An Insight into Addiction from a Genetics Perspective

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Abstract

CHRNA5 is a receptor subunit which mediates signal transmission at synapses. Defects in the gene, specifically single nucleotide polymorphisms (SNPs), have been associated with susceptibility to nicotine dependence. It is crucial to understand CHRNA5's expression in the development of therapies for individuals struggling with nicotine consumption. To date, the mechanism through which the decreased function of CHRNA5 increases nicotine intake is not well understood. As of now, there have been several GWAS studies that have evaluated the variants in CHRNA5 by utilizing statistics from different regions in the world. However, there has been minimal research done in other aspects, such as the effects of in utero nicotine exposure through maternal smoking. Maternal smoking during pregnancy exposes the developing fetus to nicotine and studies suggest that this is associated with risk factors in offspring including attention deficits, higher chance of respiratory problems, as well as a higher chance of dealing with nicotine dependency. Carriers of the risk variant may be at elevated risk of struggling with smoking cessation during pregnancy. The literature review and experimental proposal aim to contribute to the findings of the CHRNA5 gene, and specifically towards the effects of mother smokers with the risk variant on their offspring in terms of dealing with alcohol and nicotine addiction in adolescence.

The Phenotype

Although tobacco has been growing in the Americas for around 8,000 years, it was not up until around 2,000 years ago that indigenous people began chewing and smoking tobacco during cultural and religious ceremonies (Cancer Counsil NSW, n.d.). The spread of tobacco from the Americas around the world began shortly after the start of European colonization. In the 1600s, tobacco became widespread in Europe, and by the 1700s it was already established as a worldwide developed industry. Although risks associated with smoking were reported as early as the 1600s, an increase in medical reports confirming that cigarette smoking is dangerous to human health began only in the 1960s (Cancer Counsil NSW, n.d.). It is even more interesting to note that in the period between the 1930s and 1950s, advertisements in the United Stated attempted to convince citizens that doctors recommend cigarettes, especially with the famous campaign stating, "More Doctors Smoke Camels than any Other Cigarette" (Klara, 2015). Tobacco industries were thought to have manipulated scientific research efforts and marketing. For instance, people have always experienced coughing because of cigarette smoke. However, during that period, the ads suggested that it was not the cigarettes which were to blame for the coughs, but instead it was dust and germs (Klara, 2015). This brief history of tobacco and cigarettes demonstrates the long path it took for scientific research to confirm the negative risks of smoking and continues asking how much more the scientific community will learn in the future.

The predominant aspect that this paper will consider is the effect of smoking on addictive substances including nicotine and alcohol, especially in adolescents. Adolescents are of interest because this period in an individual's life is when there is an increased risk for experimentation with substance abuse. It is suggested that although starting to smoke in adolescence does not necessarily mean individuals will become smokers in adulthood, smoking as a teenager increases daily nicotine consumption and lowers the probability of quitting (O'Neill et al., 2018). Therefore, focusing on adolescents could give insight in efforts with smoking cessation at later parts in life.

Despite many research papers confirming the negative effects of cigarettes, people often still consider smoking as just a bad habit instead of an addictive substance which is difficult to quit. Nicotine has temporary pleasing effects on smokers, which is the reason why once someone finishes smoking a cigarette often wants to reach for another one (Mayo Clinic, 2022). Dopamine, which is one of the neurotransmitters released with the use of nicotine, causes feelings of pleasure and improved mood. Consequently, when an individual attempts to stop smoking, they might experience unpleasant feelings, such as strong cravings, anxiety, difficulty concentrating, depressed mood, frustration, which are all symptoms of nicotine withdrawal symptoms (Mayo Clinic, 2022). Accordingly, it is quite challenging for people that have smoked for most of their life to decide to quit, and many people require professional help.

