**Introduction**

There is a growing interest in looking at genetic components in diagnosing mental disorder and determining potential genetic risk factors in developing mental health disorders particularly when looking at schizophrenia. Schizophrenia is a neuropsychiatric disorder characterized by abnormalities of the nervous system, which coordinates voluntary and involuntary actions such as movement, various affective abnormalities such as rapids changes in mood and hallucinations are common, and molecular abnormalities associated with brain development have been recently discovered, particularly in the prefrontal cortex (Oxford 2007). Much of the interest in schizophrenia is generated by its differential diagnoses in minority patients versus non-minority patients. Where as minority patients are more likely to be diagnosed with psychotic disorders such as schizophrenia, rather then mood disorder that present similar symptoms to schizophrenia. Many explanations have been given to describe these differential diagnosing patterns such as the fact that the diagnosing mental health clinician often do not experience the socio-cultural variants experienced by minority patients, such as discrimination or poverty (Barnes 2008). Other issues such as clinician bias stemming from the prevalence of racial stereotypes of minority only adds to the issue, particularly when dealing with African American patients (Barnes 2008).

Many ethical and medicinal issues arise when an individual is incorrectly diagnosed with a mental disorder, especially mental disorders like schizophrenia that require the patient to take antipsychotics. These anti-psychotics can have mild to severe side effects that include but are not limited to blurred vision, rapid heartbeat, restlessness, tremors, and muscle spams (NIMH 2008). The diagnoses may overshadow and undermine other underlying issues such as ongoing substance abuse and dependence, which is higher in prevalence among minorities seeking medical health care at state hospitals (Mukgerjee 1983).

While initial focus has gone in to looking at target genes for schizophrenia, the movement has shifted to looking at how these genes are regulated by various molecular components. It has been suggested that the abnormalities attributed to schizophrenia are associated with inconsistencies in neurodevelopment particularly in the functions of miRNA, also called microRNAs, which are small non-coding RNA sequence thought to have a translational regulatory effect on messenger RNA (Ambros 2001).

MicroRNAs contain a RNA sequence that is partially or completely complementary to a portion of the messenger RNA sequence. MicroRNAs in conjunction with various proteins form the RNA induced silencing complex. The small miRNA strand acts as a guide, leading the protein complex to the region that it wants to regulate. The proteins are primarily responsible for the cleavage or blockage of mRNA translation. Specifically the protein Argonauate which is responsible for the silencing effect, with variants of this protein acting as endonucleases which are responsible for the cleavage of the phosphodiester bond which joins the nucleotide base pairs together (Chekanov 2008).

The miRNA attaches to the mRNA and the associated proteins in the RISC complex can either block the regions ability to translate proteins (Fig 1), acting as a translational repressor or to cleave and mark the mRNA for degradation by a ribonucleases (Fig 2) to stop further protein synthesis.

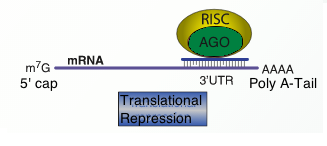


Figure 1: RNA Silencing Complex with Argonauate protein displayed (part of the protein complex displayed for emphasis) binding to an mRNA strand causing a translational repression. miRNA to mRNA sequence match does not need to be exact for the miRNA to act as a effective repressor. (Adaption)

Miller et al suggest that the dysregulation of microRNA, contributes to the development of schizophrenia. To test this idea Millet et al performed a microarray analysis measuring gene expression levels of micro-RNA associated with the dorsal lateral prefrontal cortex brain (DL-PCF), located in the upper side of the front portion of the frontal lobe of the brain, which is associated with executive function and regulation of intellectual function and action (ex. decision making), which is found to be behaviorally and neurologically underdeveloped in schizophrenia patients. Through an experiment using microarray data to determine microRNA expression levels Miller et al observed abnormal expression levels of particular microRNAs within the sample containing individuals diagnosed with schizophrenia, in comparison to the control group. Particularly miR-132, which is associated with the regulation of the NMDA receptor. The NMDA receptor is responsible for maintenance of synaptic plasticity, meaning it determines whether to prune a synapsis based on its strength determined over an interval of time. A synapsis is a gap structure that allows the nerve cells to pass signals to each other and communicate. During neurological development this receptor in conjunction with AMPA receptor is responsible for synaptic pruning; reduction in the number of synapses in order to main efficient nerve cell communication, a normal development process of brain maturation. Lack of regulation of genes related to the NMDA receptor that coordinate this process is theorized to lead to the disruption of this pruning process, leading to abnormal neurological development (Li 2009).

