How to read and understand your genetic test report

When you first receive a genetic test report, it can be overwhelming trying to figure out what all the numbers, letters, and terminology mean. You are not alone! If you have questions about your report, a genetic counselor is best suited to explain it to you. However, we know there are always more questions that arise afterward, so we hope this document will help you out!

What is Whole Exome Sequencing?

Genetic testing is the process of **sequencing** (or reading) our DNA to find **mutations** (or changes) that differ from what is common in the human population. **Whole Exome Sequencing** reads all of the **exons** to look for changes in our genome that might explain a specific phenotype (a symptom or set of symptoms). Our **genome** (all of our DNA) is separated in subunits called **genes**. Within these genes, there are even smaller subunits called **exons.** These exons contain the information for making proteins, which are the tools that cells use to do work. While exons account for only 1-2% of the genome, changes in exons have been linked to most genetic diseases, which is why we focus on them.

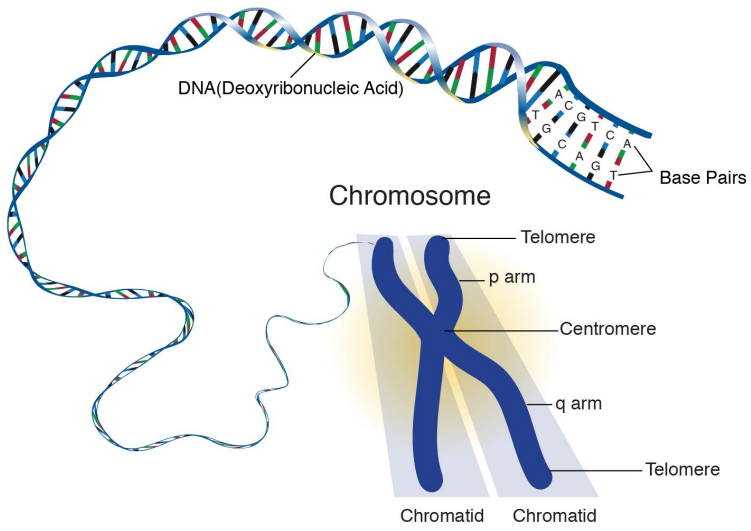
On our website, we will refer to a change in a gene as a **variant**, not a mutation. In the scientific literature, however, you will often see the word “mutation” used to describe a change.

Why is DNA important?

DNA, or deoxyribonucleic acid, contains all the information to make and maintain an entire organism. There are over 23,000 genes in the human genome and over 3 billion base pairs. On average, most genes have 3,000 nucleotides and 8.8 exons. The DLG4 gene (GenBank accession No. NC_000017) contains 22 exons that span approximately 30 kb at chromosome 17p13.1; the exon 1 contains the untranslated region and the translation start site [39]. The putative promoter of the DLG4 gene was predicted to be located between nucleotide positions –1166 and –1415 upstream from the ATG starting nucleotide using the PROSCAN.

The information in our DNA comes in the form of four **nucleotides** (or **bases**): Adenine (A), Cytosine (C), Thymine (T), and Guanine (G). These nucleotides are strung together in pairs (A-T and C-G) to form units called **base pairs**. Each base pair forms the “rungs” of a ladder between two strands of DNA, creating the recognizable double helix structure. Further coiling of the double helix packs our genome into a larger but more compact structure called a **chromosome** (see figure on next page). Humans have 23 pairs of chromosomes: 22 numbered pairs (autosomes), and a pair of sex chromosomes (XX or XY). Each pair is made up of one

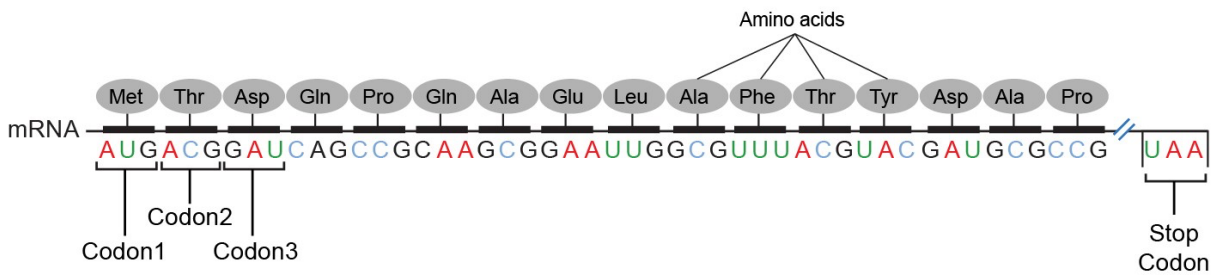
chromosome from a mother and one from a father. Each gene has a specific location on a specific chromosome. For example, the *DLG4* gene is located on the 17th chromosome.



http://www.genome.gov/sites/default/files/tg/en/illustration/chromosome.jpg

What is the significance of variants?

