The Genetics Behind Anxiety and Enzymes as a Potential Treatment?

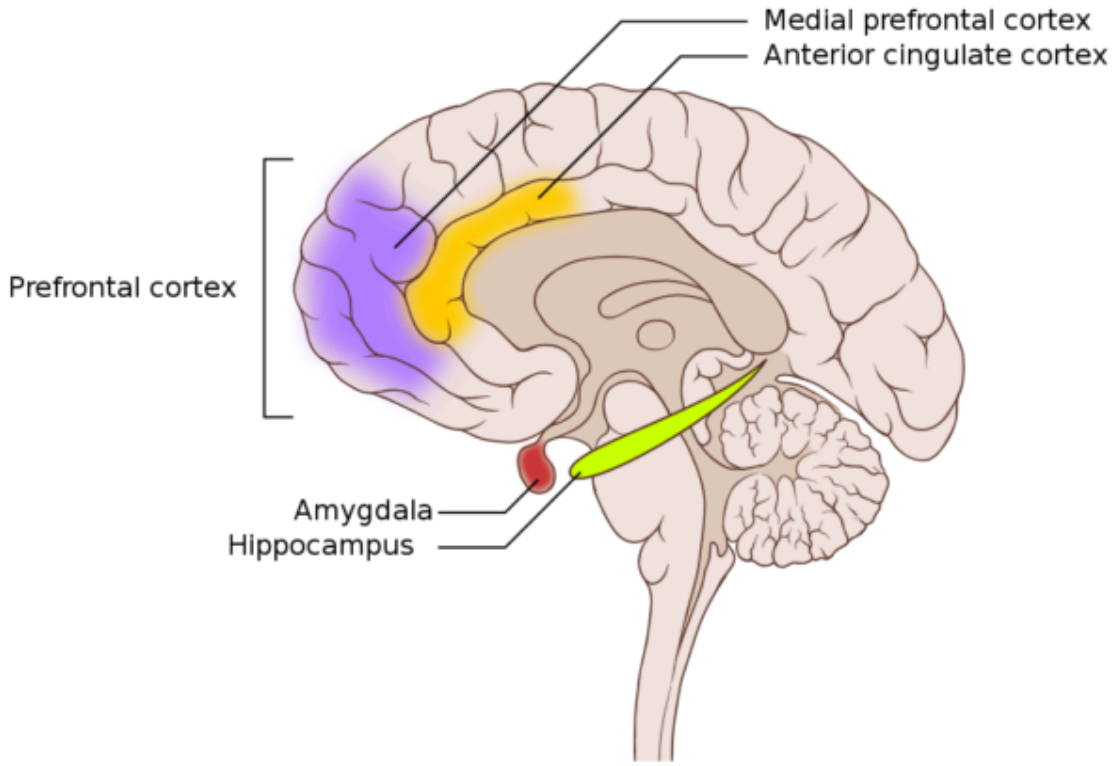
By Vasiliki Avgeri

Everyone has experienced anxiety at least once in their lifetime. Whether it was for giving a presentation, starting a new job or going on a date, our brains respond with a mixture of uneasiness and excitement to every new situation. However, what if you were to be told that each person is predisposed to certain levels of anxiety based on their genes? Imagine being able to edit the enzymes responsible for anxiety.

Understanding Anxiety

Anxiety is described as a feeling of tension or worry by the American Psychological Association (APA). Its main symptoms include a feeling of nervousness, an increased heart rate, trembling or having trouble sleeping. Severe types of anxiety can sometimes lead to anxiety disorders such as the Generalized Anxiety Disorder (GAD), Panic Disorder or Phobias. Factors such as genetics, brain chemistry and environment contribute to the development of anxiety disorders.

It is believed that a variety of different brain regions is responsible for anxiety such as the amygdala which is the emotional basis of our brains. This could work by splitting the brain into two parts: a cognitive brain and an emotional brain. Our brains also directly respond to neurotransmitters, some of which can influence how we act, feel and think causing anxiety such as Serotonin, GABA and Norepinephrine.



