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# Effects of Ethanol on Myelin Genes in the Prefrontal Cortex in Mice: A Search for a Regulatory Network

## INTRODUCTION

Alcoholism is a major health problem especially in the Western world and an understanding of the disease at the molecular level can lead to treatments in the future. The changes in gene expression in acute and chronic alcoholics are beginning to be uncovered with the help of genomics tools including DNA microarrays. DNA microarrays allow for a non-biased approach to monitoring gene expression for thousands of genes.

The mesolimbic dopamine system is the major reward system in the brain and is most targeted by alcohol. Major brain areas in this pathway include the ventral tegmental area, the nucleus accumbens, and the prefrontal cortex. The prefrontal cortex is the main cognitive processing area in the brain; it serves executive functions and has important roles in memory. This particular area has been found to display altered expression of a subset of mRNA's coding for myelin proteins in alcohol studies in both humans and mice (Lewohl et al., Kerns et al.). Contrasting results have been found: in human chronic alcohol studies, a subset of myelin genes were found to be downregulated (Lewohl et al.), while in acute mice studies a subset of myelin genes were found to be upregulated (Kerns et al.). In the study done by Lewohl et al., the genes altered included myelin-associated glycoprotein (MAG), myelin and T-cell differentiation protein (MAL), and apolipoprotein (ApoD)- all of which were downregulated. Other myelin related genes, including myelin-oligodendrocyte glycoprotein (MOG) showed no changes in gene expression. In mice studies, myelin genes found to be altered included myelin basic protein (Mbp), proteolipid protein (PLP), and myelin-associated oligodendrocytic basic protein (MOBP) (Kerns et al). The myelin gene expression results from acute ethanol in the inbred B6 and D2 mice from the Kerns et al. study differed which suggests a genetic component.

Neuropathological studies have found a reduced volume of the frontal lobes in chronic alcoholics which is mainly due to shrinkage of the cerebral white matter (Kril et al.). It has been suggested that changes in expression of myelin related genes may aid in the plasticity of the prefrontal cortex and the behavioral changes related to chronic alcohol use such as tolerance and addiction. Also disruption of these genes may aid in the shrinkage of the cerebral white matter

found in chronic alcoholics. Neuroimaging studies have discovered that myelin loss can be reversible (Shear et al.). Myelin genes are of particular interest because of their possible role in plasticity and their altered expression in the important cognitive center of the brain, the prefrontal cortex. This result suggests that there may be a region-specific regulatory mechanism for myelin genes. The differences between acute and chronic ethanol on myelin gene expression suggests that myelin genes may play an important role in the progressive development of ethanol behavior changes leading to alcoholism.

The molecular mechanism of how ethanol affects a subset of myelin related genes, specifically in the prefrontal cortex, still is yet to be determined. Major myelin-related transcription factors MyT1 and Puralpha were found not to be consistently altered in human alcoholics and so a more complicated regulation of these genes likely exists (Lewohl et al.). By using bioinformatics tools, data from various studies will be clustered and analyzed to find the relationship between myelin and myelin-related genes and see if any patterns can be found that hint at a common molecular mechanism.

## METHODS

Data will be pooled from various studies of alcohol on different strains of mice. Included in this study will be DBA/2J (D2) and C57BL/6J (B6), two inbred mouse strains that exhibit contrasting drinking behaviors. Other data will come from studies on ISS (inbred short sleep) and ILS (inbred long sleep) mice. Also data from studies on effects of Naltrexone on the myelin genes will be included. Naltrexone is a non-selective opioid antagonist and modifies ethanol induced changes in gene expression. Recent microarray analysis shows that Naltrexone prevents the upregulation of myelin genes after acute ethanol administration in mice (Kerns et. al., paper in progress). In all studies only information from the prefrontal cortex will be studied. The main goals of the research will be to 1) Compare basal expression in myelin genes between different strains of mice (B6 and D2, ISS and ILS), 2) compare alcohol induced changes in myelin and myelin-related genes in the PFC compared to controls (saline administered) in all mice strains and 3) compare expression of myelin and myelin-related genes in mice exposed to Naltrexone. The data will be analyzed using TMEV. Programs utilized in TMEV will include MCL (hierarchical clustering) and k- means clustering which are algorithms that find genes with similar expression profiles and groups them together into clusters. Multiple exploratory data

analysis will be used since different analysis can reveal different aspects of the data (Leung Y.F., Cavalieri D.). The program EASE (Expression Analysis Systematic Explorer) will further analyze the data by looking at over-represented functional categories of genes in the network. Ingenuity Pathway Analysis will help to identify biological pathways that are relevant to the genes of interest. The data will be analyzed using WebQTL which will link gene expression with behavioral data. Important specific genes found in the study will be further confirmed by real time PCR.

## POSSIBLE RESULTS AND THEIR IMPLICATIONS

A possible result of the study would be the discovery of the relation of myelin genes to one another and their regulation in a gene network. With a better understanding of what the regulation of myelin gene expression in the prefrontal cortex may involve, the next step would be to use cultured oligodendrocyte cells and silence the gene(s) believed to be involved in myelin regulation. This could be done by using a certain drug or RNA interference (RNAi). RNAi will specifically bind to the target mRNA, produce doubled stranded RNA, and then be degraded by the cell. This would silence the target gene(s) of the network and further confirm the genes responsible for the regulation of the subset of myelin genes. Further studies may include investigating how the regulation of myelin genes changes with varying doses of alcohol over time, and if these changes can be reversible.

## REFERENCES

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