alcohol precipitation. Small RNA libraries were prepared for 50 cycles of paired end multiplex sequencing.

**Table 1** Summary demographics for participants from the Australian Schizophrenia Research Bank (ASRB).

| | Non-psychiatric comparison | Schizophrenia cases | Schizophrenia cases (cognitive deficit) | Schizophrenia cases (cognitive spared) |
|---|---|---|---|---|
| Number of participants (%) | 256 (53.7) | 221 (46.3) | 111 (23.3) | 110 (23.1) |
| Male sex (%) | 43.4 | 66.5 | 75.7 | 57.3 |
| Age (years) | 44.2 ± 13.2 | 40.0 ± 10.7 | 39.4 ± 9.9 | 40.5 ± 11.6 |
| Age in years at illness onset (male/female) | N/A | 23.8 ± 6.8 / 26.1 ± 8.5 | 23.8 ± 7.0 / 25.4 ± 7.5 | 23.8 ± 6.6 / 26.4 ± 9.0 |
| Duration of illness in years (male/female) | N/A | 15.1 ± 10.2 / 16.0 ± 10.3 | 15.0 ± 9.1 / 16.1 ± 9.8 | 15.3 ± 11.7 / 16.0 ± 10.6 |
| Number of treatment resistant cases | N/A | 42 | 32 | 10 |

## References

1. Agarwal et al. Predicting effective microRNA target sites in mammalian mRNAs. *eLife* (2015) vol. 4 e05005
2. Kamburov et al. The ConsensusPathDB interaction database: 2013 update. *Nucleic Acids Res* (2013) vol. 41 (D1) pp. D793-800
3. Lenoue et al., MAGMA: Generalized gene-set analysis of GWAS data, *PLOS Computational Biology* (2015), vol. 11 (4), e1004219
4. Chen et al., ToppGene Suite for gene list enrichment analysis and candidate gene prioritization, *Nucleic Acids Res* (2009), vol. 37, pp. W305-311
5. Li et al., Erythropoietin for Cognitive Deficits Associated with Schizophrenia, Bipolar Disorder, and Major Depression: A Systematic Review, *Pharmacopsychiatry*, (2018), vol. 51(3), pp. 100-104
6. Livingston et al., Preliminary evidence for the phosphodiesterase type-4 inhibitor, roflumilast, in ameliorating cognitive flexibility deficits in patients with schizophrenia, *J Psychopharmacol*, (2021), vol. 35(9), pp. 1099-1110
7. Gould et al., Role for the M1 Muscarinic Acetylcholine Receptor in Top-Down Cognitive Processing Using a Touchscreen Visual Discrimination Task in Mice, *ACS Chem. Neurosci.*, (2015), vol. 6(10), pp. 1683-1691