The science behind anxiety, Source: The GIST

The Genetics Behind Anxiety

Anxiety is a key part of the human experience. It is believed that it served evolutionary processes and supported human's survival through enhanced preparedness and alertness. However, anxious manifestations can be exaggerated in correlation with any perceived danger that an individual is facing. Even more when they are introduced to psychological distress or when they are self-aggravating in a vicious circle. Anxiety interacts to a great extent with environmental factors and traumatic experiences. They can cause a shift in genetic expression in means of adapting to the trauma which can be passed down genetically through a process called epigenetics.

Even without trauma some people might be genetically predisposed to mental health issues. Although research is still emerging when it comes to genetic influences on mental health, it is evident that there are genetic predispositions in developing mental illness, including anxiety. It is believed that anxiety is about 30 percent inherited. For example, people who carry a variation of a gene that regulates the neurotransmitter dopamine might find it harder to regulate emotional arousal. Researchers offer a biochemical explanation to the above finding, suggesting that this sensitivity may, in combination with other hereditary and environmental factors, make them more prone to anxiety

disorders. There is not a specific “anxiety gene,” but rather many genes that interact to predispose someone to anxiety and through the biochemical process of DNA methylation, our environment can alter the expression of our genes. Doctors have also observed that anxiety is hereditary from studies in twins. Identical twins have the same set of genes and they are more likely to both develop anxiety. This suggests that anxiety may, in fact, be linked to certain genes, which makes it hereditary. Additionally, genome studies report that some genes are common in individuals with anxiety disorders.

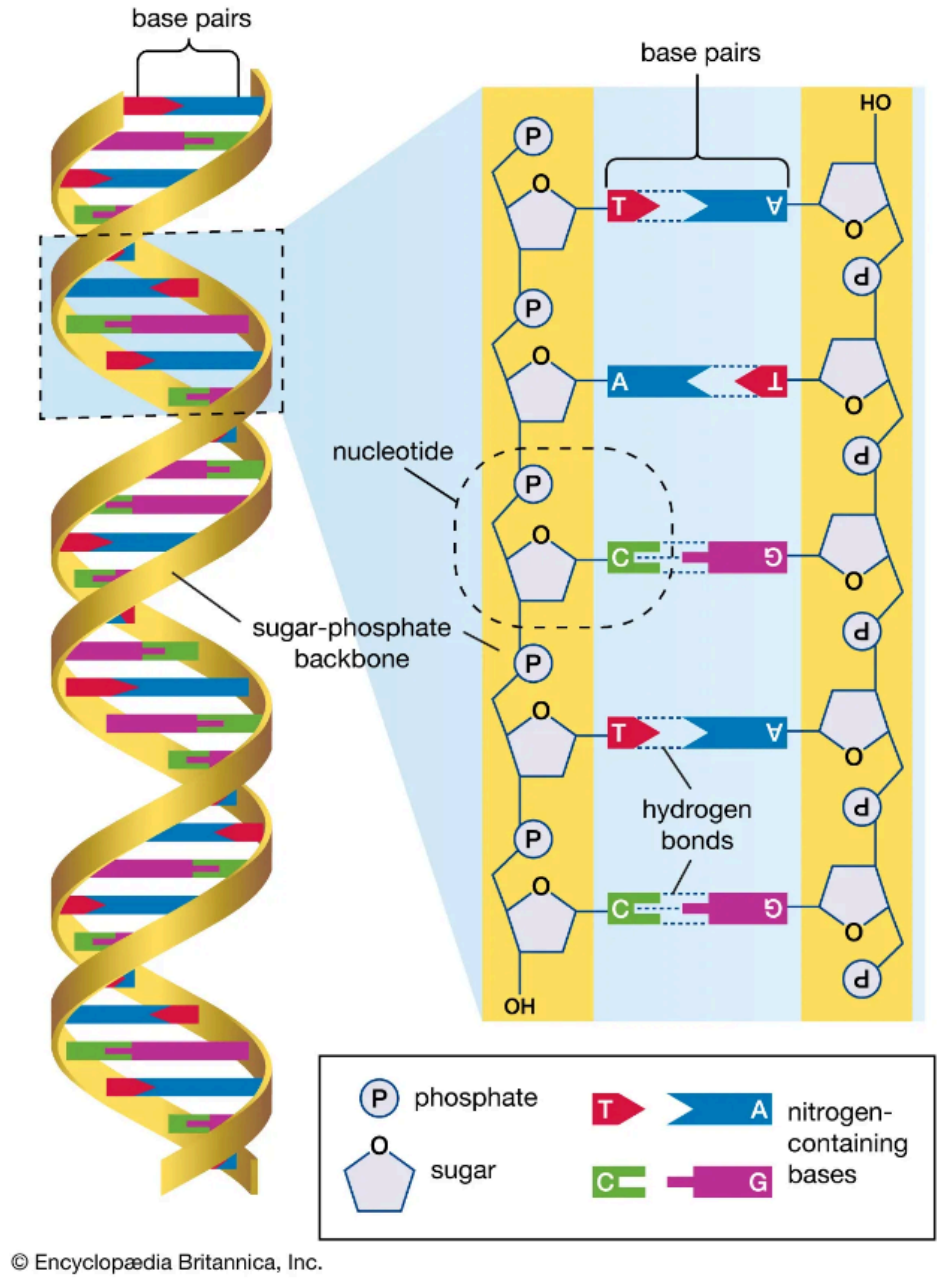
Is anxiety genetic? Source: Exodus Health