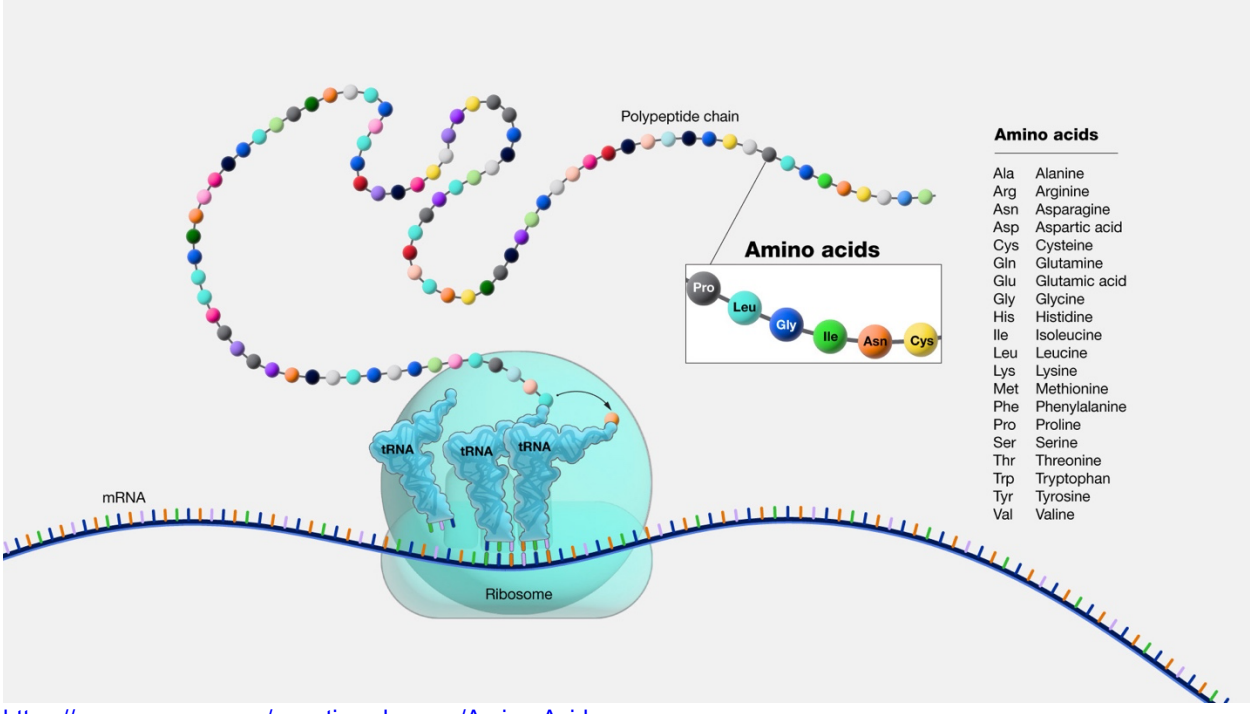
When our genetic test reports a variant in a gene, what does that actually mean and how can it affect our body? Remember that proteins are the tools that our cells use to get work done. How do we go from a gene to a protein? A gene is first transcribed, or rewritten, into **RNA (mRNA)**, an intermediate form of genetic information. The mRNA containing all the exons is used to build a protein through a process called translation. The specific information in the mRNA and exons comes in the form of **codons**, a sequence of three nucleotides that specifies an **amino acid** (shown below).



