A Cellular and Genetic Approach to Ehlers-Danlos Syndrome

LIBERTY UNIVERSITY

Parker C. Pruitt and Kristin N. Mossé, M.S.

Abstract

This paper serves as an in-depth exploration of a rare genetic disorder, Ehlers-Danlos Syndrome, discussing its genetic basis and pathology, as well as offers a unique perspective from those who are afflicted by it. Ehlers-Danlos Syndrome (EDS) is a connective tissue disorder that ultimately stems from a rare genetic mutation and disruption of the extracellular matrix. Thirteen different variations of this possibly life-threatening syndrome have been established. The types of EDS have different genes that are mutated to specifically result in the variant. The focus of this proposal is the vascular variation, otherwise referred to as vEDS. Vascular Ehlers-Danlos Syndrome can remain hidden until there is a rupture in a major artery. There are two known genetic mutations of the COL3A1 gene that result in vEDS, an amino acid substitution and deletion of multiple exons. These mutations lead to a defective production or lack of production of type III collagen from the alpha-1 chain. This mutated collagen manufacturing leads to increased tissue fragility, particularly in blood vessels. Abdominal Vascular Compression Syndromes (AVCS) all involve some sort of underlying susceptibility to compression of major vessels, but it has yet to be discovered. The umbrella-group AVCS is compiled of Median Arcuate Ligament Syndrome, May-Thurner Syndrome, Nutcracker syndrome, and more. As a new and upcoming research subject, AVCS demonstrates a correlation between each of the syndromes and now even connections with Ehlers-Danlos Syndrome. In order to better understand the contemporary relationship between EDS and AVCS, a deeper analysis of the genetics of Ehlers-Danlos Syndrome, as well as its effects, would be beneficial to patients who suffer from this syndrome and those that fall into the AVCS category.

Introduction and Research Question

Ehlers-Danlos Syndrome (EDS) is the result of a rare genetic mutation that causes a disorder of the connective tissues and affects the durability of the extracellular matrix (ECM). There are thirteen variants of EDS *(1)*, but not everyone with the syndrome has inherited it from a parent. The most prevalent indications of Ehlers-Danlos Syndrome are joint instability/hypermobility, stretchy skin, and fragile tissues. As general as these symptoms are, EDS-specific presentations still coexist within them. Of the thirteen variations, hypermobile EDS (hEDS) is the most common. About one in 3,100-5,000 people are affected with this type *(2)*.

Vascular EDS (vEDS) is one of the rarest as it occurs in about one of every 100,000-200,000 people *(1)*. Observed more frequently in women than in men *(3)*, not every type of Ehlers-Danlos Syndrome shows up the same. In vEDS, the syndrome may not be revealed until there is a rip in a major artery or an organ, but in kyphoscoliotic EDS (kEDS), scoliosis is present from birth *(1)*. To discover the root of Ehlers-Danlos Syndrome, a look at the molecular level is necessary. There are several genes known to be connected with EDS, most of them involving collagen. A mutation in the COL3A1 gene **(Figure 1)** results in the increased fragile tissue aspect, particularly in blood vessels, which is evident in vEDS *(4)*.

This evidence can be seen in vessel rupture *(1)* or compression **(Figure 2)**. Due to the severity, a further look into the COL3Al gene will help give a better comprehension of the syndrome and how to minimize the detrimental effects of the mutation. EDS is a result of two main genetic mutations: amino acid substitution that leads to misfolded proteins and exon deletion. This project focuses on the specific dysfunction of protein folding and the effect in the endoplasmic reticulum (ER) *(2)(5)*. The cell has many ways of keeping up the integrity of the proteome so that normal cellular metabolism can occur and maintain stability. The cell carries out this maintenance using chaperone proteins and ubiquitin, a protein degradation factor *(6)*. By continuing research on this particular syndrome and its cellular components, advocation for patients and expansion of knowledge in the field of rare diseases can be obtained. Rare disease research is hard to come by, especially as there are no experiments on humans, mainly just clinical observations and trends are used. There remains a gap in knowledge of dysfunction in “protein quality control” *(7)* and the resulting impacted protein folding.

