HUGH KAUL PRECISION MEDICINE INSTITUTE
The University of Alabama at Birmingham

GENETICS OVERVIEW

What is a gene?

Our bodies are composed of millions of cells. Each cell contains genetic information in the form of chromosomes, which are made of DNA and consist of smaller structures known as genes. We have 46 chromosomes in each cell, 2 pairs of 23 chromosomes. One chromosome of each pair is inherited from the father, while the other is inherited from the mother, and this is how we get the combination of our genetic information. We have approximately 25,000 genes in our cells, and given that there are 2 copies of each chromosome, our genes also come in pairs.

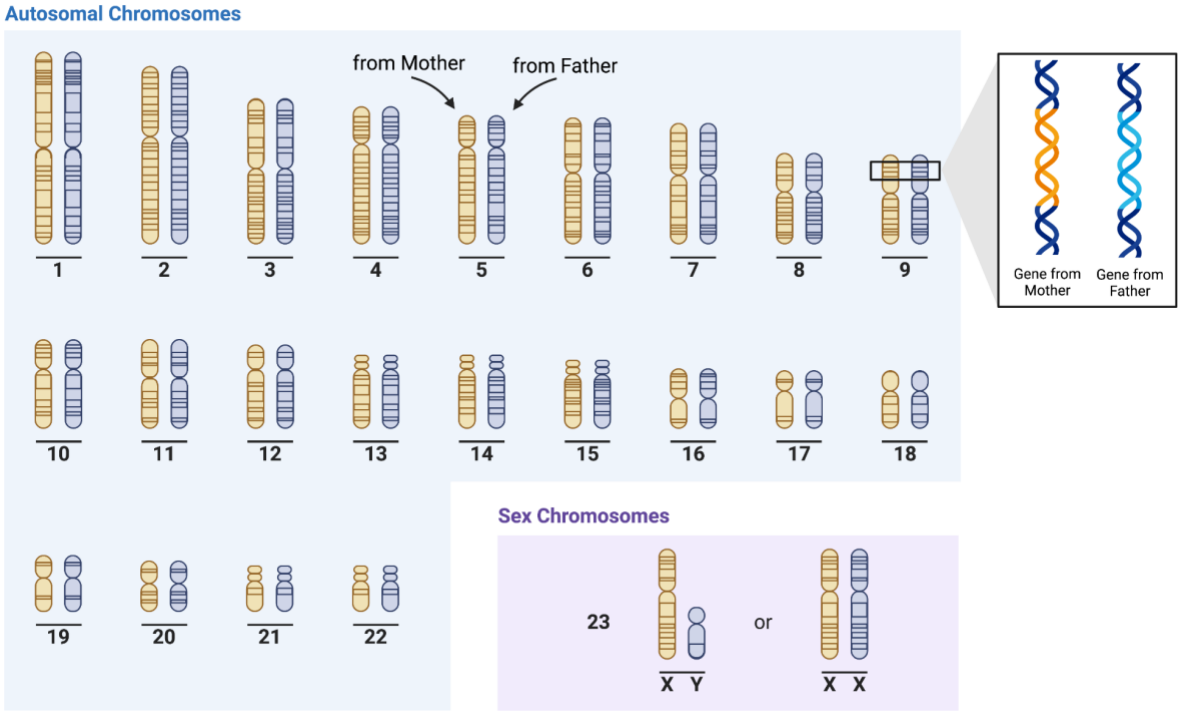

Figure 1. The Human Karyotype. A karyotype is an individual's complete set of chromosomes. We each have 23 pairs of chromosomes, one set from our mother and the other from our father. Within our chromosomes are genes, and our genes also come in pairs. Figure was made in BioRender.

How do mutations (variants) occur?

Our chromosomes are composed of genes that are made up of DNA. Each gene has a specific code made up of nucleotide bases: adenine (A), guanine (G), thymine (T), and cytosine (C). Together, these four bases make up the instructions for how to make a specific protein. Specialized structures within each cell can read this set of instructions, translating every three nucleotides (also known as a codon) into a particular amino acid, which is the building block for proteins.