https://www.genome.gov/sites/default/files/tg/en/illustration/codon.jpg

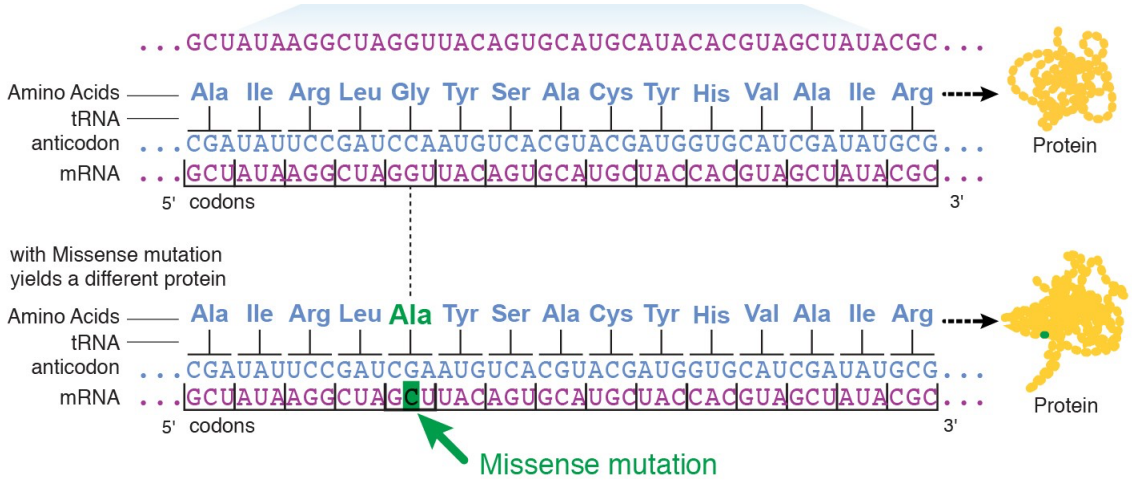
Amino acids are the building blocks of proteins. The correct sequence, or the order, of amino acids is determined by the order of codons. There are 20 amino acids in our cells (see table below). Each one has a three letter abbreviation and a single letter abbreviation. In your genetic test report, you will see both the change in the DNA sequence as well as the change in the amino acid sequence.

Name	3-letter	1-letter	Name	3-letter	1-letter
Alanine	Ala	A	Leucine	Leu	L
Arginine	Arg	R	Lysine	Lys	K
Asparagine	Asn	N	Methionine	Met	M
Aspartic Acid	Asp	D	Phenylalanine	Phe	F
Cysteine	Cys	C	Proline	Pro	P
Glutamine	Gln	E	Serine	Ser	S
Glutamic Acid	Glu	E	Threonine	Thr	T
Glycine	Gly	G	Tryptophan	Trp	W
Histidine	His	H	Tyrosine	Tyr	Y
Isoleucine	Ile	I	Valine	Val	V



https://www.genome.gov/genetics-glossary/Amino-Acids

So how does a variant affect a protein? The bottom line is that a variant in a codon can result in a different amino acid at a specific position in the protein. This matters because a protein's final 3-D structure is dependent on the correct sequence of amino acids. Furthermore, a protein's function, or what it does, is determined by its structure. Even if the variant only changes one amino acid (we call this a missense mutation shown below), the entire protein structure can still be altered! This can interfere with the protein's normal function and disrupt important cellular processes that are necessary for growth and development. When proteins, like the PSD-95, no longer function correctly this can lead to the symptoms we see with the *DLG4* variants like hypotonia, intellectual disabilities, sleep disturbances developmental delays, or epilepsy.



https://www.genome.gov/genetics-glossary/Missense-Mutation

Using what we just learned, how do we read a genetic test report?

Gene	Disease	Mode of Inheritance	Variant	Coding DNA	Zygosity	Classification
DLG4	*DLG4* Related Synaptopathy	Autosomal Dominant	p.Arg629Gln	c.1886G>A	Heterozygous	Likely Pathogenic Variant

In this sample report, a change was discovered in the *DLG4* gene.

- The **Autosomal Dominant** mode of inheritance refers to the fact that a person only needs one copy of the variant in order for the symptoms/disease to manifest. This is called a **dominant** mutation. And the change occurred on an autosome (numbered chromosome).
    - NOTE: Sometimes two copies of a variant are required to see an effect. Those are referred to as **recessive** mutations. The known variants of DLG4 typically present themselves in an **autosomal dominant** pattern.
- The specific amino acid change is **Arg629Gln**, meaning the Arginine (ARG), at position 629 in the protein, was replaced by Glutamine (Gln)

- The specific DNA change is **1886G>A**. This means that at nucleotide position 1886, the G was changed to an A, which changed the codon to one that specifies Glutamine instead of Arginine.
- The **heterozygous** zygosity means only one chromosome was detected to have the variant. In this case, the chromosome containing the variant was inherited from the father. **Mosaic** means the variant was not present in all of his cells.
- This variant has also been categorized as **likely pathogenic**, meaning there is some data linking it to specific symptoms or a disease.