Based off of personal experience and introductory research, the question posed is: **Is Ehlers-Danlos Syndrome an underlying cause of Abdominal Vascular Compression Syndromes?**

Methods

1. In order to connect to other patients with similar symptoms, I joined four groups on Facebook centered around each of the abdominal vascular compression syndromes (AVCS) that I am diagnosed with. These syndromes include May-Thurner Syndrome (MTS), Nutcracker Syndrome (NCS), Median Arcuate Ligament Syndrome (MALS), and Pelvic Congestion Syndrome (PCS).
2. Research was conducted using a poll on the groups titled “Renal Nutcracker Syndrome Support Group,” “MALS Awareness Community,” “May-Thurner Syndrome/Pelvic Congestion Awareness,” and “MALS Pals.” The poll asked each member if they were diagnosed with Ehlers-Danlos Syndrome (EDS), had it as a prognosis, or it had not been mentioned as a possibility. Members were prompted to also create a new option if the first three were not applicable.
3. Research was also conducted by reading peer reviewed, primary, and secondary articles during the process of writing research papers for both Cell Biology and Genetics.

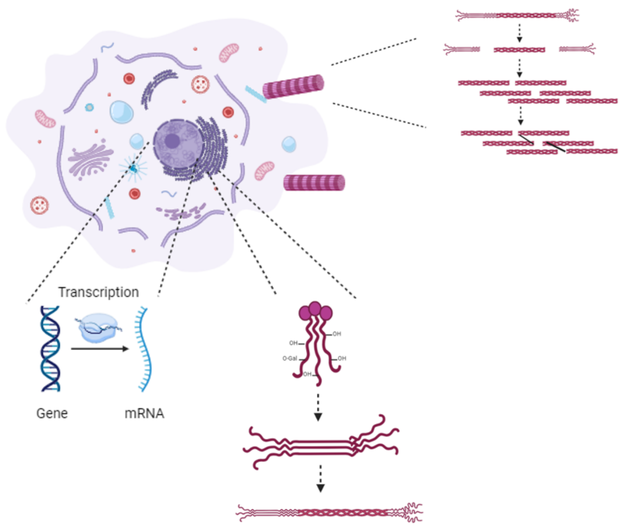

Figure 1. Visual of the summarized type III collagen synthesis starting with the COL3A1 gene. This image, made in BioRender, is the breakdown of how the COL3A1 products form to make type III collagen. The first step is the transcription, on the left side, forming a copy of the DNA sequence. The copy is then read by a messenger RNA (mRNA), the process of translation. The middle of the image demonstrates the function regulation of the protein or post-translational modifications. These convey the role of the protein, but if the protein is abnormal as in EDS, it is ineffective or defective. On the far right of the picture, three identical alpha1 chains are put together, forming the actual alpha1 chain of the protein *(8)*.

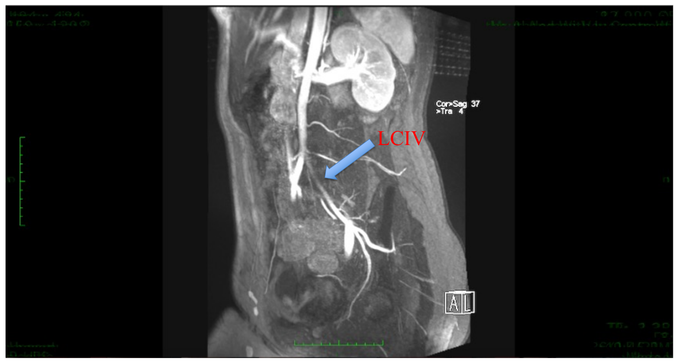

Figure 2. Pruitt, P. MR Abdomen Venogram with IV contrast. This image is from the patient’s MRV done at the Mayo Clinic in Jacksonville, Florida. It is demonstrative of how Vascular Ehlers-Danlos Syndrome (vEDS) affects the strength of the connective tissues, specifically those of the cardiovascular system. The effulgent vessels represent venous blood flow. However, there is an absence at the left common iliac vein (notated by LCIV). Under normal circumstances, the LCIV would be bright like the other veins. In this case, the overlying right common iliac artery completely compresses that vein causing an absolute lack of blood flow. The fragility of the tissues creates an opportunity for vessels, even larger ones, to be flattened. This compression is known as May-Thurner Syndrome (MTS). The symptoms presented were cold to the touch, purple discoloration, swelling, pitting edema, prolonged capillary refill time (CRT), and numbness. These symptoms only manifested in the left leg. There are several other vascular compressions found to be concurrent with EDS including Nutcracker Syndrome (NCS), Median Arcuate Ligament Syndrome (MALS), and Pelvic Congestion Syndrome (PCS). The patient has been diagnosed with all of the syndromes listed above, MTS being the most prominent.