For example, in my home country of North Macedonia, around 48.4% of the population are smokers. These are incredibly high numbers, and even though studies confirm that most smokers are aware of the risks that come with smoking, as well as the economic burden, addiction is an extremely strong tool that stops them from quitting (Analytica, 2020). Growing up in such an environment inspired me to learn more about the effects of smoking aside of my general interest in broadening the current literature review on the topic.

Furthermore, tobacco smoke can lead to a variety of health issues, including lung cancer, heart and circulatory system problems, diabetes, eye problems, infertility, tooth and gum disease and others (Mayo Clinic, 2022). Around 80% of lung cancer deaths are assumed to be a result from smoking (American Cancer Society, 2019). This paper, however, will focus on one specific condition, which is the effects of smoking during pregnancy. It is already known that women who smoke have a more difficult time becoming pregnant, and furthermore, women who smoke during pregnancy can cause tissue damage in the lung and brain of an unborn baby (CDC, 2020). Moreover, smoking mothers usually deliver their babies earlier, which comes with risk of disability, disease, and even death. Babies whose mothers are smokers could also have weaker lungs compared to other newborns, which can lead to many health problems in the future. The long extensive list of negative effects of mothers who smoke on the offspring is the reason why doctors always recommend for pregnant women to cease smoking during pregnancy. However, once again, even though women are aware of the consequences of smoking on their newborns, it is reported that ten percent of women in the United States smoke traditional cigarettes during pregnancy and fourteen percent smoke electronic cigarettes (Oncken et al., 2017). Current research should aim to find methods that will support mothers ceasing smoking for the benefit of their own newborns.

In order to contribute to the scientific conversation of the effects of smoking during pregnancy, this paper will look into the genetic aspect of nicotine dependency; which is associated with the reason some people struggle more than others with smoking cessation. Genetic factors influence how the receptors on brain's nerve cells respond to nicotine. So far, there has been significant research done on the harmful effects of cigarette smoking, however there is not as much research on the genetic background that makes some people more susceptible. There are many genes that are thought to affect the risk to nicotine dependency, however the one that will be discussed in this paper is CHRNA5. In its regular function, this gene controls the communication of our brain cells, or neurons, but when there are mutations in the gene, it can affect an individual's susceptibility to nicotine dependency and lung cancer.

This paper aims to contribute to the ongoing literature review by determining the impact of genetics on addiction. It is already known that CHRNA5 increases the susceptibility to nicotine dependency; however, there is very little research done on its effect on alcohol. Alcohol and tobacco use are highly correlated in humans, since smokers are more likely to drink heavily compared to non-smokers, and alcoholics have a higher probability of experiencing nicotine withdrawal symptoms compared to non-alcoholics (Wang et al., 2009). By narrowing the focus of this experiment to in utero nicotine exposure during pregnancy, and the genetic component behind, these findings can offer better assistance to women smokers and protect newborns from the negative consequences.

The Genotype

In its regular function, CHRNA5 is a nicotinic acetylcholine receptor, a member of ligand-gated ion channels that mediate fast signal transmis-

sion at synapses (Kniffin, 2012). However, single nucleotide polymorphism (SNP) variants in the gene are associated with an increased risk for tobacco dependence. The way that polymorphisms in this case work is that they alter the receptor composition and the binding of nicotine to the receptor. This, in fact, leads to chances in functional and behavioral outcomes (Chaity et al., 2022). Therefore, investigating into the CHRNA5 gene is of crucial interest in learning more about human nicotine dependency.

To date, there have been many human genome-wide association studies (GWAS's) which have helped in identifying the difference in SNP variants in the gene coding for the α5 subunit of nicotinic acetylcholine receptors (Lassi et al., 2016). These studies have contributed to determining the risks of nicotine dependency depending on the location where people live. In a research paper by Hong et al. (2020), the results indicated a significant association between the D398N variant and smoking. This SNP is extremely frequent in the population, being present in 35% of Europeans and 50% of Middle Eastern populations. These populations are at a higher risk of nicotine dependency and experience a more difficult time with smoking cessation (Forget B., 2018). For the purpose of this paper, we will only focus on the D398N variant, although there are many more that could also possibly offer insight into nicotine dependency.