Gene	Mode of Inheritance	Variant	Coding DNA	Zygosity	Inherited From	Classification
DLG4	Autosomal Dominant	p.(E347Rfs*12)	c.1039del	Heterozygous	De novo	Likely pathogenic

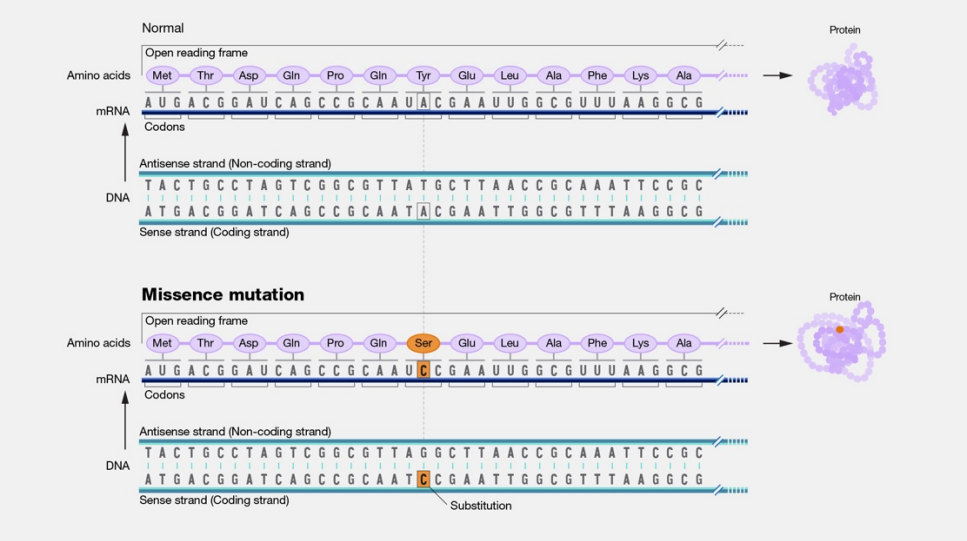
In this sample report, a variant was also discovered in the *DLG4* gene.

- The amino acid change is **Glu347Argfs*12**. The Glutamic Acid (Glu) at position 347 in the protein was replaced by an Arginine (Arg).
- **1039del** means the nucleotide change occurred at the 1039th position. The new codon specifies Arginine instead of Glutamic Acid.
- The mode of inheritance is **autosomal dominant**, meaning only one copy of the variant is needed to see an effect. The **heterozygous** zygosity means only one of the chromosomes has the variant.
- The inheritance pattern was deemed "***de novo***" meaning the variant arose by itself as a new change that was not inherited from either parent.
- The variant is classified as **likely pathogenic** because there is some data linking it to specific symptoms or a disease.

What are the different types of Variants and how to understand them?
Missense, Nonsense and Frameshift Mutations.

What is a missense mutation?
A missense mutation occurs when there is a mistake in the DNA code and one of the DNA base pairs is changed, for example, A is swapped for C.

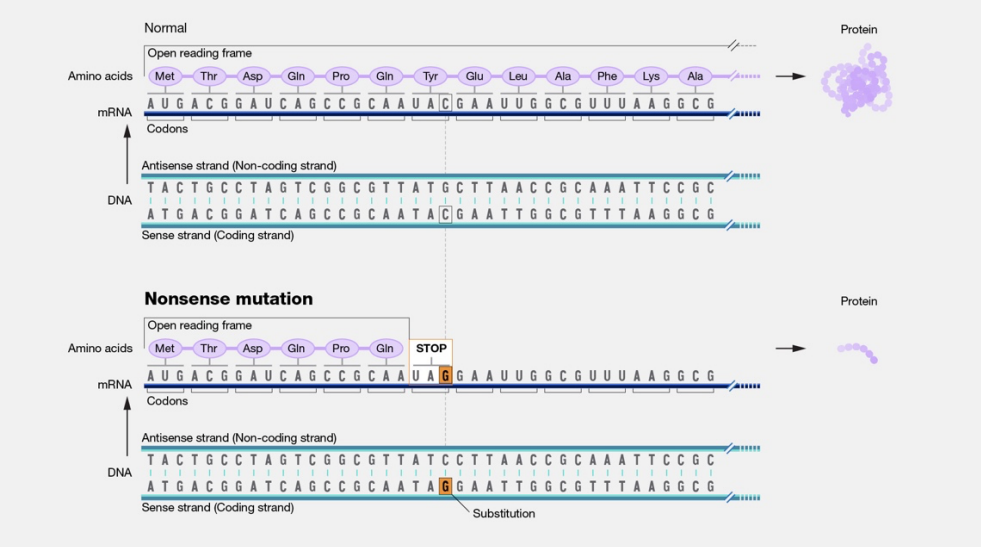
This single change means that the DNA now encodes for a different amino acid, known as a substitution. Sometimes a change in the amino acid has no effect on the resulting protein's function at all. On some occasions, the change in amino acid enhances the protein's function, but in other cases it can ultimately render the protein as "faulty".