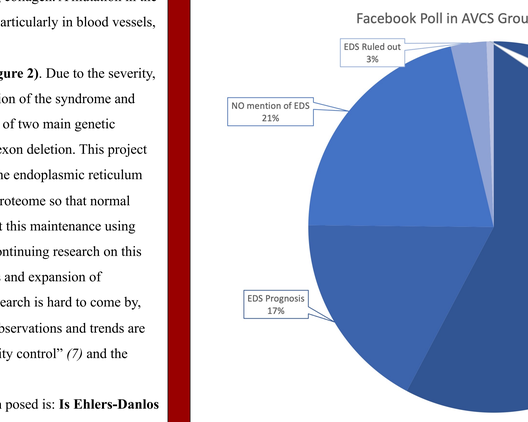

Figure 3. Result of the Facebook Group Polls. As seen above, most of the population surveyed responded with a diagnosis of Ehlers-Danlos Syndrome (EDS). The next largest part of the pie chart is representative of patients who had not heard of EDS for a possibility of a diagnosis. It appears that it is most common for someone with any of the main abdominal vascular compression syndromes to have EDS as well. While further research is still necessary, a correlation between EDS and AVCS seems to be present.

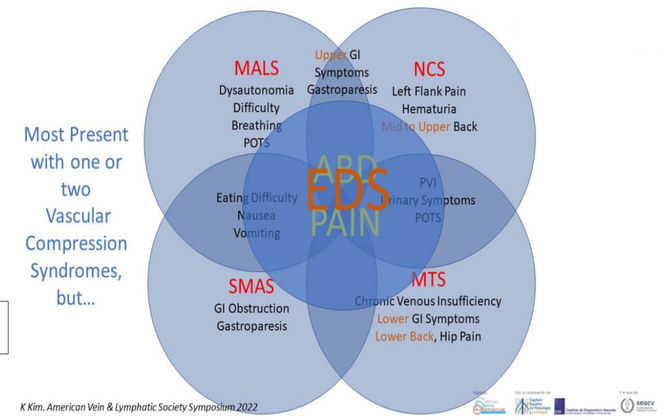

Figure 4. Overlapping Symptoms of Abdominal Vascular Compression Syndromes (AVCS) The above Venn diagram was created by Dr. Kurtis Kim who treats patients with multiple abdominal vascular compressions syndromes (AVCS) *(9)*. The symptoms of each individual syndrome can overlap as shown. Often times patients experience issues that are seemingly unrelated. As Dr. Kim has continued his research, he has discovered that his patients are for the most part also diagnosed with Ehlers-Danlos Syndrome (EDS). This information would indicate a common denominator behind the symptoms, EDS.

Acknowledgements

This research project was inspired by my personal experience with Ehlers-Danlos Syndrome and its effects on my quality of life. A huge thanks to Dr. Kurtis Kim and Dr. Elizabeth Brooke Spencer for taking a chance on me. These doctors played a monumental role in my life, my story, and the lives of others. Another thanks to Professor Mossé who encouraged me to pursue this project.

Thank You, Lord, for this life and my testimony that glorifies You.

Results and Conclusion

Results:

After two weeks, there were 161 total votes across all groups. Tallying the results, a percentage was obtained for each option, and subsequently, a pie chart was made demonstrating the collected observations **(Figure 3)**.

Using Abdominal Vascular Compression Syndromes (AVCS) as the independent variable, reason being those surveyed have a confirmed diagnosis of one or more vascular compressions, EDS was the dependent variable as it is what is being observed and determines the results. The results of the collected data indicate that EDS is found more often with AVCS than not.

Conclusions:

Despite the limited knowledge of AVCS and EDS, patients that present with either symptoms of EDS or AVCS should be evaluated for both syndromes. Further research must be done to verify this conclusion, but it is apparent that EDS is a common finding and is likely heavily correlated with AVCS.

