**Introduction**

There is a growing interest in looking 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, and molecular abnormalities associated with brain development, 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. Given, that the diagnosing mental health clinician often does not have hands on experience on socio-cultural variants experienced by minority patients, such as discrimination (Barnes 2008). 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 issues arise when a individual is incorrectly diagnosed with schizophrenia, often the biggest issue stems from the fact that the individual will most likely be placed on medication meant for schizophrenics, which often includes antipsychotics. These anti-psychotics can have sides effects that include but are not limited to blurred vision, rapid heartbeat, restlessness, tremors, and muscle spams(NIMH 2008). Which presents a litany of medical, legal, and mutual trust issues surrounding the client and clinician. This also may hinder the progress on dealing with 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. Particularly, mircroRNAs, which are known for the capacity to regulate mRNA translation of proteins. It has been suggested that the abnormalities attributed to schizophrenia are associated with neurodevelopment particularly in the functions of miRNA, also called microRNAs, which are small non-coding RNA sequence thought to have a regulatory effect during early neurodevelopment, through regulating the activity of various genes in biological networks (Ambros 2001).

MicroRNAs are small noncoding regions of RNA, which contain a RNA sequence that is a close match to a complementary messanger RNA sequences. MicroRNAs are attached to a RNA induced silencing complex which is composed of the miRNA strand and the proteins responsible for the cleavage or blockage of mRNA, specifically the protein Argonauate which is responsible for silencing effect of the miRNA and variants of this protein act as endonucleases which are responsible for the the cleavage of the phosphodiester bond linking the nucleotides together in the messenger RNA(Chekanov 2008).

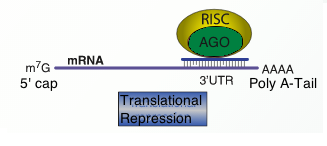


Figure 1: RNA Silencing Complex with Argonauate protein displayed (part of the protein complex displayed for emphasis) binding to an mRNA strand causing a translational repression. miRNA to mRNA sequence match does not need to be exact for the miRNA to act as a effective repressor. (Adaption)

The miRNA attaches to the complementary strand of mRNA and the associated proteins in the RISC complex can either block the regions ability to translate proteins (Fig 1), acting as a translation repressor or to cleave and degradesthe mRNA (Fig 2) to stop further protein synthesis. This process produces the regulatory effect, because miRNA has the ability to target specific region of the mRNA to modulate protein production, creating a “target gene” phenomena ().

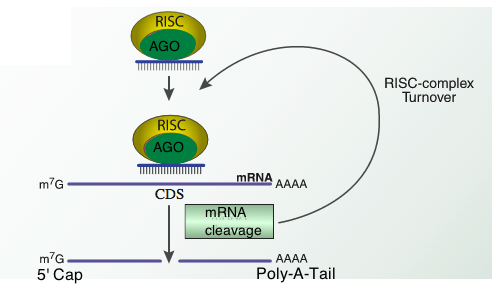


Figure 2: RNA Silencing Complex with Argonauat (AGO) protein displayed being led by an miRNA to a mRNA coding sequence site (CDS) where the miRNA will bind and the AGO protein will initiate cleavage degrading the mRNA. The miRNA to mRNA sequence match must be exact in order for the mRNA to be cleaved.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

Miller et.al suggest that the dysregulation of microRNA, contribute 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 underdevelopment in schizophrenia patients(). Through this experiment Mill et al. saw abnormal expression levels in particular microRNA within the sample containing individuals diagnosed with schizophrenia. Particularly miR-132, which is associated with NMDAR signaling, a type of signaling involving the NMDA receptor, which controls synaptic plastic, and memory functions (Li 2009). Miller also noticed that the genes associated with miR-132 had abnormal expression levels.

In the broad sense 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? In the narrow sense can we used microarray analysis to effectively track microRNA expression levels in schizophrenic individuals. If, we can do this can we find target microRNAs whose abnormal expression levels are specific to schizophrenia in further experiments?

**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 NMDAR receptor, particularly look at confirmation of abnormal regulation of miRNA-132 schizophrenia associated miRNA in patients diagnosed with schizophrenia who have a family history of schizophrenia, and patients without schizophrenia and who do not have a family history of schizophrenia. We focus on the NMDAR receptor given that…Identify MicroRNAs expressed above or below threshold in schizophrenic subjects in comparison to those with out schizophrenia.

*Microarray*

To analyze the gene expression levels of the miRNA I want to do a microarray analysis using the produce developed by Shingara et. al. for specifically assaying microRNA, using RNA samples take from tissues sample of patients with schizophrenia and those without schizophrenia 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. Probes can normally be ordered for the a company, but the process entails isolating the target DNA sequence and replicating in and pipetting each particular well of the microarray (which is fairly mechanized) which 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 NMDAR receptor. One sample must come form a “normal population” without schizophrenia (including family history) and another sample from individuals with schizophrenia.

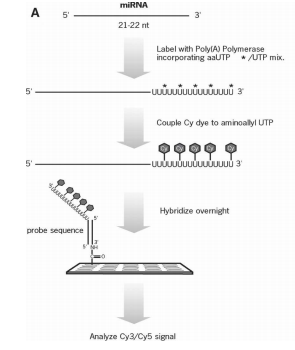


Figure 3: Have the Initial miRNA sequence without any attachment but with the addition of a Poly (A) Polymerase with get the attachment of a poly U tail. To this tail is where are fluorescent probes will attach before they are hybridized to a glass slide and analyzed. (Taken for Shingara et. al.)

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 miRNA 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 stop and the miRNAs will be recovered by the glass fiber filtering method used above.

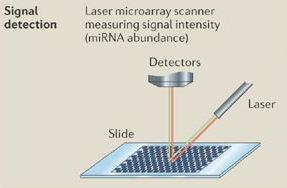


Figure 4: Image of a laser scanning a particular well in a microarray present on a glass slide and the fluorescent signal being picked up by the detector. Microarray can contain dozens of wells with multiple copies of target DNA sequences. (Taken for Clarin 2006)

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 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 expression of MIR141. Given that we see no fluorescence.

**Discussion**

If everything goes well there should be a confirmation of abnormal expression levels in our schizophrenia cohort of miR-132 and other microRNAs in the tissue samples taken form the NMDAR region.

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.

Although I factored in a family history of schizophrenia, individuals in the “control” cohort may have incidences of family mental disorders that share the same abnormal regulation in the same microRNAs. In particular it was noted in Miller et. al. that bipolar disorder shares the same abnormalities in miR-132 as schizophrenia. If the purpose of this new direction is to create a improved diagnostic method for schizophrenia there would need to be more than just miR-132 being dysregulated for this to be an effective diagnostic mechanism. There need to be a focus on a group of microRNA that are schizophrenia related and dysregulation in similar way (up or down regulated) in all tissue samples.

Another direction for this research to go in is to look at performed gene modifications in model organisms and observing behavioral changes. Such as changed the effect of the target genes on the organism or administering antipsychotics to model organism and seeing how that effects their gene expression and behavior.

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