The Influence of DRD2 Polymorphism Exon 8 C/T (rs6276) on Manifestations of Delirium Tremens & Alcohol Withdrawal Seizures

Naomi Schneider
University of New Hampshire, Durham

Follow this and additional works at: https://scholars.unh.edu/honors

Part of the Genomics Commons, Medical Genetics Commons, and the Nervous System Diseases Commons

Recommended Citation
Schneider, Naomi, "The Influence of DRD2 Polymorphism Exon 8 C/T (rs6276) on Manifestations of Delirium Tremens & Alcohol Withdrawal Seizures" (2024). Honors Theses and Capstones. 861.
https://scholars.unh.edu/honors/861

This Senior Honors Thesis is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Honors Theses and Capstones by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact Scholarly.Communication@unh.edu.
Abstract

This study explores the correlation between the DRD2 Polymorphism exon 8 C/T (rs6276) and manifestations of delirium tremens (DT). DT is a condition that is clinically diagnosed utilizing two characteristic symptom manifestations: the presence of delirium and severe alcohol withdrawal. It is not entirely understood why DT can occur in some patients, but evidence has suggested that genetic predisposition can play a role. Utilizing the National Institutes of Health (NIH) All of Us Research database and performing a secondary analysis of existing genomic data, this candidate gene association study aims to determine the genotype frequencies within three cohorts: a healthy control group (n = 554), an alcohol-dependent group (n = 577), and the delirium tremens case group (n = 350). This study is seeking to replicate and confirm findings from a previous study by Grzywacz et al. (2012) that utilized a smaller and less diverse sample population. Basic statistical analysis methods are utilized to validate the findings within this study. This study was able to reproduce the findings from the Grzywacz et al. (2012) study, in which the authors found the T/T genotype to be a positive predictive factor for the presence or lack of seizures in alcohol withdrawal. The C/T genotype may be a protective factor for alcohol dependence. The findings from this study will accelerate future research that can utilize traditional epidemiological methods to confirm the correlation.

Keywords: dopamine receptor, alcohol withdrawal-induced convulsions, alcohol dependence, genomics, candidate gene association study
Delirium Tremens

Delirium tremens (DT) is a condition that ranks as one of the most severe forms of alcohol withdrawal and is clinically diagnosed by two distinct aspects: the presence of delirium and severe alcohol withdrawal (Grover & Ghosh, 2018). Delirium involves a rapid, fluctuating onset and course of symptoms, including a notable change in level of consciousness, cognition, psychomotor activity, and sleep-wake cycle. In the case of delirium tremens, the cause can be attributed to sudden cessation of heavy and prolonged consumption of alcohol and can have several distinctive associated symptoms: autonomic hyperactivity, hand tremors, nausea, transient hallucinations, increased psychomotor activity/agitation, and generalized seizures. Alcohol withdrawal-induced seizures are classified as generalized tonic-clonic convulsions that generally occur within 12 to 48 hours after the patient’s last alcoholic beverage but can develop as quickly as two hours following the last consumption (Hoffman & Weinhouse, 2024). Delirium tremens typically develops 48 to 72 hours after the cessation of heavy and prolonged alcohol use. It is a short-lasting condition with an average duration of three to four days but can last up to eight days. Other symptoms associated with delirium tremens are tachycardia, hypertension, hyperthermia, and diaphoresis.

Typically, all patients who develop delirium tremens experience minor withdrawal symptoms before severe withdrawal. Symptoms of mild alcohol withdrawal can include insomnia, tremulousness, mild anxiety, gastrointestinal upset, anorexia, headache, diaphoresis, and palpitations. Delirium tremens is also associated with significantly elevated cardiac indices, oxygen delivery, and oxygen consumption (Hoffman & Weinhouse, 2024). Life-threatening fluid and electrolyte balances can also occur as a result of severe alcohol withdrawal. If a patient with delirium tremens is left untreated, hyperthermia, cardiac arrhythmias, and complications of
withdrawal-induced seizures can occur, potentially resulting in death. Current rates of mortality range from 1 to 4% in patients with this condition, which can be further reduced by effective and timely medical intervention. This figure has fallen from the 37% mortality rate reported in the early 20th century through earlier diagnosis, improvements in treatment and pharmacologic therapies, and improved treatment of comorbid illnesses (Hoffman & Weinhouse, 2024).

Few studies have been completed regarding the prevalence of delirium tremens in the general population. Studies from Germany and Finland have shown the prevalence rate to be 0.7% and 0.2%, respectively (Grover & Ghosh, 2018). In patients with alcohol use disorder (AUD), the occurrence of delirium tremens tends to be higher, with studies reporting 5 to 12% prevalence in alcohol-dependent individuals receiving treatment. There are an estimated eight million alcohol-dependent individuals living in the United States alone, with approximately 500,000 cases of severe alcohol withdrawal requiring pharmacologic intervention each year. A further smaller subset of those individuals requiring medical intervention will develop delirium tremens. Prevalence of this condition increases with the severity of alcohol dependence in patients with alcohol use disorder. According to Hoffman & Weinhouse (2024), risk factors involved in the development of DT include the following:

- Age greater than 65;
- Concurrent illness, comorbid medical or surgical conditions (especially traumatic brain injury);
- Physiologic dependence on gamma-aminobutyric (GABA)-ergic agents (e.g., benzodiazepine);
- Long duration of heavy and regular alcohol consumption;
- Numerous prior episodes of alcohol withdrawal;
• History of alcohol withdrawal delirium, alcohol withdrawal-induced seizures, or delirium tremens;
• Significant symptoms and signs of alcohol withdrawal in the presence of an elevated blood alcohol concentration;
• A longer period since the last drink (i.e., patients who present with alcohol withdrawal more than two days after their last drink are more likely to experience DT than those who present within two days);
• Seizures during the current withdrawal period;
• Marked autonomic hyperactivity on presentation.

While it is not entirely understood why certain individuals experience more severe withdrawal symptoms and others do not, there is evidence that suggests that genetic predisposition plays a role. Few candidate gene studies have somewhat consistently reported associations between glutamate- and dopamine neurotransmission-related genes and the development of DT.

The pathophysiology of DT involves an understanding of how alcohol, specifically ethanol, affects the central nervous system (CNS). While acute alcohol consumption produces CNS depression because of increased GABAergic neurotransmission and reduced glutamatergic activity, chronic heavy alcohol use causes a down regulation of Gamma-Amino Butyric Acid (GABA) and up-regulation of the glutamate (NMDA receptor) neurotransmission (Grover & Ghosh, 2018). When a patient withdraws after alcohol use cessation, the neurotransmitter imbalance reveals itself, and the unopposed glutamate activity leads to excitotoxicity. This neuronal excitation is caused by calcium influx and binding to glycine receptors on the NMDA complex, which results from glutamate binding to the NMDA receptor. Ethanol inhibits glutamate-induced excitation, which prevents this calcium influx and subsequent withdrawal
symptomatology (Hoffman & Weinhouse, 2024). Dopamine is also involved in both alcohol dependency and the manifestations of withdrawal, with increases in dopamine occurring during alcohol withdrawal contributing to hyperarousal. DT has also been conjectured to involve a process called “kindling,” which is defined by sensitization and neuronal excitability enhancement of the nervous system that occurs after repeated episodes of withdrawal. It has been found that kindling could explain greater excitotoxicity which is required for patients to reach severe alcohol withdrawal. As previously mentioned, genetic associations have been made that buttress the point of vulnerability for excitotoxicity for the pathogenesis of DT, specifically involving the glutamate neurotransmission gene (Hoffman & Weinhouse, 2024).