Moreover, increased research on this topic began using animal models such as mice. In an experiment by Fowler et al. (2011), the researchers managed to increase nicotine intake in mice with a null mutation in the CHRNA5 gene. Afterwards, they reintroduced the alpha-5 subunits in knockout mice. The results from this study were instrumental in learning that the CHRNA5 gene is highly responsible for nicotine dependency in mice. For this experiment, it was of interest to determine whether animal models can be used to explore maternal tobacco smoking during pregnancy. Fortunately, Buck et al. (2021) determined that animal models can also experience nicotine dependence ad neurodevelopmental disorders in children and grandchildren after being exposed to nicotine as a result of maternal tobacco smoking. This research confirmed the possibility of exploring my interest in using an animal model to test addiction after in utero nicotine exposure.

The second aspect of my research was determining whether there is an actual connection between alcohol and nicotine dependency, especially when considering the CHRNA5 gene. In my favor, evidence from electrophysiological, pharmacological, and neurochemical studies confirmed that polymorphisms within the CHRNA5 can be related to alcohol dependence as well as nicotine dependence (Wang et al., 2009). Unfortunately, however, there hasn't been much research done on this topic yet, hence the results from this study can offer possible insight into areas that should be explored more.

Lastly, I went through past literature review to consider the age group that should be used for the experiment. I decided that adolescent smoking might be the most useful group to study, initially because of their higher chance of experimentation with substance abuse, and secondly because it can help in determining how early should smoking cessation efforts begin and whether adolescence is instrumental for smokers, especially ones that have experience in utero nicotine exposure. Furthermore, although previous research determined that genetics plays a role in nicotine dependence, the contribution of genetics into who will become a smoker after nicotine exposure in utero is still unknown. Therefore, even though it can be assumed that determining who will become a smoker is not solely up to genetics, and should include environmental factors, it is significant to learn what is the percentage that genetics plays in this part. Understanding more on this topic can enhance our knowledge and development of therapies that could help smokers in the future.

Experiment

Background and Significance

The abovementioned literature has already determined, through a variety of scientific experiments, that a variant in the CHRNA5 gene is associated an increased risk of nicotine dependency. In an experiment in a research paper by O'Neill et al. (2018), the researchers examine the effect of in utero nicotine exposure during pregnancy on the nicotine intake in offspring during adolescence. This research paper specifically considers rs16969968, a SNP in CRHRNA5 where there is a change from an aspartic acid [D] to asparagine [N] at position 398 of the human α5 nicotinic acetylcholine receptor subunit. O'Neill and the other researchers aim to analyze whether nicotine exposure alone or in the presence of the risk allele would affect consumption of nicotine in adolescence.

Their results indicated that the offspring with the variant and nicotine exposure consumed the most nicotine, which confirmed the researchers' hypothesis. The offspring with the variant but no exposure to nicotine had the second most consumption, followed by offspring with no variant but with exposure to nicotine. The offspring that consumed the least nicotine across all their tested concentrations was the one without the variant but with exposure to nicotine. Their results solidified the assumption that the risk allele is responsible for nicotine dependency more than nicotine exposure alone.

Based on these results, the experiment proposed in this paper is attempting to continue examining the impact of the risk variant referred to as [N] in overall adolescent addiction. Since nicotine is an addictive substance, this future experiment pursuits to consider the effect of alcohol intake on offspring with the risk variant [N]. The results from this study are instrumental in studying more on the topic of addiction, specifically in the young and malleable adolescent population. Nicotine and alcohol are often connected, and investigating the genetic connection between the two through the risk variant [N] in CHRNA5 is of incredible significance.