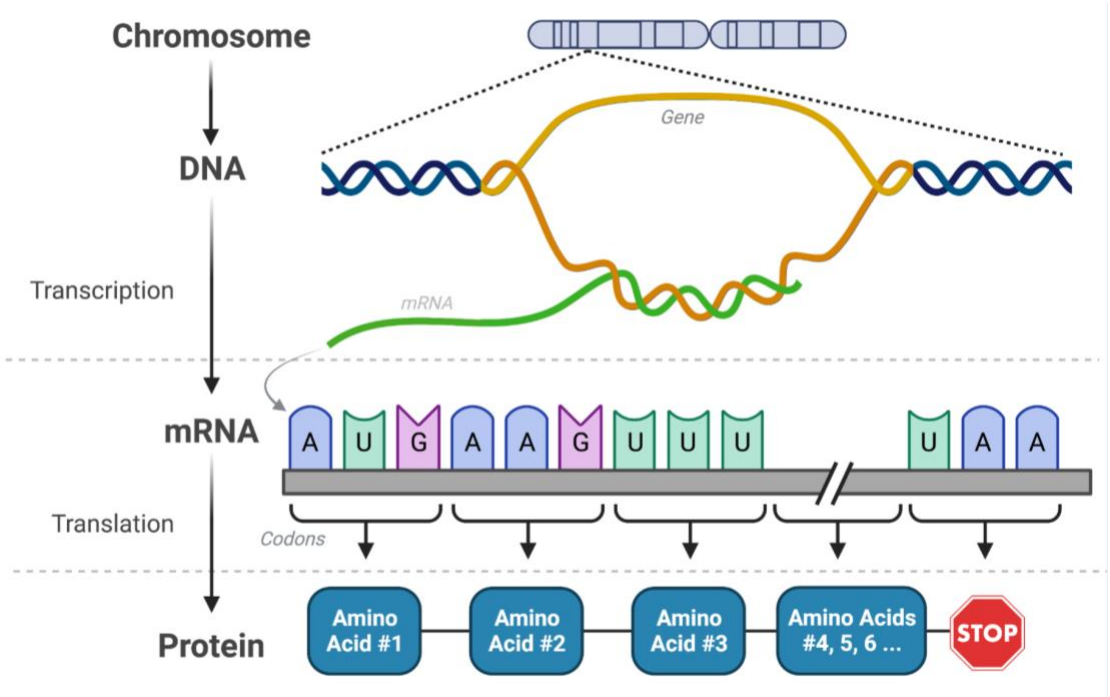

Figure 2. The Central Dogma of Biology. The central dogma of molecular biology is an explanation of the flow of genetic information within a biological system. Chromosomes contain DNA, which encodes genes. Genes can be transcribed into messenger RNA (mRNA), which contains nucleotides that encode amino acids. Every three nucleotides (also known as a codon) translate into one amino acid, with the exception of the stop codons, which signal the end of the protein.

Mutations, or variants, occur when a nucleotide is changed from one base to another, and this may cause a change in the amino acid that is encoded. Proteins are composed of many amino acids, but one alteration in the amino acid sequence could lead to dramatic effects on the protein's function. Variants in our genes typically occur during the DNA replication process when our cells grow and divide. Most of these variants are caught by the cell and corrected, however, some variants remain and typically do not affect the health of the individual.

How are these variants inherited?

For most genes, there are two copies, one inherited from the mother and one from the father. Therefore, each child has four possible genetic combinations for a gene.