How are the proteins responsible for anxiety built?

🧬What is DNA?

Before understanding how anxiety relates to inheritance factors it is crucial to comprehend how genetics work. Genes are segments of DNA that are responsible for shaping physical characteristics. DNA, or deoxyribonucleic acid, is the hereditary material in humans and is more commonly located in the cell nucleus. It is made of chemical building blocks called nucleotides which consist

of three parts: a phosphate molecule, a sugar molecule and a nitrogen base. There are four types of nitrogen bases found in nucleotides. They are called adenine [A], thymine [T], guanine [G] and cytosine [C] and can only be paired up in certain ways to form units. A is paired up with T and C with G giving nucleotides the ability to get arranged in two long strands that form a spiral called a double helix. The structure of the double helix looks like a ladder, with the base pairs representing the ladder's rungs and the sugar and phosphate molecules forming the vertical side pieces of the ladder.



DNA and human genome, Source: *Encyclopædia Britannica, Inc.*

The full DNA, or genome, for humans comprises about 3 billion bases and about 20,000 genes. The order, or sequence, of these bases determines the information responsible for building and maintaining the organism. Each DNA sequence needs to be converted into a chemical message that can be later used to produce proteins. Going back to what genes are, they actually consist of the instructions that are going to create those proteins. 99.9% percent of all humans are identical, the rest 0.1% is what makes each one of us special and holds the important traces that cause diseases.

Why is DNA Sequencing So Important?

DNA sequencing refers to the technique of decoding the exact order of nucleotides in a DNA molecule. Formulating the sequence of DNA is essential in understanding genes' functions and has actually revolutionized our grasp of biology. DNA can now be collected directly from living, dead, and even extinct species enabling us to read (sequence), write (synthesize), and edit (mutate) it.

What is DNA Synthesis?

