Polymorphisms in alcoholism

Bo Greg Hanson

UT EID: bgh555

Alcohol addiction affects numerous people all over the world. More than 50% of the adult American population drinks alcohol socially, but only 7% develop alcohol addiction or are considered dependent (Newman *et al.,* 2016). Alcoholism is known to run in families and it is estimated that 50–60% of dependency risk is genetically based (Sahni *et al.,* 2018). Rather than only one gene being solely responsible, the polygenic nature of addiction means that a culture of multiple genes contributes to alcohol dependence. As researchers hunt for the cause of these genetic variations, polymorphisms have emerged to be a common thread in all of them (Edenberg & Foroud, 2014). Polymorphisms such as single nucleotide polymorphisms (SNPs) and variable number tandem repeats (VNTRs) change the base pairs, or the number of base pair repeats within DNA sequences. Most polymorphisms are harmless but if they appear on certain genes or receptors, they can have a detrimental effect on their function. Two genes, GABA(a) and SLC6A4 have been linked to alcohol addiction and are vulnerable to polymorphisms (Edenberg & Foroud, 2014). For example, VNTRs in SLC6A4, which code for serotonin receptors, cause inadequate uptake of the neurotransmitter serotonin. Individuals affected by this VNTR need to consume more alcohol to get the same amount of serotonin absorption in their synapses (Sahni *et al.,* 2018). GABA(a) receptors are responsible for inhibition, which works in tandem with excitation like a tug of war in the brain to create spikes of electrical signals. SNPs in GABA(a) receptors decrease inhibition which in turn causes over-excitation in the neurons of those affected. (Boyd, Schacht, Prisciandaro, Voronin, & Anton, 2016). This over-excitation makes people prone to dependence as they are more highly stimulated by alcohol than others and the SNPs in GABA(a) receptors are responsible for this imbalance. Genetic polymorphisms, specifically SNPs and VNTRs, are the cause of variance in the GABA(a) and SLC6A4 genes found in alcoholism.

In 2014, a research study was conducted reviewing the genetic contributions to alcoholism. It concluded that alcohol dependency, although reliant on the environment, has a high affinity towards inheritance (Edenberg & Foroud, 2014). In order to separate the two factors, researchers looked at studies in adopted children to find distinctions between the effect of genetics and environment. In a primary study of 913 adopted young Swedish girls, the researchers found that the adoptees and their biological parents shared a similar risk of dependency as opposed to the adoptees and their adoptive parents (Edenberg & Foroud, 2014). The study compared and recorded the frequency of who developed alcoholism as adults. They found that the number of alcohol dependency cases in the daughters who had alcoholic mothers was three times more frequent than those who did not have alcoholic mothers. To pinpoint possible genetic contributions, the researchers also reviewed a study centered around the GABA(a) receptor genes in the prefrontal cortex that have a high sensitivity to ethanol due to SNPs in the alleles (Edenberg & Foroud, 2014). They found that individuals with one or more SNPs had a history of alcohol abuse in their family. The effect just one SNP can have on these GABA(a) receptors is a substantial indicator of how polymorphisms play a major role in genetic alcoholism.

The GABA(a) receptors in the brain are special because they are the receptors involved in synaptic inhibition (Newman *et al.,* 2016). The brain has both excitatory and inhibitory receptors that drive and mediate signals through action potentials which are spikes of current that stimulate neurons. Excitatory receptors elicit action potentials, and inhibitory receptors bring these action potentials back down after the flow of current peaks. Inhibitory synapses calm the neurons down allowing the brain to be balanced and not overstimulated. If these inhibitory GABA(a) receptors are not functioning properly, the brain will be overloaded with excitatory signals and cause

overstimulation because it cannot inhibit the neurons as fast as they are being excited (Boyd *et al.*, 2016). These GABA receptors are very important and subsequently an SNP causing the slightest malfunction could be critical.