**Literature Review**

**Methods**

To conduct a comprehensive literature review on the topic of genomic and genetic associations with delirium tremens and alcohol withdrawal-induced seizures, the following databases were utilized through EBSCOhost: Academic Search Alumni Edition, Academic Search Complete, Academic Search Premier, Academic Search Ultimate, APA PsycArticles, APA PsycInfo, APA PsycTests, Applied Science & Technology Abstracts (H.W. Wilson), Cochrane Central Register of Controlled Trials, Cochrane Clinical Answers, Cochrane Database of Systematic Reviews, Education Source, Health Source - Consumer Edition, Health Source: Nursing/Academic Edition, MEDLINE, and eBook Open Access (OA) Collection. Google Scholar was also utilized in this comprehensive literature review. The terms, ‘alcohol withdrawal’, ‘withdrawal-induced*’, ‘delirium tremens’, ‘genome wide association’, ‘polymorphism’, ‘gene*’, ‘ethanol withdrawal’, ‘genom*’, and ‘variant’ were used in the search. Articles in this review needed to be (1) focused on delirium tremens or alcohol withdrawal-
induced seizures, (2) available as full text in English, (3) focused on discovering genetic associations with delirium tremens or alcohol withdrawal-induced seizures.

This literature review did not exclude articles based on published date. Articles that focused solely on genetic associations with alcohol dependence were excluded. Article titles and their abstracts were reviewed for verification of these inclusion criteria. If inclusion criteria requirements were met or unclear, the full text was read, and if the article’s full text did not meet these requirements, they were excluded. Additional articles were found by searching references in the manuscript, utilizing the snowball method. 20 articles were found utilizing the inclusion and exclusion criteria within each database. The findings from each article can be found in Appendix A.

Findings

There are few genotype association studies that have been published thus far that have found direct associations between delirium tremens and specific genetic polymorphisms, and even fewer that have replicated results in multiple study populations. The candidate genes involved in dopamine (dopamine receptor D2, COMT gene, solute carrier protein [SLC6A3]) and glutamate transmission (glutamate ionotropic receptor kainite 3, NMDA glutamate receptor gene), as well as neuropeptide (cholecystokinin gene) and cannabinoid (CNR1 gene) receptor genes, have been shown to be involved in the pathogenesis of DT in multiple studies. However, these genes have also been found to be associated with alcohol dependence, which also has a significant genetic predisposition aspect. Due to this, it is unknown whether there is a direct causation effect between these candidate genes and DT, or if their association is spurious, as all patients who develop DT also experience some degree of alcohol dependence (Malhotra et al., 2018, pp. 137-138). Most studies that have found associations with these candidate genes have
specifically requested that further research be done in alternative sample populations to reconfirm results and potentially prove causation effects. It is also essential that further genetic association studies have a case-control design to improve the reliability and validity of results; this has been emphasized in multiple research studies’ limitations.

After reviewing the selected studies, it was determined that there were three genes/polymorphisms of interest that have evidence of replicated findings. The neuropeptide Y (NPY) gene, the dopamine transporter (DAT) gene, and a specific polymorphism located in the dopamine receptor D2 (DRD2) gene called “rs6276”. Due to the limitations of this study and the All of Us genomic database, the polymorphism DRD2 rs6276 was chosen as the novel focus. Previous research has shown correlations between this polymorphism, alcohol dependence, and the presence of alcohol withdrawal seizures. Specifically, two articles included in this literature review have discovered these correlations: Grzywacz et al. (2012) and Karpyak et al. (2010).

In an article published by Grzywacz et al. (2012), the DRD2 polymorphism in exon 8 C/T (known as A/G in these studies due to them being published before the recent genomic assembly change in 2013) is described as a substitution polymorphism that affects gene expression and has previously been associated with more frequent relapse in alcohol-dependent individuals. The results of this study state that:

The [C/C] genotype in exon 8 [C/T] polymorphism seems to be a positive predictive factor for the presence or the lack of seizures in alcohol withdrawal syndrome. The [C/T] genotype is possibly a protective factor for this [alcohol-dependent] phenotype (p.1130, para. 5).
The correlation between specific genotypes and the presence of alcohol withdrawal seizures can be replicated within this study utilizing a larger, more diverse population to potentially establish a causative relationship.

An article published by Karpyak et al. (2010) also studied the correlation between polymorphism rs6276, alcohol withdrawal seizures, and DT. They discovered that the combination of at least one DRD2 rs6276 G allele with another polymorphism located in the SLC6A4 gene is associated with decreased odds of DT in alcohol-dependent participants. While this study focused on the correlation between DT and the interaction effect of the DRD2 and SLC6A4 genes, it does not discount the fact that the rs6276 polymorphism is found to play a role in the expression of the alcohol withdrawal seizure phenotype. Both studies called for future researchers to study the effects of the DRD2 (rs6276) polymorphism on the presence of delirium tremens in a larger, more diverse population.

The correlational effect of rs6276 on DT in All of Us participants will be explored in this genotype association study, and reconfirmation of previous findings will be attempted across a broad and diverse population. To further reduce mortality rates and improve health outcomes in patients experiencing DT, it is important to understand the full extent of pathophysiology, risk factors, and potential genetic predispositions. If this is accomplished, primary prevention efforts can be aimed toward populations who are at a higher risk of developing severe alcohol withdrawal, and treatment therapies for those who do develop DT can be further improved upon. With the knowledge and understanding associated with decades of genetic research regarding DT, the present study aims to reconfirm previous research findings involving a polymorphism located in the DRD2 gene; specifically, the DRD2 Polymorphism exon 8 C/T (rs6276).
National Institutes of Health (NIH) All of Us Research Hub

The National Institutes of Health (NIH) has developed a robust research program in the United States that is working to accelerate medical and health research and improve individualized prevention and treatment methods for patients. The *All of Us Research Program* aims to gather data from one million or more people who are living in the U.S. and make one of the largest, richest biomedical datasets broadly available and secure for researchers to access. They emphasize diversity and inclusion within their program, stating that health care is more effective when people from all backgrounds are included in health research; expressing a commitment to recruiting a participant pool with members of groups that are historically left out of past research.

*All of Us* offers participants that voluntarily provide biosamples, such as blood or saliva, information about their genetic ancestry, risk factors for certain hereditary diseases, and their body’s reaction to various medicines. Participants who go to an *All of Us* partner center will be physically measured and can provide blood, saliva, or urine samples. Those participants will also receive a one-time compensation of USD 25 in the form of cash, gift card, or electronic voucher for their time. Participants are required to answer basic health surveys and share electronic health records to provide further medical and demographic information. Those with wearable devices can also connect them to the database so researchers can access the data collected by each participant’s device. As of May 8th, 2024, *All of Us* has collected 565,000 biosamples from fully enrolled participants, and more than 800,000 participants have completed the consent process (National Institutes of Health, 2023).

The *All of Us* Researcher Workbench is not accessible to the public, however, it can be accessed by researchers at academic, not-for-profit, and healthcare organizations. These
researchers must apply for the *All of Us* Data Use and Registration Agreement and complete a comprehensive training program that reviews the data use policies, procedures, and guidelines that must be followed. The organization that each researcher belongs to must approve their access to the program. Two training modules are required for researchers who wish to obtain access to “controlled tier” data, which includes genomic data and additional demographic details. The first module is named “*All of Us* Responsible Conduct of Research Training” and gives the researcher access to participants’ electronic health records, survey responses, physical measurements, and wearable device data. The second module is named “*All of Us* Controlled Tier Training” and gives researchers access to participants’ genomic data within the controlled tier. Once the training modules are completed, researchers must sign the “Data User Code of Conduct”, which is a document outlining the agreement between the researcher and the program which establishes that the data will not be misused or misrepresented in a way that violates the privacy of participants. After these steps are completed, the full participant database is then made available to the researcher to use appropriately for the purposes of health research.

