The Search for Potential Causative Gene(s) associated with Hypermobility Ehlers-Danlos Syndrome

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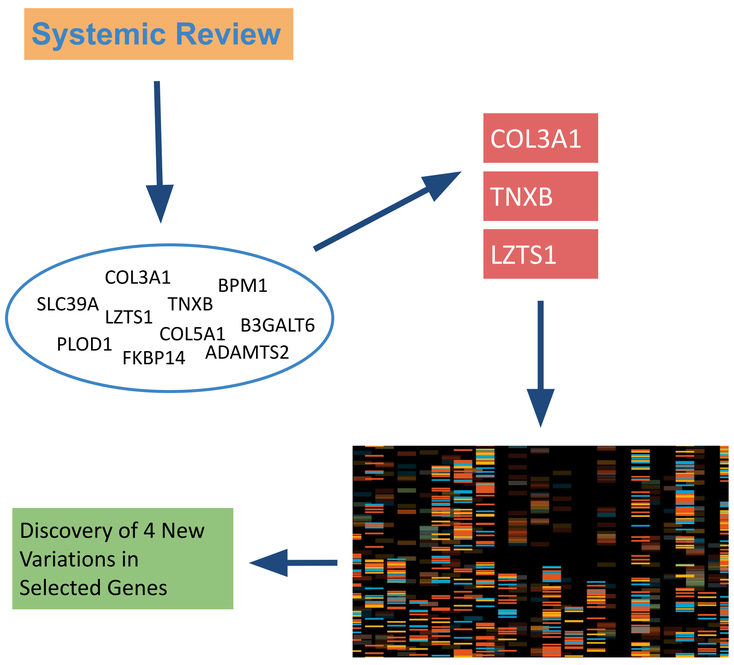
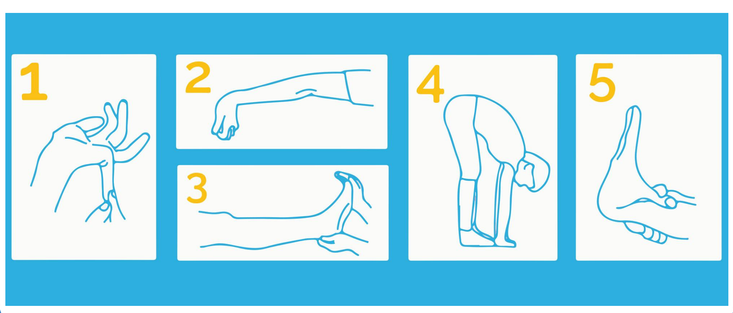
Introduction

Ehlers-Danlos Syndrome (EDS) is a group of inherited conditions mainly affecting connective tissue. This causes larger problems down the line, primarily with skin tissue, joints, and blood vessel walls. Out of the 13 subtypes, hypermobile EDS (hEDS) is one of particular interest as it's both the most common and the only one without genetic markers. Some common symptoms of hEDS include but aren't limited to: mast cell hypersensitivity, bone fragility, easy bruising, vascular fragility, muscle hypotonia, and poor wound healing. However, it is mostly characterized by joint hypermobility and skin hyperextensibility. The lack of biomarkers available for hEDS results in a severe lack of diagnosis in the general population. This usually leads to patients seeing various medical practitioners in what appears to be distinct and unconnected problems when in fact they are all symptoms of hEDS.



Another prevailing problem associated with hEDS is the misidentification as child abuse. Occurring mostly in infants, bone fragility and joint hypermobility leads to many fractures and injuries even with normal handling. However, the lack of awareness surrounding this disease has led to many pediatricians separating families and putting upstanding parents behind bars. **The aim of this study is to reaffirm and corroborate existing potential gene(s) by further analysis with a 20 patient full-genome database.**



The Beighton Test: A easy test for hypermobility on a 9-point scale

Results

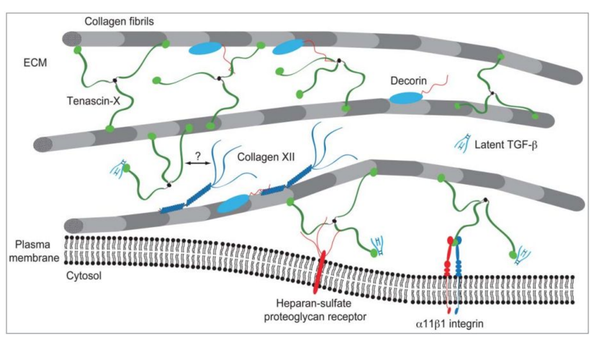
Gene:	SNP	Zygosity of Variant:	HGVSC	HGVSP	Allele Frequency	Allele	Clinical Significance	Consequence
LZTS1	rs112782644	Heterozygous	c.49C>G*	His17Asp	0.000326	T>C	Not Reported	Missense Variant
COL3A1	rs35795890	Heterozygous	c.1804C>A	Pro602Thr	0.006699	C>A/C>G	Benign/Likely-Benign	Missense Variant
TNXB (wINT V)	rs1257331554	Heterozygous	nt 85C-->T*	Arg29Trp	0.00007	T>C	Not Reported	Missense Variant
TNXB	rs60738846	Heterozygous	nt 85C-->T*	Arg29Trp	0.00193	C>T	Not Reported	Missense Variant