In a preclinical study, researchers tested to see if mice with variant SNP GABA(a) receptors changed their sensitivity to alcohol and showed binge-like behavior on the part of the affected mice (Newman *et al.*, 2016). They used three types of mutant mice that had been selectively bred, along with wild-type mice to control for the differences. For three days prior to the test, the mice had unlimited access to food and water to acclimate them to their new environment before a sign stimulus was introduced. The test was four days long. During the first three days, the mice had their water replaced with 20% ethanol for two hours, and then back to water for twenty-two hours. On the last day, they got four hours of 20% ethanol. Directly after this period, they collected blood samples from the mice to see if they were binging on the alcohol; the parameters being a blood ethanol concentration (BEC) of more than 80 mg/dl. The results showed the mice with GABA(a) SNPs drank more volume of ethanol than the wild-type mice during the four-hour binge period. After running two-way ANOVA tests, statistically, the p-value was less than 0.001 ($p<0.001$). This presented a significant difference in drinking patterns from the mutant to wild-type mice (Newman *et al.*, 2016). The mutant mice that carried the variant SNP alleles were observed to binge drink significantly more than the other mice. This study provides evidence that polymorphisms contribute to alcoholism and that SNPs account for the variance in the GABA(a) receptors of the mutant mice.

Polymorphisms in GABA(a) receptors have drawn much attention in the genetic alcoholism field due to its strong correlation with addiction. Another study in 2016 specifically focused on the GABA(a) SNP named rs279858 in humans (Boyd *et al.*, 2016). Blood samples

were collected from the total of 63 subjects that were participating. These blood samples were then genotyped by the TaqMan nuclease assay which uses PCR (polymerase chain reaction) to find the region for the SNPs and their alleles. It uses primers from known controls The SNP rs279858 region has two alleles C and T which stand for cytosine and thymine respectively. Individuals can either be homozygous C, heterozygous, or homozygous for T in their base pairs. Everyone has this SNP region where there are variations in the nucleotides, but the alleles are what matters. Based on prior studies the researchers suspected the homozygous TT alleles to be associated with overstimulation to alcohol. The structure of the experiment had two different drinking sessions eight days apart from each other. To provide for the control, participants were randomly assigned placebo or alcoholic drinks on one of the two days. Subjects filled out a questionnaire on both days after finishing the placebo or reaching a BAC level of 0.02–0.03 g%. All the participants were breathalyzed regardless on both days and the placebo drinks also had ethanol flavor, so they could not taste or tell the difference between the alcoholic drinks. During the drinking session the participants filled out the survey and rated the stimulation they felt on a scale of 1-11. The subjects homozygous for the T allele consistently reported greater stimulation in their questionnaires. The difference between their response and those with CC and CT alleles was significant to a p-value of 0.082. This data suggested those with the rs279858 SNP TT alleles felt subjectively greater stimulation from the alcohol even though all the subjects were kept at the same BAC when not given the placebo (Boyd *et al.*, 2016). It showed the SNP had a noteworthy effect on the perceived stimulation felt by the participants and this polymorphism is a contributor to alcohol dependence.

When studying addiction influences, the pleasure-reward system in our brain is crucial in forming bad habits that start this cycle. None more so crucial than serotonin, an important

neurotransmitter in this process. A 2013 study zoomed in on SLC6A4, the serotonin transporter gene where, once again, polymorphisms in affected individuals were found and studied. The researchers looked at a VNTR polymorphism 5-HTTLPR which has been shown to cause slower and less efficient absorption of serotonin (Cao, Hudziak, & Li, 2013). This study aimed to determine if there is indeed a correlation between these polymorphisms and alcohol dependency in adults from different ethnicities. The scientists obtained 5-HTTLPR allelic frequencies for normal people in different ethnic backgrounds and in patients with alcohol abuse history. They took numbers from over 66 studies and well over 2,000 samples. They analyzed these frequencies to find a trend and see if they were associated with alcoholic patient data. In European populations, the frequency of variant 5-HTTLPR of was at an average of 57% but remained lower in Asian populations at 27%. This strongly correlated with the average frequencies of patients in alcohol rehab, Europeans at 55% and Asians having 25% frequency. The overall p-value from combining both populations was $p=0.037$ which proves a significant result and not one merely generated by chance or coincidence (Cao *et al.,* 2013). This study once again shows that polymorphisms, in this case, VNTRs, are a significant contributor to eliciting alcohol addiction.