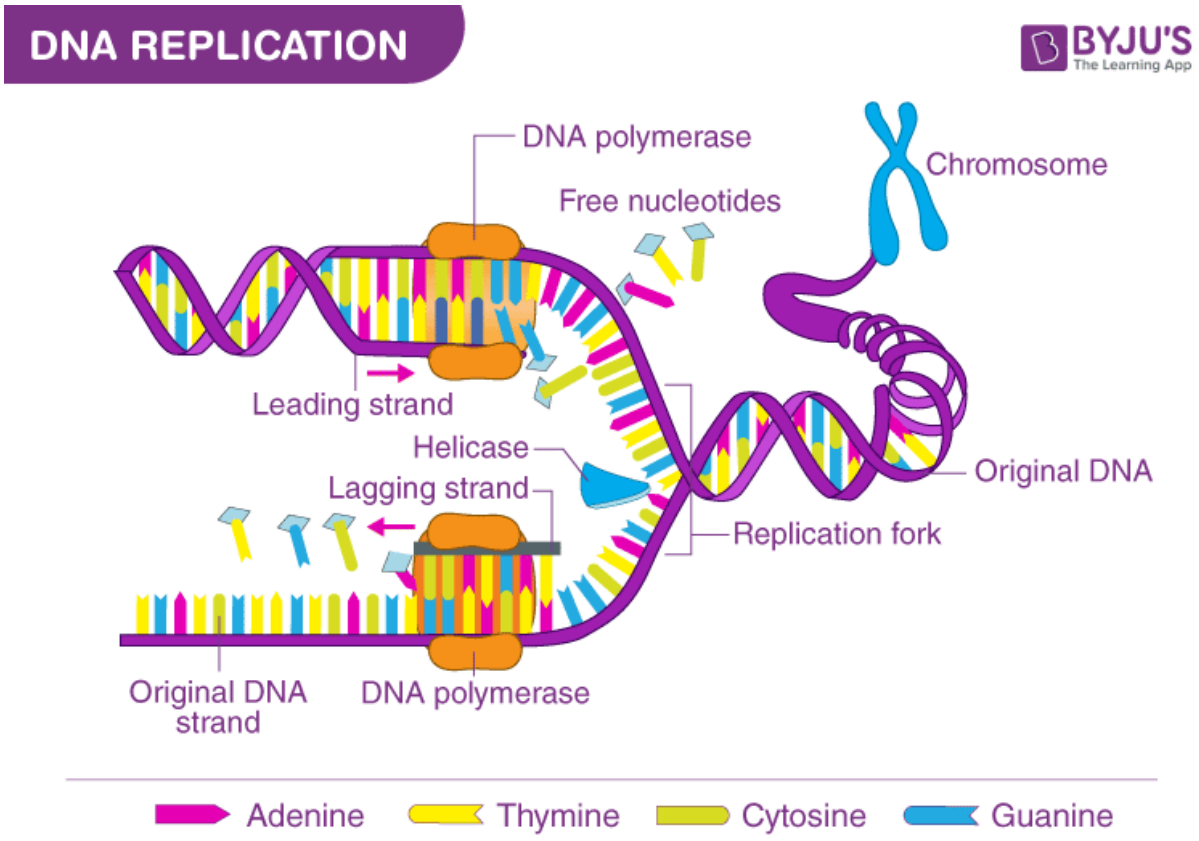
DNA synthesis is the natural (*in vivo*) or artificial (*in vitro*) process of creating new deoxyribonucleic acid (DNA) molecules. DNA synthesis methods started being invented in the 1980s. Back then, the chemical method of phosphoramidite synthesis allowed scientists to create a DNA strand by sequencing attached nucleotides. However, this type of synthesis is costly since it requires the use of toxic chemicals which are only stable for a week or two. Furthermore, these methods can add single nucleotides precisely but by increasing the DNA length, potential mistakes will accumulate. However, in recent years alternatives to chemical DNA synthesis have started to emerge. The potential uses for DNA synthesis technologies appear to be endless. They might be used for manufacturing textiles, enhancing agricultural production, developing diagnostic methods, engineering microorganisms for industrial purposes, recycling debris and decontaminating polluted environments. Additional DNA synthesis applications may not even be known yet. Using DNA to store data is one of the most intriguing ideas that is currently being explored. There are several DNA synthesis methods that have been developed in synthetic biology research. Bellow are described some of the most common DNA synthesis methods:

DNA Replication: It is actually an *in vivo* type of DNA biosynthesis. DNA replication is the process of copying and duplicating a DNA molecule. It is a semi-conservative mechanism which means that the new DNA molecule will

consist of two strands: one newly created and one originated from the initial strand.

Before a cell divides, it must first replicate its entire genome so that resulting daughter cells consist of the same genetic content.

1. The first step is unwinding the DNA strands through the action of an enzyme called helicase that breaks the hydrogen bonds holding the complementary bases together.
2. The enzyme primase will then attach a piece of RNA called a primer that will act as the starting point for DNA synthesis.
3. Another group of enzymes called polymerase will bind to the strand and start adding the complementary bases.



DNA replication, Source: BYJU'S

Polymerase Chain Reaction (PCR): PCR is a technique used to rapidly produce (amplify) copies of a specific segment of DNA which can be later studied thoroughly. PCR consists of a template which contains the region that the researcher wants to amplify; primers, or oligonucleotides, which are short

strands of single-strand complementary DNA and DNA polymerase which is the enzyme that naturally performs DNA replication.

⛓ PCR uses DNA's complementary base pairing and double-stranded nature combined with the melting temperature of DNA molecules. The processes includes 3 types of temperature-dependant reactions:

1. DNA melting (denaturation) that is going to break the hydrogen bonds between the two strands of template DNA.
2. The second reaction is going to carry out the annealing of complementary base pairs.
3. Enzyme-driven DNA replication, or elongation, is assembled with a temperature reaction that optimizes the activity of DNA polymerase. DNA polymerase then synthesizes a new DNA strand which is identical to the template strand.