A major benefit of utilizing the NIH’s *All of Us* Research Program is that the researcher does not have to acquire IRB approval to perform a secondary analysis of the existing data within the database. *All of Us* is a single IRB, and the organization is charged with reviewing protocols, obtaining informed consent, and providing participant-facing materials for the program (National Institutes of Health, 2021). Due to this, the lengthy process of applying to the IRB for performing human subject-based research is no longer necessary. This allows for even fewer barriers to entry for researchers to take part in revolutionary and groundbreaking health research.
Study Design

To explore the correlation between the DRD2 polymorphism and manifestations of delirium tremens, previous studies have found success in performing a candidate gene association study. A genetic association study examines a single polymorphism or a set of polymorphisms near a single gene; in this case, DRD2 polymorphism (rs6276). The goal of genetic association studies is to establish statistical significance regarding correlations between polymorphisms and specific phenotypes or disease states so that genetic risk factors can be identified and studied further using traditional epidemiological methods (Lunetta, 2008). While this study focuses on a specific candidate gene, some genetic association studies take a “genome-wide” approach, with hundreds of thousands to millions of polymorphisms being studied, which has only become feasible in the past two decades by rapidly improving genotyping technologies. According to Lunetta (2008), there are two main pitfalls to genetic association studies that must be addressed: multiple testing and population stratification.

Multiple testing occurs in studies that report the results of multiple, often correlated phenotypes or the results of multiple genetic models or covariate adjustments (Lunetta, 2008). For example, in a genome-wide association study that tests many hypotheses (in this case, SNPs), a type I error may occur if the type I error rate, $\alpha$, is not adjusted downward to not reject too many hypotheses. Traditionally, when only 1 or a few tests are performed, the $\alpha$ value is set to 0.05; in this study, only one single nucleotide polymorphism is being tested against three participant groups, which minimizes the effect of multiplicity significantly.

Population stratification occurs when participants in a study differ by ethnic background or another potentially confounding factor for the studied phenotype. For example, if a study’s case population has a greater proportion of Hispanic participants than that of the control
population, spurious associations may be made if both the phenotype and genotype distribution differ among the subpopulations (Lunetta, 2008). Due to the limitations of this study and the *All of Us* research database, not all confounding factors can be accounted for. However, a properly designed candidate gene association study of demographically matched individuals will have a much lower probability of population stratification at a single locus than that of stratification at any locus across the genome in a genome-wide association study (Anderson et al., 2010). In this study, each cohort of participants has been demographically matched according to age, race, ethnicity, and sex at birth.

**Figure 1**

*Race Distribution by Cohort*

**Figure 2**

*Age Distribution by Cohort*
This candidate gene association study involves the DRD2 gene, specifically the exon 8 C/T polymorphism (rs6276). The expression of each genotype was determined within three demographically matched case and control groups: the healthy control group (n = 554), the alcohol-dependent group (n = 577), and the delirium tremens group (n = 350). For the healthy control group, inclusion criteria required the presence of electronic health records and short-read whole genome sequence data and required that participants are not currently prescribed medications or treatment for alcohol use disorders. Exclusion criteria for this healthy control group included various demographic data to best match participants in the delirium tremens case group, history of delirium tremens or alcohol withdrawal-induced convulsions found in electronic health records, prescriptions or treatment for epilepsy/seizures found in electronic health records, indicating “self” or “skip” on a survey question asking who in their family has had epilepsy or seizures, history or observations of seizures or epilepsy in their medical records, or history of medications or treatment for alcohol dependence. These criteria yielded a cohort size of 554 participants.

For the alcohol-dependent cohort, inclusion criteria required participants to have electronic health record and short-read whole genome sequence data available, have
“uncomplicated alcohol withdrawal” as a listed condition in medical history, have observations of alcoholism, alcohol abuse, or unhealthy alcohol drinking behavior listed in the electronic health record, and have answered “daily or almost daily” on a survey question that asks how often in the past month do they have six or more drinks containing alcohol on one occasion. Exclusion criteria included various demographic data to best match participants in the delirium tremens case group, history of delirium tremens or alcohol withdrawal-induced convulsions found in electronic health records, prescriptions or treatment for epilepsy/seizures found in electronic health records, indicating “self” or “skip” on a survey question asking who in their family has had epilepsy or seizures, history or observations of seizures or epilepsy in their medical records, or history of medications case group, or treatment for alcohol dependence. These criteria yielded a cohort size of 577 participants.

For the delirium tremens case group, inclusion criteria required participants to have electronic health records, short-read genome sequence data, and documented history of delirium tremens or alcohol-withdrawal convulsions. Exclusion criteria required the participants in this group to have clear, documented demographic data; excluding “skip”, “none indicated”, and “prefer not to answer” answers in demographics surveys. These criteria yielded a cohort size of 350 participants.

Data Collection Methods

The All of Us Research database contains both short- and long-read whole genome sequence data from participants who have submitted a biosample to the program. This data can be accessed in various formats through the Jupyter Notebook which is located on the cloud analysis servers. A Jupyter Notebook is created for each cohort, and blocks of code input can be utilized to manipulate and view the variant data in various formats. The researcher can choose
the main coding language used within the notebook, and for the purposes of this study, Python was chosen as the primary language. Genomic extraction will occur once the notebook is created, and the dataset is analyzed. Once this process is completed, the data is available to manipulate. The resulting variant-call format (VCF) files can be accessed within the Jupyter Notebook directory as well.

Using Jupyter’s “playground mode” allows for the manipulation of data through the appropriate input of code. In this study, a Hail Matrix table that allows visualization of genotype distribution across a cohort was created. The following code is used to create the Hail Matrix table:

```python
workspace_bucket = os.environ['WORKSPACE_BUCKET']

vcf_dir = os.environ['DATASET_***_VCF_DIR']

hail_matrix_table_gcs = f'{workspace_bucket}/dataset_***.mt'

if not os.exists(hail_matrix_table_gcs):
    print('writing matrix table')

    hl.import_vcf(f'{vcf_dir}/*.vcf.gz', force_bgz=True, array_elements+required=False).write(hail_matrix_table_gcs)
```

Then, the following code must be inputted to allow the Jupyter Notebook to “read” the Hail Matrix table:

```python
mt_*** = hl.read_matrix_table(hail_matrix_table_gcs)
```
Defining a genomic region of interest based on the chromosomal position of the SNP is necessary to isolate the specific rs6276 variant. The code required to complete this step is as follows:

```python
test_intervals=['chr11:113410675-113410676']

mt = hl.filter_intervals(mt,
    [hl.parse_locus_interval(x,) For x in test_intervals])
```

Finally, to visualize the Hail Matrix Table data and subsequently gather the genotype frequencies of each cohort participant:

```python
mt = mt.annotate_entries(n_alt = mt.GT.n_alt_alleles())

mt.n.alt.show(n_cols = *)
```

The resulting Hail Matrix Table contains a list of participant IDs and the corresponding number of “alternate alleles” present within the chromosomal position that was chosen. With C being the reference allele and T being the alternate allele for this SNP, if the participant has a “1” listed in the table (indicating the presence of one alternate allele), they have the C/T genotype. If the participant has a “2” listed in the table (indicating the presence of two alternate alleles), they have the T/T genotype. If the participant has a “0” listed in the table (indicating the lack of alternate alleles), they have the C/C genotype. If a participant has the C/T or T/T genotype, then it is established that they have the variant genotype; the C/C genotype does not contain the variant allele/SNP.
Statistical Analysis Methods

Multiple analysis tests can be performed depending on the scope of the study and its correlating dataset to determine the statistical significance and reliability of the data collected. Pearson’s Chi-Square test, also known as the “goodness of fit” test, can analyze the genotype frequency tables that were created during the data collection process. This hypothesis testing procedure measures how well the observed data distribution fits with the expected distribution if the variables are independent. The following formula is used to calculate the “chi-squared” or $X^2$ value:

$$x^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Within this formula, $O_i$ is the observed count in each cell within the contingency table, and $E_i$ is the expected count in each cell. The resulting value can then be utilized to calculate a p-value, which is used to measure the data’s statistical significance. A p-value of 0.05 or lower is generally considered statistically significant (Anderson et al., 2011). The p-value can be calculated in Microsoft Excel utilizing the function “=CHISQ.DIST.RT($X^2$, degrees of freedom)”.