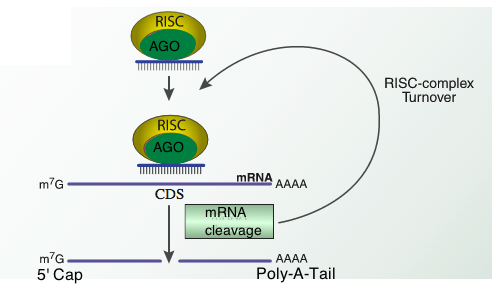


Figure 2: RNA Silencing Complex with Argonauat (AGO) protein displayed being led by a miRNA to an mRNA coding sequence site (CDS) where the miRNA will bind and the AGO protein will initiate cleavage degrading the mRNA. The miRNA to mRNA sequence match must be exact in order for the mRNA to be cleaved. (Adaption)

There is a lot of focus on what the microRNAs are doing to the genes and how microRNA effect gene expression. Is mircoRNA regulation a response to external stimuli? Or are the microRNAs functioning as regulator without any external stimuli? I would like to look at expression of miR-212 which is a NMDA receptor related microRNA which was not found to have an abnormal expression level in Miller et. al. ANOVA analysis, although it is co-transcribed with miR-132 as they are in the same microRNA family, and they share sequence similarities(Mellios 2012 ). I want to use NMDA receptor antagonist dizocilpine, which produces symptoms similar to schizophrenia in humans by blocking the ion channel of the NMDA receptor, in model organisms to see if the microRNA will respond to the antagonist in a similar fashion as miR-132 and if expression levels will be affected (Mellios 2012). I want to observe whether there is a relationship between changes in the NMDA receptor and changes in microRNA associated with the NMDA receptor and if miR-212 expression levels can be synthetically regulated using NMDA antagonists. I am theorizing that if this microRNA behaves similarly to miR-132 we should see a change in expression levels when dizocilpine is introduced, indicating a possible relationship between this miRNA and the receptor (Miller 2012).

In the narrow sense do specific targeted pathways regulate microRNAs, and knowing this can microRNAs be used as markers to identify whether an individual is susceptible to developing schizophrenia and can it be used to differentiate schizophrenia from other neurological diseases?

**Experiment**

The goal of this experiment is to measure the expression levels of miRNAs found in the dorsal lateral pre-frontal cortex associated with the NMDA receptor, particularly looking at confirmation of abnormal regulation of miRNA-212 schizophrenia associated miRNA in model organisms administered dizocipine .The tissues samples taken form the model organism control and dizocipine cohorts will be harvest and measured using a miRNA microarray.

*Model Organisms*

For consistency C57BL/6 lab strain mice will be used and 0.1 mg/kg of dizocilpine dissolved in saline will be administered to pups daily from 4 days of birth to 17 days of birth through the abdominal region, after which the mice will be allowed to mature. A control group of mice will be set up to received saline injections at the same interval as the mice receiving dizocilpine as a control. After 60 days all mice will be killed using by carbon dioxide exposure, and their tissue samples from the pre-frontal cortex will be harvested for microarray analysis.

*Microarray*

To analyze the gene expression levels of the miRNA I want to do a microarray analysis using the procedure developed by Shingara et. al. for specifically assaying microRNA, using RNA samples take from tissues sample of model organisms with administered dizocilpine and those without dizocilpine (administered saline) as a control. A microarray is a solid surface (usually a chip) covered with extremely small DNA wells where each well represents a gene and contains a DNA probe for those particular genes. Normally a large microarray would contain a whole human genome, but for this experiment we only want DNA probes that a miRNA specific. The process entails isolating the target DNA sequence and replicating in and pipetting each particular well of the microarray where it is bound to the solid surface on the array. In order to do this I would first have to collect a tissues samples from the region of interest in this case that would be the dorsal lateral pre-frontal cortex near the NMDA receptor.

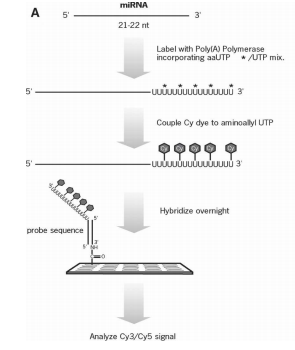


Figure 3: Displayed above is the initial miRNA sequence without any attachments but with the addition of a Poly (A) Polymerase we get the attachment of a poly U tail. The fluorescent probes will attach to the poly-U tail before the miRNA is hybridized to a glass slide and analyzed. (Taken for Shingara et. al.)