Future Work

There has been a new development of a mouse with vascular Ehlers-Danlos Syndrome (vEDS) by the research team at Johns Hopkins University, the genetic mutation of which is the gly-to-ser amino acid substitution *(9)*. This mouse model can be used to exhibit the most prominent physical presentations of vEDS to find new treatments and solutions for patients affected by this connective tissue disorder.

- An antibody-based enzyme-linked immunosorbent assay (ELISA) can be used in the quantification of ubiquitin linkages on tagged misfolded proteins.
- Fluorescence microscopy can be used to establish the half-life of the misfolded proteins that would in-turn influence cell mechanism regulation.

There are few preventative treatments for Ehlers-Danlos Syndrome, yet research continues to provide new information. The patient whose image was used in **Figure 2** was made aware of an EDS clinic being held at the Mayo Clinic in Jacksonville, Florida. At this clinic, patients undergo genetic testing as well as evaluation of symptoms. After evaluation and screening, doctors are assigned to the patient’s care team and the offer of participating in clinical trials is made available. Even with these options a cure is still unknown *(11)*. Although EDS cannot be cured, management of symptoms allows for the best opportunity for treatment. A doctor who specializes in each body system can help treat symptoms within their area of expertise *(12)*. Despite the complications that arise from the mutation of the COL3A1 gene, patients with Ehlers-Danlos Syndrome are constantly being advocated for by doctors around the world.

References

1. What is EDS? https://www.ehlers-danlos.com/what-is-eds/
2. Chiarelli, N., M. Ritelli, N. Zoppi, and M. Colombi. 2019a. Cellular and molecular mechanisms in the pathogenesis of classical, vascular, and hypermobile ehlers–danlos syndromes. *Genes.* 10:609.
3. Justine Hugon-Rodin, Geraldine Lebegue, Stephanie Becourt, Calude Hamonet & Anne Gompel, Gynecologic symptoms and the influence on reproductive life in 386 women with hypermobility type ehlers-danlos syndrome: a cohort study | Orphanet Journal of Rare Diseases | Full Text. *Orphanet Journal of Rare Diseases.*(2016). https://ojrd.biomedcentral.com/articles/10.1186/s13023-016-0511-2
4. T. Miklovic, V. C. Sieg, in StatPearls (StatPearls Publishing, Treasure Island (FL), 2023). https://www.ncbi.nlm.nih.gov/books/NBK549814/
5. Welihinda, A.A., W. Tirasophon, and R.J. Kaufman. 1999. The cellular response to protein misfolding in the endoplasmic reticulum. 7:293-300.
6. Sun, Z., and J.L. Brodsky. 2019. Protein quality control in the secretory pathway. *J.Cell Biol.* 218:3171-3187.
7. Samant, R.S., and J. Frydman. 2019a. Chapter Two - Methods for measuring misfolded protein clearance in the budding yeast Saccharomyces cerevisiae. *Meth.Enzymol.* 619:27-45. doi: 10.1016/bs.mie.2018.12.039.
8. H. Kuivaniemi, G. Tromp, Type III collagen (COL3A1): Gene and protein structure, tissue distribution, and associated diseases. *Gene.* **707**, 151-171 (2019). https://www.sciencedirect.com/science/article/pii/S0378111919304652
9. Johns Hopkins Medicine. Director: Dietz, Hal M. *The Center for Vascular Ehlers-Danlos Syndrome Research.* https://www.hopkinsmedicine.org/genetic-medicine/research/research-centers
10. Kim, K. 2023. Abdominal Vascular Compression Syndromes: Current and Future of Management of Abdominal Vascular Compression Syndromes that Includes Median Arcuate Ligament Syndrome, Nutcracker Syndrome, Superior Mesenteric Artery Syndrome, May-Thurner Syndrome, and Associated Pelvic Venous Insufficiency. *Journal of Vascular Surgery.* 77:50S.
11. Ehlers-Danlos Syndrome Clinic in Florida. (2023). https://www.mayoclinic.org/departments-centers/ehlers-danlos-syndrome-clinic-florida/overview/ovc-20522975
12. T. Miklovic, V. C. Sieg, Ehlers danlos syndrome. (2019). https://www.ncbi.nlm.nih.gov/books/NBK549814/