The second statistical analysis test that can be performed is called an odds ratio (OR) test, which is a common association analysis test in case-control studies. This test calculates the odds of disease occurrence with and without a specific genotype when compared to a control group without disease occurrence. 95% confidence intervals can also be calculated, and all calculated results can be visualized in a forest plot. The formulas to calculate the odds ratio and confidence intervals are further discussed in an article published by Anderson et al. (2011) which outlines basic statistical analysis methods in genetic case-control studies.
Results

When analyzing the C/C genotype in Table 1 across all three cohorts, the highest correlation was found when comparing alcohol-dependent participants to a healthy control group. According to the calculated OR value found in Figure 4, participants with this genotype have approximately 5.1 times increased odds of developing alcohol dependence than those with the C/T or T/T genotype. The p-value of $2.90404 \times 10^{-33}$ supports this association as well. Increased odds of DT with the C/C genotype that can be seen within the odds ratio chart are likely due to alcohol dependence being a confounding factor in patients with DT.

When analyzing the C/T genotype in Table 2 across all three cohorts, the highest correlation was found when comparing alcohol-dependent participants to a healthy control group. According to the calculated OR value found in Figure 4, participants with this genotype have approximately 0.51 times decreased odds of developing alcohol dependence than those with the C/C or T/T genotype. This indicates that the C/T genotype might be a protective factor against developing alcohol dependence. The p-value of $1.83331 \times 10^{-7}$ supports this association as well. There is some association between alcohol dependence and those with DT, with 1.14 times increased odds of developing DT with the C/T genotype compared to alcohol-dependent control participants.

When analyzing the T/T genotype in Table 3 across all three cohorts, the highest correlation was found when comparing participants with DT to an alcohol-dependent control group. According to the calculated OR value found in Figure 4, participants with this genotype have approximately 1.18 times increased odds of developing DT than those with the C/C or C/T genotype. This suggests that the T/T genotype is a predictive factor for the presence of DT or alcohol withdrawal seizures. The p-value of $3.06343 \times 10^{-61}$ supports this association as well.
Table 1

*The Frequency and Distribution of Delirium Tremens and Alcohol Dependence Phenotypes in DRD2 exon 8 C/C Genotype Carriers*

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups Studied</th>
<th>C/C (+)</th>
<th>C/C (-)</th>
<th>Total Group Participants</th>
<th>Total Study Participants</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N₁</td>
<td>% of N₁ + N₂</td>
<td>N₂</td>
<td>% of N₁ + N₂</td>
<td>(N₁+N₂)</td>
</tr>
<tr>
<td>DT Relative to Healthy Control</td>
<td>Healthy Control</td>
<td>92</td>
<td>16.6</td>
<td>462</td>
<td>83.4</td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>156</td>
<td>44.6</td>
<td>194</td>
<td>55.4</td>
<td>350</td>
</tr>
<tr>
<td>Alcohol Dependence Relative to Healthy Control</td>
<td>Healthy Control</td>
<td>92</td>
<td>16.6</td>
<td>462</td>
<td>83.4</td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Alcohol Dependent</td>
<td>291</td>
<td>50.4</td>
<td>286</td>
<td>49.6</td>
<td>577</td>
</tr>
<tr>
<td>DT Relative to Alcohol Dependent Control</td>
<td>Alcohol Dependent</td>
<td>291</td>
<td>50.4</td>
<td>286</td>
<td>49.6</td>
<td>577</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>156</td>
<td>44.6</td>
<td>194</td>
<td>55.4</td>
<td>350</td>
</tr>
</tbody>
</table>
### Table 2

**The Frequency and Distribution of Delirium Tremens and Alcohol Dependence Phenotypes in DRD2 exon 8 C/T Genotype Carriers**

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups Studied</th>
<th>C/T (+)</th>
<th>C/T (-)</th>
<th>Total Group Participants (N₁+N₂)</th>
<th>Total Study Participants</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>DT Relative to Healthy Control</td>
<td>Healthy Control</td>
<td></td>
<td></td>
<td>226</td>
<td></td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>101</td>
<td>328</td>
<td>59.2</td>
<td></td>
<td>904</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.8</td>
<td>71.2</td>
<td></td>
<td></td>
<td>346</td>
</tr>
<tr>
<td>Alcohol Dependence Relative to Healthy Control</td>
<td>Healthy Control</td>
<td></td>
<td></td>
<td>226</td>
<td></td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Alcohol Dependent</td>
<td>151</td>
<td>426</td>
<td>73.8</td>
<td></td>
<td>1131</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.2</td>
<td>73.8</td>
<td></td>
<td></td>
<td>331</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E-07</td>
</tr>
<tr>
<td>DT Relative to Alcohol Dependent Control</td>
<td>Alcohol Dependent</td>
<td>151</td>
<td>426</td>
<td>73.8</td>
<td></td>
<td>577</td>
</tr>
<tr>
<td></td>
<td></td>
<td>26.2</td>
<td>73.8</td>
<td></td>
<td></td>
<td>927</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2656</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>101</td>
<td>249</td>
<td>71.2</td>
<td></td>
<td>350</td>
</tr>
<tr>
<td></td>
<td></td>
<td>28.8</td>
<td>71.2</td>
<td></td>
<td></td>
<td>307</td>
</tr>
</tbody>
</table>
Table 3

*The Frequency and Distribution of Delirium Tremens and Alcohol Dependence Phenotypes in DRD2 exon 8 T/T Genotype Carriers*

<table>
<thead>
<tr>
<th>Study</th>
<th>Groups Studied</th>
<th>T/T (+)</th>
<th>T/T (-)</th>
<th>Total Group Participants (N₁+N₂)</th>
<th>Total Study Participants</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N₁</td>
<td>N₂</td>
<td>% of N₁+N₂</td>
<td>% of N₁+N₂</td>
<td></td>
</tr>
<tr>
<td>DT Relative to Healthy Control</td>
<td>Healthy Control</td>
<td>236</td>
<td>318</td>
<td>42.6</td>
<td>57.4</td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>93</td>
<td>257</td>
<td>26.6</td>
<td>73.4</td>
<td>350</td>
</tr>
<tr>
<td>Alcohol Dependence Relative to Healthy Control</td>
<td>Healthy Control</td>
<td>236</td>
<td>318</td>
<td>42.6</td>
<td>57.4</td>
<td>554</td>
</tr>
<tr>
<td></td>
<td>Alcohol Dependent</td>
<td>135</td>
<td>442</td>
<td>23.4</td>
<td>76.6</td>
<td>577</td>
</tr>
<tr>
<td>DT Relative to Alcohol Dependent Control</td>
<td>Alcohol Dependent</td>
<td>135</td>
<td>442</td>
<td>23.4</td>
<td>76.6</td>
<td>577</td>
</tr>
<tr>
<td></td>
<td>Delirium Tremens</td>
<td>93</td>
<td>257</td>
<td>26.6</td>
<td>73.4</td>
<td>350</td>
</tr>
</tbody>
</table>
When referencing previous research regarding the DRD2 polymorphism exon 8 C/T (rs6276), it should be noted that authors refer to the reference allele as A, and the alternate allele as G. This is due to the GRCh37 to GRCh38 assembly change in 2013. The NIH acknowledged the change in December 2013, which caused the location of the SNP to change as chromosomal positions were shifted. Alleles found in research that utilized the GRCh37 assembly must be flipped to match those within the GRCh38 assembly. In previous studies that utilize the GRCh37 assembly, the C allele is called a G allele, and the T allele is called an A allele (Cariaso, 2019).