If a variant occurs in only one gene copy and affects the individual, it is called a dominant condition; if an individual is affected but carries two variants, one on each gene copy, then it is a recessive condition. In an autosomal dominant disease, such as Developmental And Epileptic Encephalopathy 56 (DEE56), the child may have inherited an altered gene copy from one parent. In rare situations, a variant can occur spontaneously with no family history of the condition. This is called a *de novo* variant, and it is not present in either of the individual's parents (Figure 3). On average, the genome of an individual contains 44 to 82 *de novo* single-

nucleotide variants, and these variants can have a wide range of effects, from having no detectable impact to causing developmental abnormalities or genetic disorders.[13]

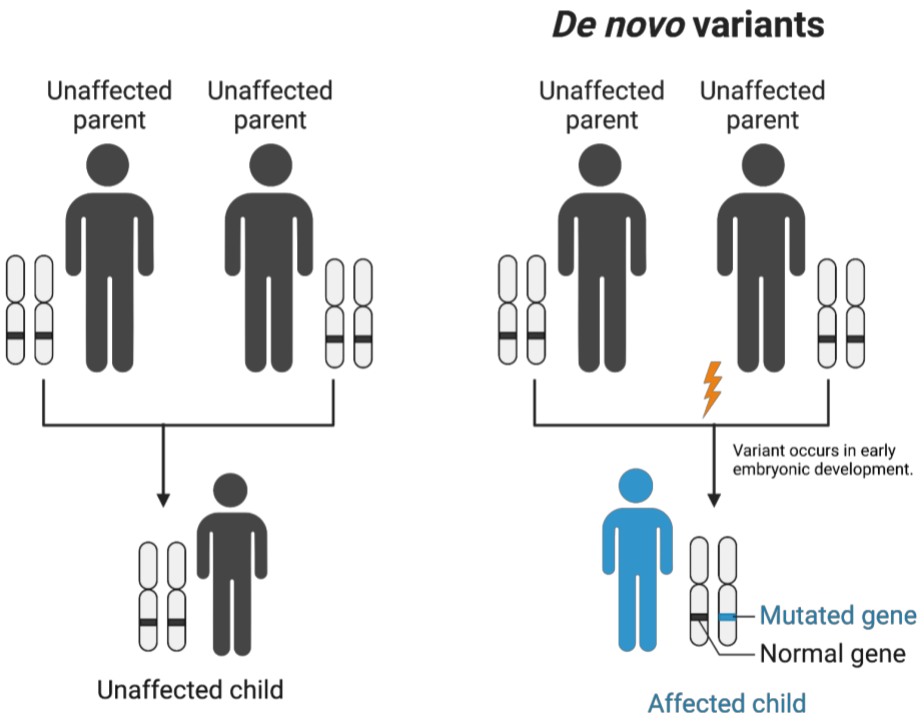

Figure 3. *De novo* variants. *De novo* variants can occur at any point during embryonic development, can affect any gene in the genome, and are not inherited from either parent.

Why do variants cause disease?

Some genes are essential for proper development and function, and they need to be expressed at a certain level for the organism to survive and function normally. In these cases, it is typically necessary for both copies of the gene to be functional in order to produce enough of the protein encoded by the gene to support the necessary processes. Haploinsufficiency occurs when one copy of a gene is lost or non-functional (also known as a “loss-of-function” variant), usually due to a variant that disrupts the gene’s function or a deletion that disrupts a region of the gene, and a single (healthy) gene copy is not sufficient to produce the amount needed.

Dominant negative variants are variants that alter a protein in such a way that it interferes with the normal function of the protein. These variants typically result in the production of a protein that is not functional or that has reduced function, and they cause problems even when only one copy of the mutated gene is present. Dominant negative variants can have a range of effects, depending on the function of the protein.

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GENETIC ANALYSIS

How can I interpret James's genetic report?

In a genetics report, you may see the terms "heterozygous" or "homozygous". Heterozygous means that only one gene copy contains a particular variant, whereas homozygous means that both gene copies contain the same variant. Determining how many gene copies are affected is essential in making a molecular diagnosis.

Multiple variants have been reported to cause Developmental and epileptic encephalopathy 56 (DEE56). In James's case, the genetic report revealed one heterozygous pathogenic variant in the *YWHAG* gene.

The variant is located at the 394th nucleotide of the gene. A gene without the variant would have a cytosine (C) base in the DNA sequence, however, the genetic code is altered and there is a thymine (T) instead of the cytosine (C) at the 394th position. This is designated by the notation "394C>T" in the genetic report. Once the cells begin protein production, the coding change induces a corresponding amino acid change from arginine (Arg) to cysteine (Cys) in the 132nd amino acid, shown as "Arg132Cys" in the genetic report (Figure 4). Since this variant is a single base substitution that produces a different amino acid from what is normally produced, it is called a missense variant.

