

Tayab Waseem

Will mutations in the *nmr-1* Gene Modify Ethanol Responses in *C. elegans*

Alcoholism is a problem that affects over 140 million people worldwide. Alcohol misuse is associated with more than 100,000 deaths annually in the United States alone. It is associated with many social and legal problems, acts of violence, and accidents (Alcoholism Statistics). Alcoholism is among the most common psychiatric disorders in the general population (NIAAA). Susceptibility to alcoholism has a significant genetic component, shown by multiple experiments. By analyzing the differences in people's genes, we can find clues as to why there is such a difference in the human population for ethanol sensitivity and alcoholism. Because you can't change a gene in humans, the model organism *C. elegans* is used.

Ethanol is the main psychoactive constituent in alcoholic drinks and affects the body in many ways. The NMDA (N-methyl-D-aspartate) receptor (NMDAR) is a major target of alcohol (ethanol) in the brain and has been implicated in ethanol-associated phenotypes such as tolerance, dependence, withdrawal, craving, and relapse (Wang). Many experiments have been done that show that ethanol acutely inhibits NMDA activated ion currents (Lovinger). It has already been tested that over time the sensitivity of NMDAR to ethanol decreases over time (Grover). Due to the fact that everyone has variations in their genes and thus different alcohol tolerance levels; we can find that if someone has a mutated version of a gene whether or not they can be at a higher risk for alcoholism. Knowing if variation in the NMDAR gene can change ethanol sensitivity might enable you to know if someone is more at risk of developing alcoholism and thus we can take precautions to minimize this risk. *nmr-1* which encodes for an NMDA type glutamate receptor affects the duration of forward movement which is important for

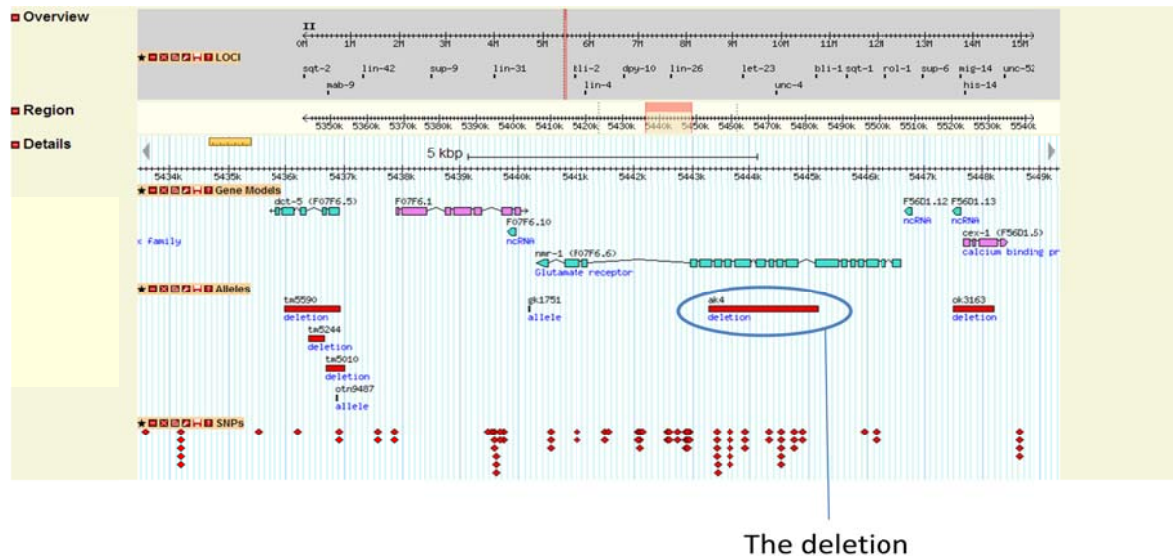
when the *C. elegans* is in search of food. (Brockie). If *nmr-1* does turn out to be important in the effects of ethanol then we can possibly start to better understand other effects of alcohol such as tolerance and withdrawal.

At the molecular level function of proteins, such as NMDAR is conserved among species and therefore we may learn something about human alcohol response by conducting experiments in organisms other than humans to see how modifying certain genes will affect the body's response to ethanol. The organism that we are using to better understand ethanol effects on humans is the worm *C. elegans*. The same concentrations of ethanol that produce intoxication in humans have the same neurodepressive effects on behavior in *C. elegans* (A central role of the BK potassium channel in behavioral responses to ethanol in *C. elegans* (Davies).