This study utilized three cohorts that are substantially larger and more diverse than past studies that explore the association between rs6276 and DT. The results from this study suggest that the T/T genotype is a positive predictive factor for the presence or lack of seizures in alcohol withdrawal, which reconfirms the findings from Grzywacz et al. (2012) which utilized a cohort of 213 alcohol-dependent participants.
The author states that the T/T genotype is more common in participants who have experienced severe alcohol withdrawal. As this study was able to replicate the findings from the original study by Grzywacz et al. (2012) utilizing a larger, more diverse sample as well as a control cohort, the association between the T/T genotype and higher odds of developing alcohol withdrawal seizures can be confirmed. Further research utilizing traditional epidemiological methods that account for population stratification should be completed to verify the correlation between this genotype and DT.

Considering the findings regarding the C/T genotype being a protective factor against developing alcohol dependence, it could be inferred that the presence of this SNP may protect a participant from developing alcohol dependence; if alcohol withdrawal does occur within this population, DT is more likely to occur when compared to other genotypes in alcohol-dependent participants. These findings conflict with the findings from research mentioned by Grzywacz et al. (2012):

According to Finckh et al. [12] A/A genotype carriers in exon 8 DRD2 polymorphism predispose to severe forms of alcohol addiction. The results reported by Samochowiec et al. [43] indicate to the association between the DRD2 gene exon 8 polymorphism and severe AD (p. 1131, paras. 2-3).

Further research regarding the role of rs6276 in manifestations of alcohol dependence needs to be explored. While this was not the focus of this study, these results are valuable to future research regarding the DRD2 Polymorphism exon 8 C/T (rs6276).
Limitations

Limitations for this study may include but are not limited to the inability to account for all modulating factors, the inability to complete further statistical analysis to adjust for population stratification (e.g., logistic regression), and the inability to account for phenotypes of delirium tremens, alcohol withdrawal seizures, or alcohol dependence within “healthy” control group participants. Environmental and other genetic factors that are involved in the metabolism and pharmacodynamic effects of alcohol may play a role in modulation, which can skew the results of this study. Logistic regression and other statistical analyses in future research should be completed to ensure that population stratification is not affecting the results of this study. Due to the limitations of the All of Us research database, only so much could be done to ensure that the cohorts did not contain duplicate participants or participants from the same family, which may also affect results.

Conclusion

This study has explored the correlation between the DRD2 Polymorphism exon 8 C/T (rs6276) and manifestations of DT and alcohol withdrawal seizures. Understanding a disease's genetic component can revolutionize the way providers treat and educate their patients and how we utilize primary prevention methods. The future of genomics is here, and it can be seen through companies that offer at-home genetic testing and basic health risk assessments that are discovered through each biosample.

DT is a life-threatening condition that affects those who are at their most vulnerable during alcohol withdrawal, and it is not even entirely understood why it occurs in some patients and not others. If it is discovered that an individual’s genetics predispose them to severe alcohol
withdrawal manifestations, this may be a significant deterrent from developing severe alcohol dependence which may lead to those manifestations. Considering it is already understood that alcohol dependence can be hereditary in nature, this fact alone has given people a motive to avoid alcohol as a risk factor for potentially developing dependence.

The findings in this study will accelerate future research that can utilize traditional epidemiological methods to confirm or rebuke the correlation between the rs6276 polymorphism and DT. The All of Us Research Program is a unique effort on the NIH’s behalf to promote genomics research and further understand genetic predisposition behind various disease processes. This program can and should be utilized by future researchers to explore other genetic associations with DT, alcohol withdrawal seizures, and alcohol dependence.
## Appendix A

### Comprehensive Literature Review Findings

<table>
<thead>
<tr>
<th>Name of Article</th>
<th>Authors/Date</th>
<th>Polymorphism(s)/Genes studied</th>
<th>Background</th>
<th>Methods</th>
<th>Findings</th>
<th>DOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allelic Association of a Dopamine Transporter Gene Polymorphism in Alcohol Dependence with Withdrawal Seizures or Delirium</td>
<td>Thomas Sander, Helmut Harms, Jan Podschus, Ulrich Finckh, Bernd Nickel, Arndt Rolfs, Hans Rommelspacher, and Lutz G. Schmidt (1997)</td>
<td>DAT1 – dopamine transporter gene</td>
<td>The role of the dopamine transporter in terminating dopaminergic activity in synaptic neurotransmission suggests that variants of the dopamine transporter gene (DAT1) might contribute to individual differences in vulnerability to addictive behavior.</td>
<td>A population-based association study investigated whether variants of DAT1 confer susceptibility to alcohol dependence in 293 alcoholic subjects.</td>
<td>Analyzing a VNTR polymorphism in the 3’ untranslated region of DAT1, a significantly increased prevalence of the nine-repeat allele was found in 93 alcoholic subjects displaying withdrawal-induced seizures/delirium. Data provides evidence that a major genetic determinant of DAT1 influences vulnerability to severe withdrawal symptoms.</td>
<td><a href="https://doi.org/10.1016/S0000-63223(96)00044-3">https://doi.org/10.1016/S0000-63223(96)00044-3</a></td>
</tr>
<tr>
<td>Modification of Alcohol Withdrawal by the A9 Allele of the Dopamine Transporter Gene</td>
<td>Lutz G. Schmidt, M.D., Helmut Harms, Ph.D., Silke Kuhn, M.A., Hans Rommelspacher, M.D., and Thomas Sander, M.D. (1997)</td>
<td>DAT1 (SLC6A3) - dopamine transporter gene</td>
<td>Recent studies have found a significantly higher prevalence of the nine-repeat (A9) allele of a variable-number tandem repeat in the 3’ untranslated region of the DAT1 gene in alcoholics who reported histories of</td>
<td>In 48 chronically alcohol-intoxicated subjects, withdrawal symptoms were examined and the presence of a variable-number tandem repeat in the 3’ untranslated region of the DAT1 gene was</td>
<td>The A9 allele of the dopamine transporter gene is associated with more severe effects of alcohol withdrawals, possibly because of the modifications of the brain’s capacity to compensate for long-</td>
<td><a href="https://doi.org/10.1176/ajp.155.4.474">https://doi.org/10.1176/ajp.155.4.474</a></td>
</tr>
</tbody>
</table>
alcohol withdrawal-induced seizures or delirium tremens than in comparison subjects.

Upregulation of the glutamergic neurotransmission from chronic ethanol intoxication may cause a hyperexcitable state during alcohol withdrawal that may cause alcohol withdrawal-induced seizures and delirium tremens.

Evaluates the association between a history of alcohol withdrawal-induced seizures and delirium tremens and mGluR7 (Tyr433Phe) and mGluR8 (C2756T) metabotropic glutamate receptor polymorphisms.