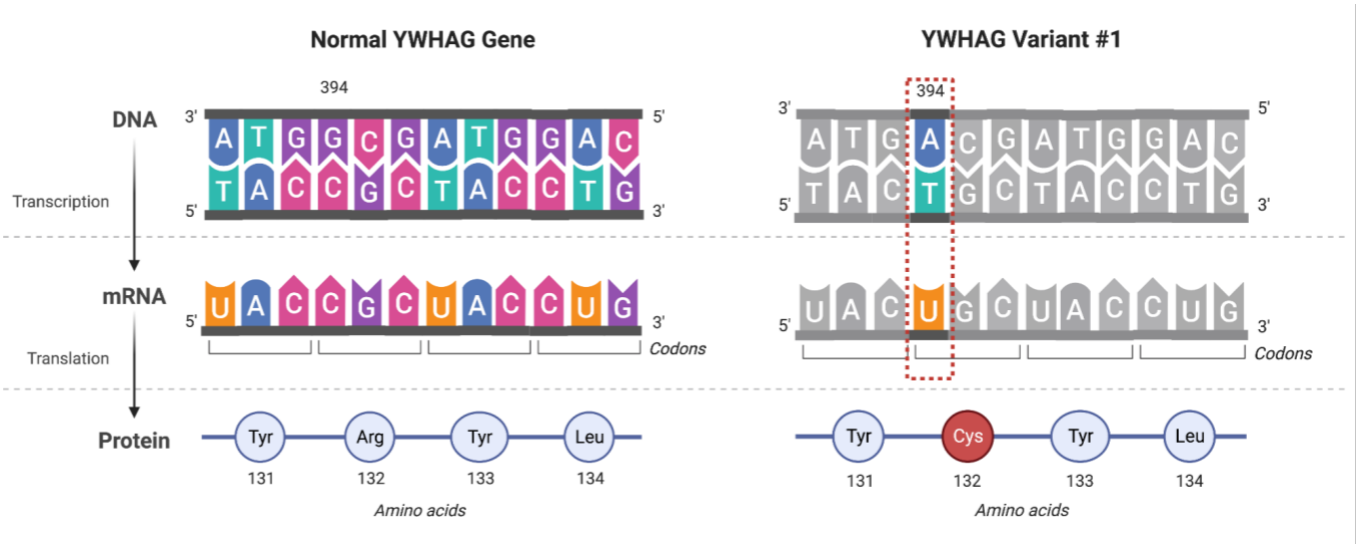

Figure 4. Visual of the *YWHAG* variant c.394C>T (p.Arg132Cys).

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DISORDER INFORMATION

What is Developmental and Epileptic Encephalopathy 56 (DEE56)?

Developmental and epileptic encephalopathy (DEE) refers to a group of severe epilepsy syndromes associated with seizures and developmental delay or developmental regression.[1] Symptoms of DEE may appear in infancy or early childhood.[2]

Most DEEs are caused by a *de novo* variant.[2] Multiple genes contribute to DEE, and the *YWHAG* gene was added to the list of known DEE genes in 2017 based on a publication by Guella et al. (2017). *YWHAG* gene variants are specifically associated with developmental and epileptic encephalopathy 56 (DEE56). There have been eleven variants reported to cause DEE56.

What causes Developmental and Epileptic Encephalopathy 56 (DEE56)?

Developmental and epileptic encephalopathy 56 (DEE56) is a specific type of DEE caused by a genetic variant of the *YWHAG* gene.[2] The number 56 stands for being the 56th gene identified to cause DEE. *YWHAG* encodes for YWHAG, a member of the 14-3-3 protein family.[3] 14-3-3 proteins are involved in multiple cellular functions, such as cell survival, cell growth, and cell signaling.[4] They are highly expressed in the brain, heart, and skeletal muscle.[3] There are seven different types of 14-3-3 proteins, each named using a letter from the Greek alphabet: β, γ, ε, σ, ζ, τ, and η. YWHAG stands for the gamma (γ) 14-3-3 protein.