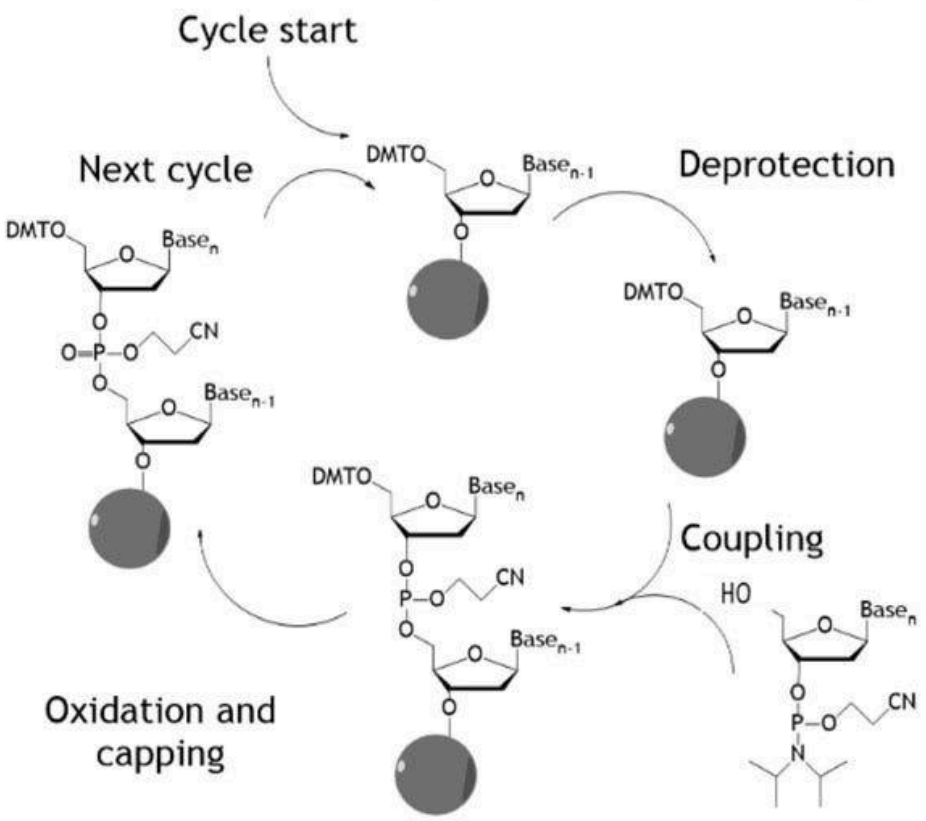
Oligonucleotide Synthesis: Oligonucleotide synthesis, sometimes referred to oligo synthesis, is the method of defined sequences of single-strand nucleic acids. Traditional oligonucleotide synthesis uses phosphoramidites which can be either normal or modified nucleotides that have groups to ensure that their amines, hydroxyl groups, and phosphate groups are cooperating incorrectly.

It is decoded to a repeating sequence of four reactions (deprotection, coupling, capping and oxidation) with single nucleotides added sequentially.

1. Firstly, acidic catalysts immobilize the dimethoxytrityl group of the nucleotide on a solid phase.
2. Secondly, the hydroxyl group of the nucleotide aimed to be added is activated by phosphoramidite and mixed with the tetrazole activator. The obtained nucleoside-phosphite activator is basically hydroxyl-activated nucleotides.
3. Thirdly, a small number of hydroxyl-activated nucleotides not involved in the condensation reaction are prevented from participating in the reaction by acetylation.
4. Lastly, an iodine solution prevents oxidation of trivalent phosphotriester to pentavalent phosphotriester using. After addition of all nucleosides in series, the obtained oligo is released.

Currently, the length of oligos synthesized by the phosphoramidite chemistry-based method is limited to 200 nucleotides and cannot exceed 300 nucleotides theoretically. However, the widespread and cheap oligos produced

on such a large scale have a variety of applications. For example, the oligos can be used as molecular tools for the construction of large cell populations with various genotypes.