After the samples have been collect the RNA can be extracted by adding a mixture of organic solvents, which causes the separation of RNA, DNA, proteins and other cellular components from one another. In the case of RNA a lysate needs to be prepared with reagents that inactivate that has ability of ribonuclease enzymes to degrade the RNA into its smaller components. After the lysate is added you can either vortex or homogenize (carefully crush the sample in a mortar and pestle fashion) the sample in order to degrade the tissue, then add acid phenol chloroform extract to the sample in order to separate the RNA components from the other cellular components. The RNA components should be semi-pure at this point. The solution is then purified by the addition of ethanol and the filtrate through a glass fiber filter, which prevents the movement of the RNA. This washing is repeated as necessary and then the solution is eluted. The sample containing the total RNA is then fractioned (separated) using gel electrophoresis (filled with a denaturing gel) with dye added to track the movement of smaller RNA fragments. Once the gel reaches the bottom of the column, electrophoresis will be stopped and the miRNAs will be extracted from the gel and purified by the glass fiber filtering method used above.

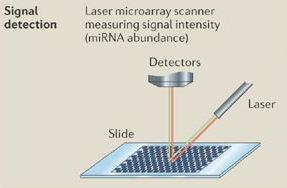


Figure 4: Image of a laser scanning a particular well in a microarray present on a glass slide and the fluorescent signal being picked up by the detector. Microarray can contain dozens of wells with multiple copies of target DNA sequences. (Taken for Clarin 2006)

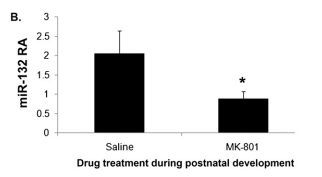
MicroRNA are then labeled with a poly-A polymerase and amine modified nucleotides, which adds a poly-U tail to the 3’ end of the miRNA the amine reactive dye fluorescently labels the miRNA. The now labeled miRNA are hybridized to a glass slide (microarray) where DNA probes that are miRNA specific have been arrayed. During this process the miRNA will attach to their complementary or partially complementary DNA strands, where the DNA and miRNA are heated up to high temperatures causing them to anneal to each other this is done by placing the sample into a hot water bath. The array is them scanned by a laser the measures the signal intensity being given off by the probes, which represents the amount of miRNA which are bound to a particular well in the microarray. An example of a microarray heat map output is seen in Figure 6.



Figure 6: Small sample output of a microarray results where we see that in the Brain and Heat and Sk. Muscle there is no or very little change in expression of MIR141 abnormal tissue samples and normal tissue samples. Given that we see no fluorescence.

**Discussion**

If everything goes well there should be a confirmation of abnormal expression levels (in this case down regulation) in our dizocipine cohort of miR-212 in the presence of dizocilpine similar to what was seen with miR-132 in the Miller et al (Figure 7). If this is the case a future direction for this study could look at ways NMDAR different antagonists affect overall miRNA expression levels, not just on microRNA determined to be mathematically significant. Another direction would be to use targeted NMDA receptor antagonist to observe if specific microRNA respond differently to the introduction of different types of inhibitors.



A negative but even more interesting result would be if the miR-212 expression levels do not respond similarly to miR-132. This would lead to another interesting question of how these two very functionally similar microRNAs have completely divergent expression levels in the presence of dizocilpine, whether the change boils down to something more structural (form and function) in nature.

Figure 7: Postnatal development drug treatment sample for mice administered saline solution and mice administered dizocilpine (also called MK-801). There is significant downregulation of miR-132 in mice sample given 0.1 mg/kg of dizocilpine daily from four days of birth to seventeen days of birth.

Although Microarrays are used to capture the expression levels of certain genes, the results often need to be confirmed by more reliable methods such as real-time polymerase chain reaction, which produces more accurate results. Microarrays also present a unique difficulty in the fact that a microarray can be run multiple times on similar tissue samples and has very different results from what is expected. This will most definitely bring into the question the accuracy of the original results if not confirmed with another method.

A huge limitation to this study is the it can not be reproduce in humans given that the antagonist used causes brain damage in humans, and model organism data while useful has it limitations in its applications to humans. Moving forward it may be useful to instead look at how the NMDA receptor signaling patterns and how this affects different types of microRNA in different variations of schizophrenia.

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