Two monomers, or individual YWHAG proteins, bind together to fold into a 3D structure.[5] YWHAG almost always exists in this folded and bound state. The bound protein complex is able to connect to various different target proteins. Among the target proteins are cytoskeleton proteins responsible for maintaining the cell's shape and signal-creating proteins responsible for cell growth and cell death.

YWHAG haploinsufficiency, which means one copy (the one with the variant) of the gene is underactive but the activity of the other (healthy) copy is not enough, is the mechanism behind DEE56.[5] Proteins are made of numerous building blocks called amino acids. In YWHAG, there are three amino acids, two arginines, and one tyrosine, that form a positively charged pocket within a binding groove.[2] The groove attracts and binds negatively charged proteins. When a variant changes one of the amino acids in the binding groove, the ability of YWHAG to connect to other proteins is impaired, negatively affecting the protein's overall function (Figure 5). YWHAG is most expressed in the brain, and the protein is believed to play a large role in neuronal signaling.[5] The inability to bind to target proteins among neurons in the brain is expected to cause delayed brain development, reduced brain size, and infantile seizures characteristic of DEE56.

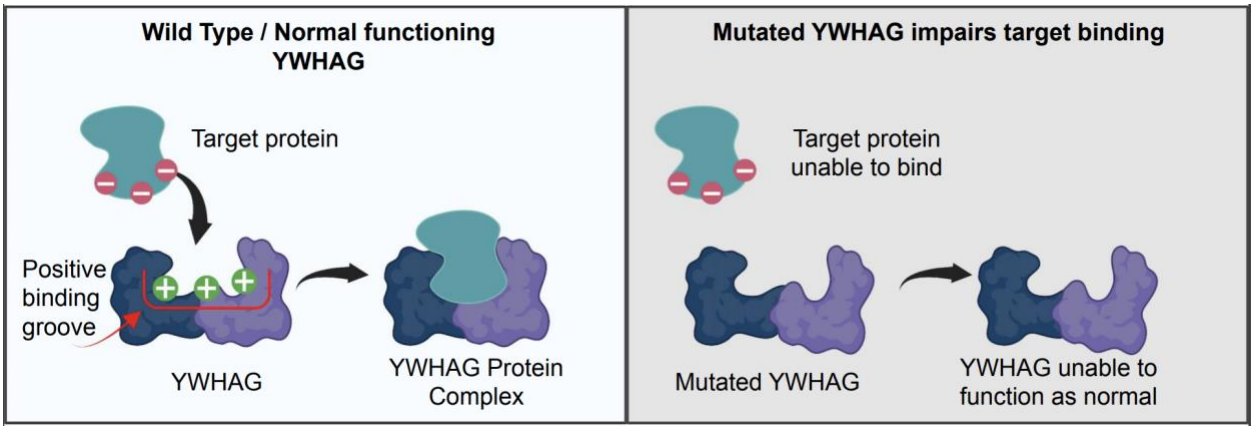

Figure 5. Loss of function variant, p.Arg132Cys, in *YWHAG* leads to Developmental and Epileptic Encephalopathy 56 (DEE56). Left - the YWHAG protein has a positively charged binding groove that allows target proteins to bind and perform various cellular functions. Right - In patients with the variant p.Arg132Cys, the amino acid substitution of arginine for cysteine destroys the bonds that normally form between YWHAG and target proteins.