With both SNPs and VNTRs in GABA(a) and SLC6A4 genes having a reputation for alcoholism, researchers naturally want to study the polymorphisms together in the same human subjects. In 2018 a research project combined SLC6A4 and GABA(a) polymorphic gene data to determine the association between them and alcohol dependence. In the SLC6A4 gene researchers specifically examined the notorious 5-HTTLPR VNTR (Sahni *et al.,* 2018). They genotyped healthy subjects and those diagnosed with alcohol dependence to collect data. Those with alcoholism had to provide statistics about how many drinks they have in one day and how

many years they have been alcohol dependent. The scientists then studied the different genotypes and deciphered the results to see if there is a correlation between alcoholism and these two polymorphic regions. They collected 5mL blood samples from 141 alcoholics and 110 healthy participants to use as a control. Using PCR designed with the select primers for both polymorphic regions they would amplify the target sequences and report allele frequencies among both groups of participants. For the VNTR in the 5-HTTLPR region, there are three possible genotypes LL, LS, and SS. The S allele is the shorter VNTR that has more deletions and is associated with reduced transcription in the SLC6A4 gene that causes slower, less effective uptake of serotonin in its receptors. The specific SS genotype had a higher frequency in the alcoholic subjects compared to the control subjects. What was even more significant however was that those with the SS genotype also consumed significantly higher amounts of drinks per day compared to other genotypes with a p-value < 0.0001. The GABA(a) SNP also has three different genotypes, CC, CT, and TT that showed similar results to the previous study mentioned in this paper. The TT genotype had a much higher rate of alcohol consumption per day and showed a longer duration of dependence among the alcoholic group. The differences once again showed a $p<0.0001$ for both factors which are highly significant (Sahni *et al.*, 2018). This provides more tangible evidence for polymorphisms in alcohol dependence.

In all these studies on genetic alcoholism, the common theme is polymorphisms. We see how specific genes contribute to risk factors in alcohol dependence and more importantly how SNPs and VNTRs are significantly associated with that risk (Edenberg & Foroud, 2014). Although the two genes GABA(a) and SLC6A4 act on different receptors and have contrasting roles and mechanisms, they both have polymorphic tendencies that manifest in affected individuals. In mice, it was found that higher levels of binge drinking were significantly

correlated with the mutant mice who had the GABA(a) SNPs (Edenberg & Foroud, 2014). In humans the allelic TT SNPs in GABA(a) receptors showed how this genotype has a higher sensitivity to alcohol and how individuals experience higher stimulation (Boyd *et al.,* 2016). Such a strong stimulation the first time someone drinks gives them a greater chance of repeating the behavior and subsequently needing more alcohol volume to achieve the same effects later in life (Newman *et al.,* 2016). This explains the long years of addiction and a higher rate of alcohol consumption found in those with the TT genotype. Just one SNP polymorphism can have these detrimental effects and the same goes for VNTRs in SLC6A4 (Cao *et al.,* 2013). The VNTR polymorphism 5-HTTLPR in the SLC6A4 gene showed a reduced efficiency in the absorption of serotonin due to a shorter number of repeats in this DNA sequence (Sahni *et al.,* 2018). Individuals with this VNTR consume more alcohol to achieve the same feeling of serotonin they crave. It explains why those affected have a higher average number of drinks per day. It gives insight into why some people are casual drinkers while others are compulsive drinkers (Boyd *et al.,* 2016). The importance of these polymorphisms cannot be overlooked. SNPs and VNTRs in GABA(a) and SLC6A4 genes not only increase the risk of addiction but also support to explain the phenomenon of genetic alcoholism.

Work Cited

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