Experimental proposal

The first part of the experiment will be repeated almost identically as the methodology in the research paper by O'Neill et al. (2018). At first, female mice will be exposed to either drinking water with 0.2% saccharin or drinking water with 0.2% saccharin and nicotine for 30 days before breeding. In this process only females will be exposed to nicotine and not their male partners, since the experiment is interested in investigating in utero exposure. The mice will be maintained on a standard 12-hour light/dark cycle and given food and water at the same time up until they are about to give birth.

For the second part of the methodology, the offspring would be left to grow for approximately 30 days and both sexes would be utilized. The offspring might be homozygous for either the [D] or the [N], and both groups will be used for the second part of the experiment. It would be expected that around 100 offspring are a part in the study. Firstly, the offspring will be weaned for 30 days. After this period passes, there will be 4 testing groups in the experimental part:

Group 1: Offspring with the risk variant [N] and *in utero* nicotine exposure.
Group 2: Offspring with the risk variant [N] and no nicotine exposure.
Group 3: Offspring without the risk variant [D] and *in utero* nicotine exposure.
Group 4: Offspring without the risk variant [D] and no nicotine exposure.

The difference between the groups is firstly whether they have the risk variant [N] or not [D], and secondly whether they have been exposed to nicotine or not. After the 30 days when the mice have reached adolescence, they will be presented with three bottles in the same room where they have been spending their time since birth. One of the bottles will contain water serving as a control, and the other two will be containing different concentrations of ethanol: one at 100 μg/mL and the other one at 400 μg/mL. The reasoning behind the concentrations is to observe if there will be any differences in alcohol consumption depending on the concentration. The water bottles will be weighed at the same time at the end of every day to observe the overall consumption of each one.

It is assumed that this experiment would support one of more of the following hypotheses:

H1: Adolescent mice N with in utero nicotine exposure will consume more alcohol at both concentrations.
H2: Adolescent mice N without in utero nicotine exposure will consume the second most alcohol at both concentrations.

H3: Adolescent mice D with in utero nicotine exposure will consume the third most alcohol at both concentrations.
H4: Adolescent mice D without in utero nicotine exposure will consume the least alcohol at both concentrations.
H5: Adolescent mice N with in utero nicotine exposure will consume significantly more alcohol at 400 µg/mL.
H0: Alcohol exposure will have no effect on adolescent mice with or without in utero exposure of nicotine.

As of current literature review and my personal research, there has not been an experiment that has tested the effect of alcohol consumption in offspring with the N variant. Therefore, this experiment is novel and contributes to the topic of adolescent addiction. The results of this experiment will help to better understand the mechanism of the CHRNA5 gene and its impact on addiction in mice. The results of this experiment could be possibly used to observe the effect in the human population.

Analysis:

After collecting the data of the consumption from the three different bottles, the results will be averaged and analyzed using an ANOVA test, allowing to compare the four different experimental groups. The goal of the analysis will be to examine the relation between the groups and to observe whether it will be statistically confirmed that nicotine and alcohol dependency can be correlated. The results of this study could potentially give insight in a new direction on how researchers investigate the genetic background of addiction.

Conclusion

The desired outcome of this study is to gain better understanding of the effects of maternal smoking on addiction in the second generation, or offspring. Furthermore, the overall goal is to learn about the genetic aspect of nicotine dependency, and its connection with alcohol. Developing better skills on this topic can help professionals support women during their pregnancy, especially ones with the risk variant [N]. Moreover, learning more about the mechanism of addiction after in utero nicotine exposure can help professionals in efforts of smoking cessation during pregnancy. The study aims to suggest that offspring of mothers with the CHRNA5 variant will have a higher chance of becoming addicted to substances such as nicotine and alcohol. Further research could explore whether the nicotine or alcohol consumption continues in adulthood or is more common among adolescents. As of now, understanding how adolescent in utero nicotine exposure affects alcohol consumption is just one step in the development of cessation therapies for individuals who are struggling with substance abuse.

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