https://www.genome.gov/genetics-glossary/Missense-Mutation

What is a nonsense mutation?

Like a missense mutation, a nonsense mutation also involves a single alteration to the DNA base pair. However, in the case of a nonsense mutation, this single change results in the production of a stop codon, thereby terminating protein synthesis prematurely. The result? A shortened protein that may function, but also may not.

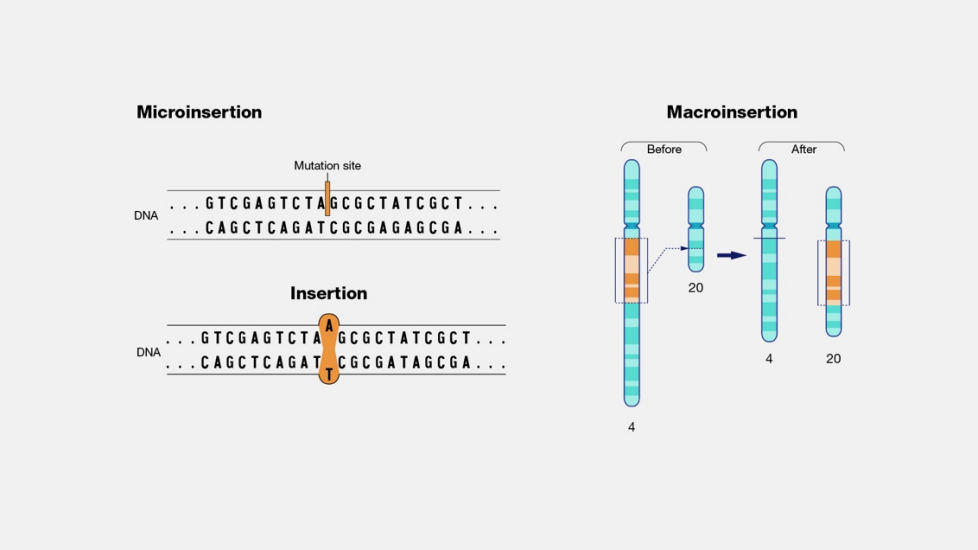


https://www.genome.gov/genetics-glossary/Nonsense-Mutation

What is an insertion mutation?

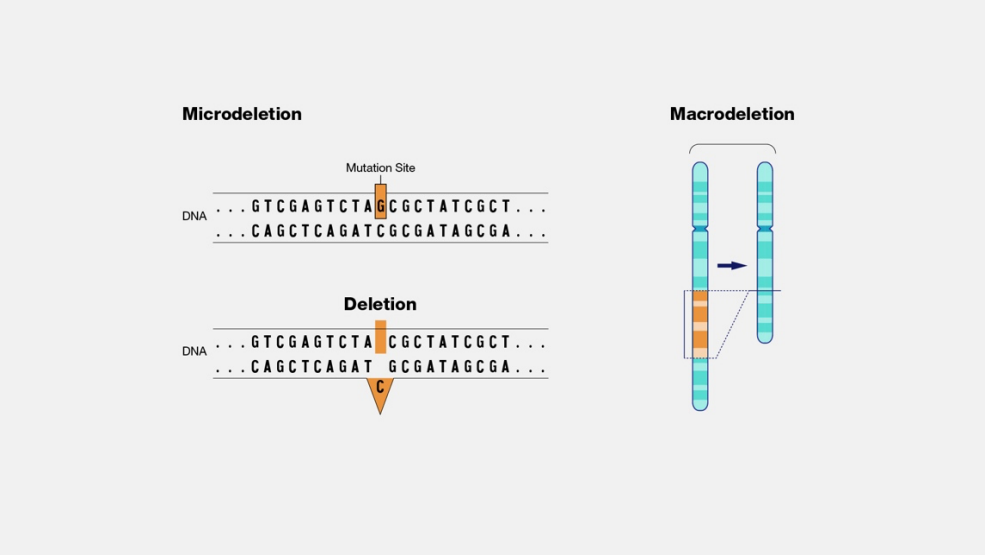
An insertion mutation involves the addition of one (or more) nucleotide base pairs into the DNA sequence. Insertion mutations can vary in size, ranging from the insertion of just one base pair into the DNA sequence to the insertion of a section of a chromosome into another chromosome.