No significant associations were obtained between both receptor polymorphism and alcohol withdrawal-induced seizures/delirium tremens.

Polymorphism of the Neuropeptide Y Gene: An Association Study with Alcohol Withdrawal

Takehito Okubo & Shoji Harada (2001)  
Neuropeptide Y, a 36-amino acid neurotransmitter, is widely distributed in the nervous system and is one of the most abundant peptides within the body. Thought to modulate fundamental human functions, recent studies revealed that NPY-

Neuropeptide Y, a 36-amino acid neurotransmitter, is widely distributed in the nervous system and is one of the most abundant peptides within the body. Thought to modulate fundamental human functions, recent studies revealed that NPY-

No significant associations were obtained between both receptor polymorphism and alcohol withdrawal-induced seizures/delirium tremens.

https://doi.org/10.1093/alcalc/acb174
deficient mice consumed large amounts of ethanol compared to wild-type and NPY-overexpressed mice. Seizures, 49 with hallucinations) and 98 Japanese male control subjects. This mutation itself is not involved with the etiology of seizure in alcohol withdrawal symptoms, but it might be associated with other unknown functional mutations. Further study of the relationship with the NPY receptors is necessary to elucidate the etiology and symptomatology of alcoholism.

| Alcoholism-related phenotypes and genetic variants of the CB1 receptor | U. W. Preuss, G. Koller, P. Zill, B. Bondy, and M. Soyka (2003) | CNR1 polymorphism – biallelic cannabinoid receptor gene Tested the potential influence of a biallelic cannabinoid receptor gene (CNR1) polymorphism on severe alcohol withdrawal syndromes. | Neurotransmitter release of GABAergic and glutamatergic neurons may be significantly influenced by cannabinoid CB1 receptors. GABA and glutamate have been reported to be involved in the pathogenesis of severe alcohol withdrawal-induced seizures and delirium tremens (DT). After correcting for multiple testing, no association of the A-OR G-allele of CNR1 G1359A polymorphism with a history of alcohol withdrawal-induced seizures was detected. In addition, no significant relationships with other alcoholism-related phenotypes were found. Failed to confirm an earlier report of a potential role of a | https://doi.org/10.1007/s00406-003-0440-7 |
| Pharmacogenetic studies of alcohol self-administration and withdrawal | John C. Crabbe & Tamara J. Phillips (2003) | Comprehensive literature review of all previously studied targeted genes. Those involving withdrawal severity include:

- **Dopamine D1 receptor gene**
- **GABA receptor subunit gene**
- **PKC epsilon gene**
- **Protein tyrosine kinase Fyn gene**
- **Adenosine A2A receptor gene**

This literature review examines research that has used rodent genetic animal models to identify genetic influences on two broadly defined alcohol-related traits: alcohol self-administration and alcohol withdrawal. Although this review does not detail the results of research using human populations, some of the relevant correspondences between human and animal studies for the genetics of alcohol self-administration and withdrawal are discussed.

- **CNR1 polymorphism in the pathogenesis of DT.**
- **Using polymerase chain reaction.**

<table>
<thead>
<tr>
<th>Phenotypes</th>
<th>Genes</th>
<th>Genes</th>
<th>Phenotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol abuse disorders are influenced by multiple factors, both genetic and environmental. Advances in genetic technologies have focused attention in recent years on genetic risk factors, although a long and rich history of genetic investigation exists. Genes, and behavioral or physiological traits have been identified that appear useful in the prediction of alcoholism risk.</td>
<td><strong>Dopamine D1 receptor gene</strong></td>
<td>increased withdrawal severity in knockout mice.</td>
<td><strong>GABA receptor subunit gene</strong></td>
</tr>
<tr>
<td>PKC epsilon gene</td>
<td></td>
<td>PKC epsilon gene</td>
<td>Prokaryotic activated inwardly rectifying potassium channel (GIRK)-2 gene</td>
</tr>
<tr>
<td><strong>G-protein activated inwardly rectifying potassium channel (GIRK)-2 gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Adenosine A2A receptor gene</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### The A9 Allele of the Dopamine Transporter Gene Is Associated with Delirium Tremens and Alcohol-Withdrawal Seizure

<table>
<thead>
<tr>
<th>Study</th>
<th>Authors</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Philip Gorwood, Frédéric Limosin, Philippe Batel, Michel Hamon, Jean Ades, and Claudette Boni (2003)</td>
<td>DAT – dopamine transporter gene plays a key role in homeostatic regulation of dopaminergic neurotransmission and could thus be involved in the variability of two severe alcohol withdrawal symptoms: alcohol withdrawal-induced seizures (AWS) and delirium tremens (DT). Performed DNA amplification by polymerase chain reaction of the 40-base pair VNTR polymorphism in the 3’ untranslated region of the DAT gene, with genotyping being carried out without knowledge of clinical status of patients. Sample population included 120 male patients with alcohol dependence and 65 control subjects without psychiatric or addictive morbidity matched for gender and French origin. The association of the A9 allele of the DAT gene with severe alcohol withdrawal complications is confirmed with this study’s results. The effect of the A9 allele appeared to be particularly, or even exclusively, observed in subsets of older patients with a longer history of alcohol dependence.</td>
<td></td>
</tr>
</tbody>
</table>

### Ionotropic glutamate receptor gene GRIK3Ser310Ala (T928G) - functional polymorphism in delirium tremens in alcoholics

<table>
<thead>
<tr>
<th>Study</th>
<th>Authors</th>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.W. Preuss, P. Zill, G. Koller, B. Bondy, V. Hesselbrock and M. Soyka (2006)</td>
<td>The upregulation of glutamatergic neurotransmission resulting from chronic ethanol intoxication may cause a hyperexcitable state during alcohol withdrawal. Evaluates association between a history of alcohol withdrawal-induced seizures and delirium tremens and a functional polymorphism. While a significant relationship between history of delirium tremens and the Ser310 allele was detected, no significant results were obtained for alcohol.</td>
<td></td>
</tr>
</tbody>
</table>

https://doi.org/10.1016/S0006-3223(02)01440-3

https://doi.org/10.1038/sj.tpj.6500343
withdrawals, which may lead to seizures and delirium tremens.

Severe alcohol withdrawal syndrome is characterized by several symptoms that can include withdrawal seizures, which are likely to be caused by a disinhibition of the gamma aminobutyric acid (GABA) system (Schmidt & Sander 2000). GABA is the major inhibitory neurotransmitter in the central nervous system and mediates many of the behavioral effects of ethanol consumption and alcohol withdrawal.

The polymorphism GABA<sub>B</sub>R1 T1974C [rs29230] of the GABA<sub>B</sub> receptor gene is not associated with the diagnosis of alcoholism or alcohol withdrawal seizures.

| Michael Köhnke, Sandra Schick, Ulrich Lutz, Annette Köhnke, Reinhard Vonthein, Werner Kolb, and Anil Batra (2006) | GABA<sub>B</sub>R1 T1974C [rs29230] of the GABA<sub>B</sub> receptor gene was assessed by using a previously described PCR-based restriction fragment length polymorphism assay. There were three groups of participants: alcoholics with a history of AWS (n = 233) well-characterized alcoholics compared to 309 control subjects, all German descent. | Genomic data was extracted from participants using standard procedures and the exonic GABA<sub>B</sub>R1 T1974C [rs29230] polymorphism of GABA<sub>B</sub> receptor gene was assessed by using a previously described PCR-based restriction fragment length polymorphism assay. There were three groups of participants: alcoholics with a history of AWS (n = 233) well-characterized alcoholics compared to 309 control subjects, all German descent. | No significant differences in allelic or genotypic distribution of GABA<sub>B</sub>R1 T1974C [rs29230] or allele C carrier status were found between the two groups. It also needs to be critically mentioned that the frequency of the CC genotype was low in all groups (<3%), making a statistical statement on the influence of this genotype more difficult due to a resulting |

Further investigation and confirmation of Ser310 and delirium tremens’ relationship is warranted.

https://doi.org/10.1111/j.1369-1600.2006.00013.x
symptoms (Fadda et al. 1985; Buck 1996).

alcoholics with only mild withdrawal symptoms (n = 97), and a healthy control group (n = 101).