Traditional oligonucleotide synthesis, Source: Manufacturing Chemist

Artificial Gene Synthesis: Also known as DNA printing is a method in synthetic biology that is used to create artificial genes. It is mainly based on solid-phase DNA synthesis, however it differs from molecular cloning and polymerase chain reaction (PCR) since it does not require pre-existing DNA sequences. Thus, it sets the potential to create a completely synthetic double-stranded DNA molecule without limitations on any nucleotide sequence or length. Most approaches combine organic chemistry and molecular biology techniques while entire genes may be synthesized *de novo*. Synthesizing nucleic acid sequences can be proven more cost-effective than usual cloning and mutation procedures. Gene synthesis is an important tool in many fields such as gene expression, vaccine development, gene therapy, and molecular engineering. Lastly, this method has previously been used to generate functional bacterial or yeast chromosomes consisting of approximately a million base pairs. Adding novel

nucleobase pairs to the pre-existing base pairs in nature could greatly expand the genetic code.

Enzymatic Synthesis: Enzymatic synthesis uses solid-phase synthesis processes. A short strand of DNA synthesized on a solid support is being extended by DNA polymerases by using nucleoside triphosphates. DNA polymerases use a template DNA strand that provides base pairing while selecting the incoming nucleotide. Although polymerases are effective in amplifying existing DNA templates, they are unable to generate de novo DNA sequences. Therefore molecular biologists needed to develop an enzyme that can synthesize DNA strands in a template-independent fashion, and most likely to have to do that is terminal deoxynucleotidyl transferase (TdT). In human immune cells, TdT extends DNA encoding areas of antibody proteins that recognize intruders allowing a better probability of identifying threats. Outside of the immune system, TdT could add about 100 bases per five minutes. Comparing it to the fact that each step of phosphoramidite chemistry takes about 10 minutes, this is considered a rapidly increasing number.

Is there a correlation between anxiety and enzymes?

The past few years there has been ongoing research on the role of enzymes in anxiety disorders. Below is an example of findings from a recent study. Oxidative imbalance appears to have an important role in anxiety forming. Studies in both humans and animals have shown a strong dependency between anxiety and oxidative stress. Specifically in humans the increased malondialdehyde levels and discrepancies in antioxidant enzymes have been observed. Studies using knockout or overexpression of antioxidant enzymes have shown a correlation between anxiety-like behavior and oxidative stress proving that it might be caused by pharmacological-induced oxidative stress. Some of the techniques used to study the above relationship between anxiety behaviors and oxidative stress are enzymes knockout and overexpression, gene deletion and polymorphisms. Another study, noted that overexpression of glutathione reductase 1 and glyoxalase 1 had a direct correlation with anxiety-like behavior in mice brain. Additionally, local inhibition of glyoxalase 1 expression by RNA interference decreased the anxiety-like behavior.

Pregabalin is a drug that is used to treat epilepsy, neuropathic pain, fibromyalgia, and generalized anxiety disorder (GAD). A process that was developed included

synthesizing a liquid enzyme to avoid cost rise and improve the overall purity of the final product accompanied with higher yields.

Buspirone is a drug used to treat anxiety and depression while producing its effects by binding to the serotonin 5HT1A receptor. It results from hydroxylation reactions which makes it extensively converted to various metabolites and blood concentrations return to low levels a few hours after dosing.

Barriers of Enzymatic Synthesis for Anxiety Treatment

There hasn't been a significant number of initiatives regarding research for enzymatic synthesis as a treatment for anxiety however some key limiting factors when it comes to developing those.

1. Enzymatic synthesis requires great expertise and specificity when it comes to any practice relevant to producing compounds with therapeutic effects even when it comes to anxiety that is so complex and impacted by a variety of elements.
2. The stability and compatibility of compounds synthesized by enzymes under certain conditions should be examined as well. Enzymes are sensitive to temperature, pH, and other environmental factors, which could affect the results.
3. As each DNA synthesis method, enzymatic synthesis raises ethical and social questions regarding gene modification and alteration both of which can be perceived as controversial aspects.

As a person who has been struggling with anxiety my whole life and only managed to feel it becoming worse, enzymatic synthesis seems like a promising solution. Nevertheless, a lot more precise research needs to be conducted in order to cover the variety of factors contributing to anxiety. Anxiety can often be manifested by external factors and environmental influences so it is crucial to understand each barrier.