## Results

### Neuronal-origin miRNA from serum EVs are altered in SZ and severe disease

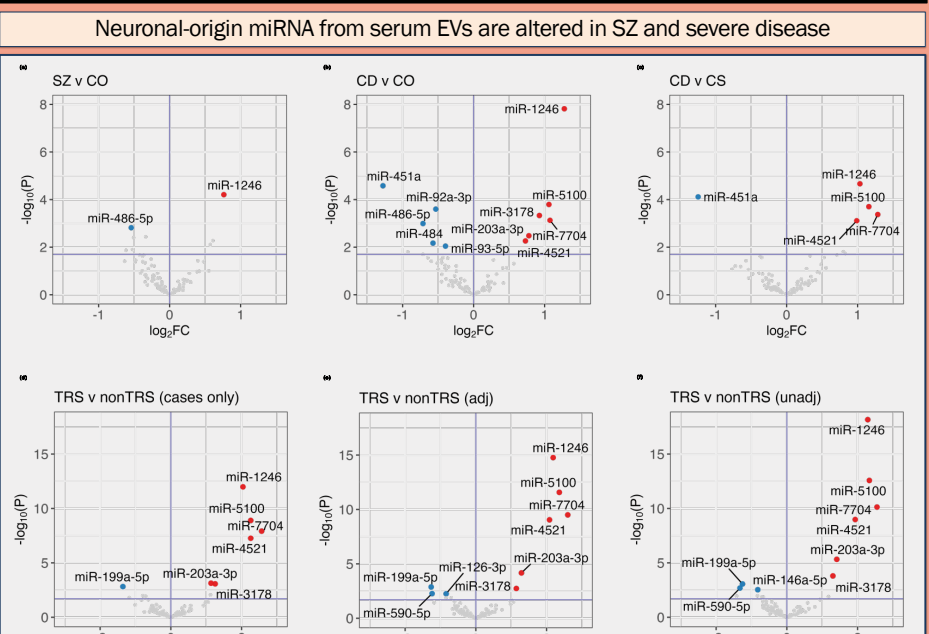


**Figure 2. Differentially expressed neuronal-origin miRNA from serum EVs of schizophrenia subjects. (a)** Schizophrenia subjects (SZ) (n=221) compared to non-psychiatric comparison subjects (CO) (n=256). **(b)** cognitive deficit schizophrenia subjects (CD) (n=111) compared to CO (n=256). **(c)** CD compared to schizophrenia subjects with spared cognition (CS) (n=110). **(d)** treatment resistant schizophrenia subjects (TRS) (n=42) compared to treatment responsive schizophrenia subjects (nonTRS, cases only (n=179). **(e-f)** TRS compared to nonTRS including CO (n=435) **(e)** with adjustment and **(f)** without adjustment for case or comparison status. The horizontal line in each plot represents $P$ <0.1 adjusted for multiple testing by FDR.

### Neuronal EV miRNA target synaptic pathways

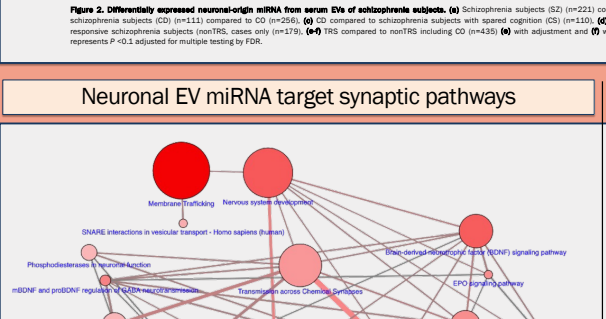


**Figure 3. Cognitive deficit miRNA targets are enriched in synaptic biology.** Predicted targets of neuronal miRNA differentially expressed in schizophrenia subjects with severe cognitive impairments were identified using Targetscan human (1) and filtered to retain genes targeted by at least two miRNA. Over-representation was determined using ConsensusPathDB-human (2). Visualisation of enriched pathways shows *EPO signaling* ($P$ = 0.013), *Cholinergic synapse* ($P$ = 0.024), *Phosphodiesterases in neuronal function* ($P$ = 0.035) and *GABAergic synapse* ($P$ = 0.035). Stated $P$ values adjusted for multiple testing by False Discovery Rate (FDR).

### Genetic enrichment

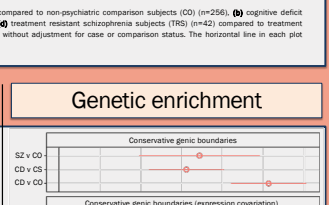


**Figure 4. Gene-set association of predicted miRNA targets with schizophrenia.** Forest plot of MAGMA [3] gene-set association results for the three sets of predicted targets for the differentially expressed miRNA between each group. SZ = schizophrenia, CD = cognitive deficit schizophrenia, CS = cognitive spared schizophrenia, CO = non-psychiatric comparison subjects. Each panel displays the results constructing a model using liberal or conservative definitions of the boundaries for each gene, as well as a model with conservative boundaries additionally covaried for cortical gene expression for each gene. Conservative boundaries extend the gene 5kb upstream and 1.5kb downstream to capture regulatory variation. Liberal boundaries are 35kb and 10kb upstream and downstream, respectively. The MAGMA beta-coefficients for the gene-set term are plotted with the error bars representing the standard error of the coefficient.

## Conclusions

- Large sample determination of circulating neuronal origin miRNA expression profiles from individuals living with schizophrenia and non-psychiatric comparison subjects.
- Greatest alteration of miRNA expression seen in severe cognitive deficit and treatment resistant schizophrenia.
- Neuronal EV miRNA are brain expressed and their predicted targets are over-represented in synaptic biology (GO:0050803, *regulation of synapse structure or activity* (Bonferroni adjusted $P$ value 0.026) [4]).
- Enriched pathways for cognitive deficit miRNA (Fig. 3) suggest treatment with EPO, PDE inhibitors and combination cholinergics may be particularly efficacious for this group, supported by clinical trials and the literature [5,6,7].
- Partitioning of neuronal miRNA, via encapsulation and release in EV, may serve to augment the synaptic regulatory environment.
- Neuronal origin miRNA from circulating EVs demonstrate potential for biomarker development; schizophrenia as a whole and subgroups.