Neuropeptide Y Receptor Genes Are Associated with Alcohol Dependence, Alcohol Withdrawal Phenotypes, and Cocaine Dependence


NPY – neuropeptide Y NPY1R, NPY2R & NPY5R - neuropeptide Y receptor genes

Several lines of evidence in both human and animal studies suggest that variation in neuropeptide Y (NPY) or its receptor genes (NPY1R, NPY2R, and NPY5R) is associated with alcohol dependence and alcohol withdrawal symptoms.

39 single nucleotide polymorphisms (SNPs) were genotyped across NPY and its three receptor genes in 1,923 subjects from 219 multiplex alcoholic families of European American descent. Family based association analyses were performed to test whether variation in these genes is associated with alcohol dependence. Secondary analyses evaluated whether there was an association of these SNPs with symptoms of alcohol withdrawal and cocaine use.

SNPs in NPY2R provided significant evidence of association with alcohol dependence and alcohol withdrawal symptoms. SNPs in NPY5R demonstrate a significant association with alcohol withdrawal-induced seizures. These results indicate that sequence variations in the NPY receptor genes are associated with alcohol dependence and withdrawal symptoms.

https://doi.org/10.1111/j.1530-0277.2008.00790.x
| Genotype polymorphisms related to delirium tremens, a systematic review | Barbara C. van Munster, Johanna C. Korevaar, Sophia E. de Rooij, Marcel Levi, Aeilko and H. Zwijnderman (2009) | A systematic literature review identified 30 polymorphisms, located in 19 different genes. Positive associations were found in three different candidate genes involved in dopamine transmission, one gene involved in the glutamate pathway, one neuropeptide gene, and one cannabinoid gene. This review examined research articles that analyzed the association between a genetic polymorphism and delirium tremens without other alcohol withdrawal symptoms. | Found 25 studies that include 30 polymorphisms located in 19 different genes. Positive associations were found in three different candidate genes involved in dopamine transmission, one gene involved in the glutamate pathway, one neuropeptide gene, and one cannabinoid gene. Two candidate genes involved in the dopamine transmission, dopamine receptor D3 and solute carrier family 6 were each associated with delirium tremens in two different study populations. The findings regarding two candidate genes involved in dopamine transmission, DRD3 and SL6A3, were confirmed in a second sample population, supporting a possible role of this neurotransmitter system in delirium tremens. | https://doi.org/10.1111/j.1530-0277.2006.00294.x |
**Interaction of SLC6A4 and DRD2 polymorphisms with a history of delirium tremens**

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Victor M. Karpyak, Joanna M. Biernacka, Mark W. Vander Weg, Susanna R. Stevens, Julia M. Cunningham, David A. Mrazek and John L. Black (2010)</td>
<td>Investigated the effects of 12 previously reported genetic variations in two groups of alcohol-dependent individuals with history of withdrawal, separated by the presence or absence of alcohol withdrawal-induced seizures and delirium tremens. Results reveal decreased likelihood of delirium tremens in alcoholics that carry the DRD2 rs6276 G allele and SLC6A4 LL genotype. Provides the first evidence to implicate the interaction between serotonin and dopamine neurotransmission in etiology of delirium tremens. Replication and confirmation are needed.</td>
</tr>
</tbody>
</table>

**SLC6A4 promoter polymorphism (5-HTTLPR)**

Several genetic polymorphisms have previously been reported to be associated with alcohol withdrawal-induced seizures and delirium tremens. Results found significant interaction effects of the SLC6A4 promoter polymorphism and DRD2 exon 8 single nucleotide polymorphism rs6276 on alcohol withdrawal-induced seizures and/or delirium tremens. Results reveal decreased likelihood of delirium tremens in alcoholics that carry the DRD2 rs6276 G allele and SLC6A4 LL genotype. Provides the first evidence to implicate the interaction between serotonin and dopamine neurotransmission in etiology of delirium tremens. Replication and confirmation are needed.

**DRD2 exon 8 single nucleotide polymorphism (rs6276)**

Several genetic polymorphisms have previously been reported to be associated with alcohol withdrawal-induced seizures and delirium tremens. Results found significant interaction effects of the SLC6A4 promoter polymorphism and DRD2 exon 8 single nucleotide polymorphism rs6276 on alcohol withdrawal-induced seizures and/or delirium tremens. Results reveal decreased likelihood of delirium tremens in alcoholics that carry the DRD2 rs6276 G allele and SLC6A4 LL genotype. Provides the first evidence to implicate the interaction between serotonin and dopamine neurotransmission in etiology of delirium tremens. Replication and confirmation are needed.
The Roles of Mpdz and Kcnj9 in Ethanol Withdrawal: Pleiotropic Effects and Potential Mechanisms

| The Roles of Mpdz and Kcnj9 in Ethanol Withdrawal: Pleiotropic Effects and Potential Mechanisms | Lauren C. Milner (2010) | MPDZ – Multiple PDZ (PSD-95/Disc-large/Zonula occludens-1) domain protein | Quantitative trait loci (QTL) mapping in mice has allowed for the identification of specific chromosomal segments harboring gene(s), called quantitative trait genes (QTGs), that influence ethanol withdrawal convulsion severity; and fine-mapping efforts have reduced these chromosomal regions to sizes amenable to discrete gene identification. | Our laboratory employed QTL mapping strategies to identify two high-quality QTGs for ethanol withdrawal severity on mouse chromosomes 1 and 4. Mpdz, located on chromosome 4, encodes the multiple PDZ domain (MPDZ) protein, which is present throughout the central nervous system and functions to influence intracellular receptor signaling. Kcnj9, located on chromosome 1, encodes a subunit of G protein-coupled inwardly rectifying potassium (GIRK) channels, which are distributed throughout the central nervous system. The results obtained provide additional support for these genes’ effects on withdrawal convulsion severity, although gene effects on other ethanol withdrawal behaviors were not consistently observed. In addition, evidence for a functional interaction was observed between MPDZ and 5HT2C receptors in vitro and in vivo, but this association did not influence ethanol withdrawal severity, at least in the models tested. | https://doi.org/10.6083/M4JM27MQ |
Genome-wide association study identifies 5q21 and 9p24.1(KDM4C) loci associated with alcohol withdrawal symptoms

Ke-Sheng Wang, Xueleng Liu, Qunyuan Zhang, Long-Yang Wu, and Min Zeng (2011)

Several genome-wide association studies of alcohol dependence and alcohol-related phenotypes have been conducted; however, little is known about genetic variants influencing alcohol withdrawal symptoms. This study is the first genome-wide association study of alcohol withdrawal syndrome. Utilizing 461 cases of alcohol dependence with alcohol withdrawal symptoms from the COGA GWA study and Australian twin-family study of alcohol use disorder datasets, this study conducted a genome-wide association study to search for novel genetic factors affecting alcohol withdrawal symptoms. This study identified 51 single nucleotide polymorphisms (SNPs) associated with alcohol withdrawal symptoms at an allelic p value less than 10−4 in the COGA sample. Especially, one SNP (rs770182) at 5q21 near EFNA5 and three SNPs (rs10975990, rs10758821, and rs1407862) in KDM4C showed strong associations with alcohol withdrawal symptoms in the COGA sample. The associations of several genes/regions were replicated in the OZALC family data. 