It has been observed in *C. elegans* that if tissue alcohol concentration is kept constant then there are behavioral adaptations that are observed within that time period alone. The *C. elegans* is comparatively less impaired after thirty minutes even though the tissue ethanol level is the same as it was twenty minutes prior. An increase in movement is correlated with a decrease in intoxication. As the worms develop tolerance they are better able to move, therefore an increase in speed is observed. The acute response to ethanol in *C. elegans* is what is going to be tested. The acute level of response is the response that occurs within thirty minutes of ethanol administration. The acute tolerance represents the plasticity of the nervous system that allows the nervous system to undergo an adaptation in the presence of ethanol.

The experiment will be done using a strain of wild-type *C. elegans* as well as a mutated strain. The mutated *C. elegans* will have a mutation at the *nmr-1* gene, specifically the mutation will consist of a deletion called *ak4*. 1880 base pairs will be deleted (see below). The mutants

have already been created and can be ordered from the stock center. The mutation in this strain has already been characterized, however in order to make sure that you are in fact using the mutated version of the gene PCR can be run to make sure there is actually a mutation. During PCR we are going to look to see that the 1880 bases between the flanking sequence “ttccagtcgagttgaatcccatat” and “gactccaattcgttcacattatca” have been deleted.



(<http://www.wormbase.org>)

The wild-type and *nmr-1* (*ak4*) mutant *C. elegans* will first be observed and recorded using the software ImagePro, and their rate of movement will be recorded. Once this data is collected the *C. elegans* will be placed onto agar plates that have ethanol concentration values of 100,200,300,400,and500 mM. The *C. elegans* will be observed and data will be recorded for 50 minutes using the Imagepro software.

The data from the movement of the wild-type *C. elegans* will be compared to the data from the mutant strain to determine if the mutated gene has an effect on the acute tolerance of the

C. elegans. To determine if there was an affect the rate and quality of behaviors will be compared. In order to minimize the error in the experiment the environment that the experiment is going to be conducted in will be kept constant. Also the frequency of direction changes can be observed with the collected data. Which we predict may be important because *nmr-1* is known to affect direction changes (Brockle).

Previous data suggests that humans that are less sensitive to alcohol or develop tolerance to ethanol faster are much more likely to become alcoholics (Schuckit). If the data shows that with the mutant gene the acute ethanol response is larger, then we can determine that those people with a mutated NMDAR gene may be at a higher risk of developing alcoholism.

Works Cited

1. Alcoholism Statistics <http://www.alcoholaddiction.info/alcoholism-statistics.htm>.
2. National Institute on Alcohol Abuse and Alcoholism Publications Distribution Center. <http://pubs.niaaa.nih.gov/publications/AA76/AA76.htm>. P.O. Box 10686, Rockville, MD 20849-0686
3. Schuckit, M.A. (2002). Vulnerability factors for alcoholism. In Neuropsychopharmacology: https://www.acnp.org/Docs/G5/CH98_1399-1412.pdf
4. Ron D, Wang J. The NMDA Receptor and Alcohol Addiction. In: Van Dongen AM, editor. Biology of the NMDA Receptor. Boca Raton (FL): CRC Press; 2009. Chapter 4. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK5284/>
5. Lovinger DM, White G, Weight FF. Ethanol inhibits NMDA-activated ion current in hippocampus neurons. Science. 1989. PMID: 2467382
6. Grover CA, Frye GD, Griffith WH. Acute tolerance to ethanol inhibition of NMDA-mediated EPSPs in the CA1 region of the rat hippocampus. 1994. PMID: 7913393
7. Brockie PJ, Mellem JE. The C. elegans glutamate receptor subunit NMR-1 is required for slow NMDA-activated currents that regulate reversal frequency during locomotion. Neuron. 2001. PMID: 11545720
8. Davies AG. A central role of the BK potassium channel in behavioral responses to ethanol in C. elegans. Cell 2003. PMID: 14675531
10. Penelope Brockle. The C. elegans Glutamate Receptor Subunit NMR-1Is Required for Slow NMDA-Activated Currents that Regulate Reversal Frequency during Locomotion. Neuron. 2001. <http://cds.unibas.ch/~hills/BrockieetalHillsNMDA.pdf>