Variant information

A study by Guella et al. (2017) showed variants in YWHAG affecting folding and protein binding abilities.[2] Your son's variant, p.Arg132Cys, was among the variants studied. Guella et al. (2017) found seven different missense variants that led to DEE56. In 2021, Xing-Guang Ye et al. (2021) found that all missense variants currently found to be associated with DEE56 are *de novo* and located near the positive binding groove.[6] Any substitution of the arginine at residue 132, as in your son's case, with a neutral or negatively charged amino acid affects the protein's ability to bind to other proteins. The reason why some changes in the protein sequence have such a strong impact on the binding of two proteins is because they stop certain molecules of the protein from sticking together. These molecules stick together using something called "hydrogen bonds," which are like tiny magnets between certain atoms in the molecules. Because of the amino acid substitutions around the binding groove, these hydrogen bonds between YWHAG and target proteins are unable to form and connect the proteins together. Xing-Guang Ye et al. (2021) found substitution of arginine with cysteine at residue 132 destroyed all hydrogen bonds that form between YWHAG and the target protein, potentially explaining the more severe form of DEE56 that develops (Figure 5).

How can DEE56 be managed or treated?

Typical disease management consists of using antiepileptic drugs to help control seizures. Sodium valproate, levetiracetam, ethosuximide, carbamazepine, and stiripentol are commonly prescribed antiepileptic drugs in patients with *YWHAG* variants.[7] There are some cases in which multiple anti-epileptic medications were prescribed before seizure control occurred.[8]

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RESEARCH AND SUPPORT

Clinical trial information

A clinical trial is a research study that involves testing a new treatment or intervention in people to evaluate its safety and effectiveness. There are a few things you should consider when reading about a clinical trial:

1. The purpose of the trial - It's important to understand the specific goals of the clinical trial and how the treatment or intervention being tested is intended to work.
2. The potential risks and benefits - All clinical trials have some risks, and it's important to understand what these risks are and how they are being managed. At the same time, clinical trials can also offer the opportunity to access new treatments or therapies that may not be available elsewhere.
3. The eligibility criteria - Clinical trials have specific eligibility criteria that outline who can participate.
4. The time and commitment involved - Clinical trials can take place over a period of weeks, months, or even years, and they may require regular visits to a hospital or research center.

As part of the process of obtaining informed consent for participation in a trial, the information above should be explained to you. It is essential that you fully understand this information before making a decision to participate in the study. Therefore, you should not be asked to make a decision until these aspects have been clearly explained.

Although there are no current clinical trials for *YWHAG*, there is an ongoing study interested in characterizing the epilepsy phenotype of patients with variants in YWHAG. There is no 'intervention' for this study, i.e., no treatment is administered as part of it. However, these types of studies provide the foundation for interventional studies in the future.

Interest	Status	Coordinating Site	Study Contact
Characterizing the epilepsy phenotype of patients with variants in YWHAG	Recruiting	Meyer Children's Hospital ITALY	Valentina Cetica, PhD Phone: +390555662844 Email:valentina.cetica@meyer.it

Forming a patient community

Connecting with other families who are going through a similar experience can be incredibly helpful. One way to do this is by forming a patient community. Starting a patient organization can allow you to connect with other families, raise awareness about the disorder, and advocate for better resources and support. Share4Rare has a free toolkit for patient advocacy.

If you are interested in starting a rare disease not-for-profit foundation or if you are looking to expand the impact of your foundation and get your organization “research-ready”, we recommend exploring NORD’s free RareLaunch program: https://learn.rarediseases.org/.

In addition to forming a patient community, creating a patient registry can be a valuable way to connect with other families and advance research efforts for your rare disease. A patient registry is a database of information about people living with a specific disorder and can be used to help researchers and clinicians better understand the disease and develop new treatments. There are a number of resources available for creating a patient registry, including the National Institutes of Health's Patient Registry (https://registries.ncats.nih.gov/), Ciitizen (https://www.ciitizen.com/), Rare X (https://rare-x.org/), and Global Genes (https://globalgenes.org/). These resources can help you get started with building your registry, including guidance on data privacy and management.

Finding others

If you are looking to find others with your specific variant, using MyGene2 can connect you with families who have the same condition or variants in the same gene. It can also connect you with researchers and clinicians who have information on your specific gene or variant. If there is no information on the specific variant or gene, you can create a profile to share any information you have to connect with other families in the future. Additionally, Dr. Matt Might has published a blog post on finding others by harnessing the power of the internet: https://matt.might.net/articles/rare-disease-internet-matchmaking/.