The end result is a potentially malfunctioning protein.



What is a deletion mutation?

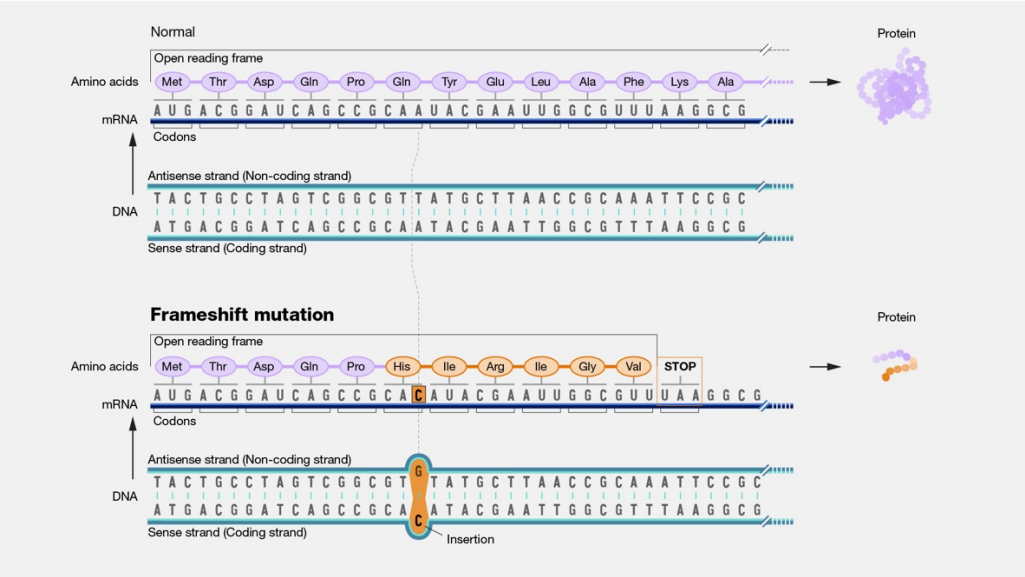
As the title may suggest, a deletion mutation occurs when there a piece of DNA is removed from the sequence. The size of the DNA that is removed can vary in length, from a single base pair to an entire gene or several consecutive genes. The removal of the DNA can, again, compromise the function of the encoded protein.



What is a frameshift mutation?

A frameshift mutation occurs when the aforementioned "addition" or "deletion" mutations result in a change to the gene's reading frame, which includes groups of three bases that encode for an amino acid. The change in the reading frame alters the grouping of the bases and

subsequently changes the amino acids that are encoded. Often, the encoded protein is non-functional.



https://www.genome.gov/genetics-glossary/Frameshift-Mutation

If your genetic test report finds a change in the *DLG4* gene, does this mean you have a *DLG4*-related Synaptopathy?

This is something your genetic counselor will discuss with you. They will consider all the symptoms that have presented themselves and decide if a *DLG4 related Synaptopathy* diagnosis is appropriate. Please keep in mind that the same variant in numerous people can also manifest itself differently. Symptoms can be similar, but each person's body can uniquely process the variant, resulting in different outcomes. Genes also often work together with many other genes in order for our bodies to function properly. It is possible that variants in other genes will affect how the *DLG4* variant behaves.

Additional references:

https://www.ncbi.nlm.nih.gov/gene/1742

https://www.genome.gov/

https://www.technologynetworks.com/applied-sciences/articles/essential-amino-acids-chart-abbreviations-and-structure-324357