Influence of DRD2 and ANKK1 polymorphisms on the manifestation of withdrawal syndrome symptoms in alcohol addiction

Anna Grzywacz, Andrzej Jasiewicz, Iwona Malecka, Aleksandra Suchanecka, Elzbieta Grochans, Beata Karakiewi

The identification of allelic variants associated with the development of addiction and alcohol dependence may be useful in A group of 213 patients with alcohol dependence were split into two subgroups based upon the DRD2 gene polymorphisms –141 C I/D (rs1799732) and exon 8 G/A (rs6276) – dopamine receptor 2 and ANKK1 gene polymorphism Taq1A. Results show significant associations between single nucleotide polymorphisms in exon 8 A/G in the DRD2 gene and alcohol

https://doi.org/10.1007/s00702-011-0729-z

https://doi.org/10.1016/S1734-1140(12)70909-X
Delirium Tremens Candidate Gene Association Study

Cz, Agnieszka Samochowiec, Przemysław Bienkowski, and Jerzy Samochowiec (2012) [rs1800497] - Ankyrin Repeat and Kinase Domain Containing 1 (ANKK1) gene polymorphisms and its association with the dopamine system. The study involved patients undergoing alcohol withdrawal therapy and aimed to identify candidate genes associated with delirium tremens.

Presence or absence of alcohol withdrawal-induced seizures and delirium tremens. Genotyping and statistical analysis were performed to determine associations between withdrawal symptoms and dopamine receptor 2 (DRD2) gene polymorphisms and ANKK1 gene polymorphisms. The A/A genotype of exon 8 A/G polymorphism seems to be a positive predictive factor for the presence or lack of seizures.

This data reconfirms that dopamine receptor 2 gene polymorphisms are associated with alcohol withdrawal syndrome.

An exploratory study of candidate gene(s) for Delirium Tremens: Adding the new cholinergic dimension to the conundrum


COMT gene

There are only a handful of genetic association studies for delirium tremens (DT) published so far. Lack of consistency amongst various previous studies proves to be an issue. Most candidate genes in these studies were also genetic association study with a case control design with 210 AD male subjects and 200 age-matched control subjects. Genomic DNA extraction and genotyping with statistical analysis

GABRA1 gene

T allele carrying status (GT + TT) [rs1824024] of muscarinic cholinergic receptor 2 (CHRM2) was found to be significantly associated with DT.

GABRA2 gene

When compared with the general population, this genetic polymorphi
found to be associated with alcohol dependence (AD), so their association with DT may be spurious. Barring one study of acetylcholine esterase, candidate genes involved in cholinergic transmission which is regarded as one of the potential pathophysiological contributors for the development of delirium was never investigated so far regarding DT.

| TSPO polymorphism in individuals with alcohol use disorder: Association with cholesterol levels and withdrawal severity | Corinde E. Wiers, Luana Martins De Carvalho, Colin A. Hodgkinson, Melanie Schwandt, Sung Won Kim, Nancy Diazgrandaos, Gene-Jack Wang, David Goldman, and Nora D. Volkow (2019) | TSPO polymorphism rs6971 – translocator protein | Evaluated whether plasma cholesterol and triglycerides are disrupted in alcohol use disorder and their association with rs6971 in participant s with alcohol use disorder (AUD) and a control group. | Results showed a significant effect of TSPO rs6971 on withdrawal scores, with higher CIWA scores in AA group compared with GG/AG group. Reports association between TSPO genotype and withdrawal severity but calls on further studies to demonstrate and

https://doi.org/10.1111/adb.12838
increases plasma high-density lipoproteins (HDLs), its effects on total cholesterol and triglycerides along with its relationship to TSPO genotype have not been assessed.

establish causality.

| Investigation of cytochrome p450 | Cytochrome p450 (CYPs): CYP1A2, CYP2D6, CYP2E1 and CYP3A4 gene expressions and polymorphisms in alcohol withdrawal | DNA and RNA isolation of blood samples from a sample of 50 alcoholic individuals at the beginning and end of alcohol dependence treatment, as well as a control group of 23 male smokers in the same age range. Gene expression was quantified by quantitative polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) technique was used for polymorphisms. No differences in expression levels of studied genes in patients before and after treatment and in control group were detected. A significant difference in the frequency of CYP1A2*1 F c.734C>A polymorphism was detected between patient and control group, indicating a possible role of this allele as a risk factor for alcohol dependence. |
| Nazife Taşçoğlu, Çetin Saatç, Rabia Emekli, Gulten Tuncel, Ertuğrul Esel, and Munis Dundar (2021) | Cytochromes 450 (CYPs) are an enzyme superfamily that are involved in the metabolism of various compounds in the body, including alcohol. Therefore, changes in the expression levels or structural changes in the protein caused by gene polymorphisms might affect alcohol tolerance, dependence, and withdrawal. | https://doi.org/10.5505/kpd.2021.60938 |
Elevated transferrin saturation in individuals with alcohol use disorder: Association with HFE polymorphism and alcohol withdrawal severity

<table>
<thead>
<tr>
<th>Authors</th>
<th>Study Design</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Danielle S. Kroll, Katherine L. McPherson, Peter Manza, Melanie L. Schwandt, Pei-Hong Shen, David Goldman, Nancy Diazgranados, Gene-Jack Wang, Corinde E. Wiers, and Nora D. Volkow (2021)</td>
<td>Conducted a cohort study to examine whether peripheral iron measures, genetic variation in HFE rs1799945 and their interaction differed between 594 inpatient participants with AUD detoxing and 472 healthy control subjects.</td>
<td>Within the AUD group, transferrin saturation significantly predicted withdrawal symptom severity. HFE rs1799945 minor allele carriers showed elevated transferrin saturation compared to non-carriers, both in AUD and control subjects. Within AUD cohort, HFA rs1799945 predicted CIWA withdrawal scores, and this relationship was significantly mediated by transferrin saturation.</td>
</tr>
</tbody>
</table>

Iron loading has been consistently reported in those with alcohol use disorder (AUD), but its effect on clinical course of disease is not yet fully understood. Approximately 50% of individuals with a history of excessive alcohol use will experience some withdrawal symptoms, while an estimated 2-8% will have delirium tremens, a more severe presentation of alcohol withdrawal with a high mortality rate when untreated. Iron overload has been consistently observed in patients with AUD, and it normalizes with detoxification. Elevated peripheral iron levels affect the clinical course of AUD including an association with liver damage, and there is some preclinical studies.
Evidence that iron loading affects withdrawal: one study in alcohol-dependent mice demonstrated that administration of iron chelators prior to detoxification, which normalizes iron levels, lowered alcohol withdrawal scores and mortality.

Genetic associations of severe alcohol withdrawal are of interest. Previous studies have revealed that the SORCS2 gene is associated with alcohol withdrawal, and the stress hormones and ethanol enhance the expression of this gene in vitro.

Cross-sectional study of 307 subjects with alcohol use disorder (AUD)/alcohol dependence (AD) was performed to determine associations between polymorphisms of MTNR1A (rs34532313) and MTNR1B (rs10830963) genes and alcohol withdrawal syndrome severity. Carriers of the GG genotype of the MTNR1B gene (rs10830963) experienced more symptoms of severe withdrawal. In general, it can be concluded that melatonin receptors are involved in the pathogenesis and severity of alcohol withdrawal syndrome (AWS).
References


