A genome wide microRNA investigation suggests new biomarkers in suicide and lithium targets

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Results from post-mortem brain studies have shown that microRNAs (miRNAs) may play a key role in suicide. MiRNAs regulate gene expression post-transcriptionally by binding to the 3' untranslated region (UTR) of target genes, leading to the degradation of messenger RNA (mRNA) and prevention of its translation into proteins. Thus, altered miRNA levels could influence the function of a large number of genes. While recent findings have suggested the involvement of specific miRNAs/genes pairs in suicide, their role as peripheral biomarkers or targets of pharmacological treatments have been scarcely investigated. In this study, we measured genome wide differences in miRNAs expression levels and tested the effect of lithium on their expression using lymphoblastoid cell lines (LCLs) from suicide completers and non-suicidal controls affected by bipolar disorder (BD).

The sample comprised 7 suicide completers (SC) and 12 non-suicidal bipolar patients with low genetic risk of suicide (LR). LCLs from these subjects (set prior to death) were grown under controlled conditions in medium with or without lithium chloride 1mM for 1 week. Global expression of miRNA was measured using nCounter® miRNA expression Assay (NanoString Technologies). Differences in miRNAs expression and lithium effects between groups were tested with t-test corrected for false discovery rate (FDR; $q<0.05$).

After correction for multiple comparisons and filtering, three miRNAs were selected for validation with quantitative reverse-transcription PCR (qRT-PCR). MiR-4286 and miR-186-5p were successfully validated. MiR-7286 was up-regulated in SC versus LR at basal, while miR-186-5p was down-regulated in lithium-treated LCLs from SC compared to LR, suggesting a potential role of this miRNA in lithium mechanism of action. However, while findings from LCLs suggest that miR-4286 and miR-186-5p could represent potential peripheral biomarkers of suicide and targets of lithium, they do not provide evidence for their role in neuronal processes involved in suicide.

To further test whether miR-4286 and miR-186-5p could constitute lithium targets in neurons, we explored the effect of therapeutic concentrations of lithium (1mM) on the expression of these two miRNAs in human neural progenitor cells (NPCs), showing a significant down-regulation by lithium of both miRNAs. Moreover, we measured the expression of miR-4286 and miR-186-5p in post-mortem anterior cingulate cortex (ACC) from 13 suicide bipolar patients and 13 healthy controls (HC). MiR-4286 was dysregulated in brains from suicide completers compared to controls, though this finding was in opposite direction than observed in LCLs.

We then performed an *in silico* analysis to predict miRNA targets using four different databases: miTG, miRDB, mirSV and TS. Targets for further analyses were selected from those present in the four databases and showing the highest scores. MiR-4286 targets 32 genes while miR-186-5p targets 200 genes. The expression of a subset of these genes is currently under investigation in LCLs, NPCs and AAC and findings will be presented at the conference.

Our study suggests that miR-4286 and miR-186-5p could constitute potential biomarkers of suicide and be involved in the mechanism of action of lithium. Lithium is the most effective treatment in suicide prevention and findings from our study could provide new insights into the understanding of lithium effects on miRNAs/mRNAs interactions and their role in suicide.