The graph above shows the 4 new heterozygous missense variants linked to hEDS, along with their respective frequencies, clinical significance, allele types, and zygosity. During the process of genomic analysis, no results were initially found with the given allele frequencies of the genes. This meant none of the patients in the 20-patient genomic pool had the exact variation as those found in the literature review. Criterias were then changed to 3% prevalence, expanding the search and finding the variants shown above. Every variation found has paved the way for substantial inquiry back towards the original study as well as all the papers that reference them.

The first variant discovered was a T to C change in LZTS1. This gene is very commonly studied because of its link to multiple human cancers, making it relatively easier to speculate its relationship with hEDS. It has a wide range of functions involving cell cycle regulation, axon growth and guidance, and neuronal development. It has also been shown to disrupt the ProSAP2 gene, which is connected to a proven syndrome linked to joint laxity. Last but not least, LZTS1's modulation of gene transcription has been speculated to affect the expression of certain genes encoding those of connective tissue.

The next variant located was a C to A change in COL3A1. This gene is especially interesting as it is typically linked to Type 4 EDS, the most severe and life-threatening of all 13 subtypes. A mutation to this gene leads to a reduced and altered collagen organization which impairs the secretion of molecules into the ECM.

The final 2 variants found were T to C changes in TNXB. This is the gene that encodes for Tenascin X, part of a still growing Tenascin family consisting of other extracellular glycoproteins. Its function consists of maintaining distance between collagen fibrils by forming bridges, which upholds the connective tissue everywhere in the body.



A model showing TNX function in between collagen fibrils

Discussion

After a much more thorough dissection of the genetic variants found, various interpretations were made based on the original case studies in the literature review. In LZTS1, 1 new variant was found in 16 patients in hEDS. While in the original study, 2 variants were found in 34 patients from one family along with 3 variants out of a 230 patient database. However, the patient database consisted of both hEDS and BJHS (Benign-joint hypermobility syndrome), indicating that the actual ratio of variant:patient could be significantly lower. **With that in mind, the variant discovered helps ascertain the prevalence of LZTS1 among hEDS patients, potentially helping it become a (albeit limited) biomarker for this disease.** It's also worth noting that the original case study only checked within the protein-coding part of the genome, which meant any variations in intronic regions and promoter sequences went unreported. Future studies should take into consideration conducting full genome-wide linkage analysis in order to ensure no stone goes unturned.

The next variant in COL3A1 is especially interesting and one of the more notable discoveries in this study. As previously mentioned, COL3A1 is typically linked to Type 4 EDS, a more severe type that typically has no symptoms of hypermobility (a hallmark trait of hEDS). Only 1 study in 1994 discovered a family with the physical symptoms of hEDS but the genetic basis of COL3A1/Type 4 EDS. As a result, literature reviews on EDS typically assert that while COL3A1 has been linked to hEDS, it was only once and has never happened since. **That makes the discovery of COL3A1 in this small patient pool (16) especially interesting as it demonstrates that this gene has a larger involvement in hEDS than most imagined.** Future studies should be conducted with larger patient populations of hEDS in order to fully flesh out this relationship along with its prevalence.

Last but not least, 2 variations found in TNXB are also set to be the first of its kind. In the original case study, no male was found with the mutation but 9 females were. Although this is likely due to the small sample size (6), the **variation found in this male infant is the first variant of TNXB located in a man.** Another point of interest is the ratio of variation to patient pool. **In both studies, 3 missense variations were found in 16 patients with hEDS.** Although this exact pairing is more likely than not a coincidence, it's interesting to help speculate its prevalence in future studies.

Although no exact biomarker was discovered accounting for all the symptoms of hEDS, this study does support existing case studies while paving way for future analysis to be performed. It has been especially beneficial in the quest to determine the prevalence of this disease, in hopes that it will eventually reach the awareness it deserves. With that in mind, further research should be conducted to narrow down promising prospects so millions around the world can finally be made aware of the disease immuring them in their day-to-day routine.

Methods

A broad systematic literature review was conducted in search of potential candidate genes linked to hEDS. Keywords used under databases PubMed and Google Scholar were "biomarkers" and "hypermobile Ehlers-Danlos Syndrome" (OR "Ehlers-Danlos Type 3" OR "Ehlers-Danlos Hypermobility type"). Articles were selected on the basis of sample size, type of genetic analysis, and relevance. Appropriate data was then collected from each article: diagnostic criteria (< 2017), allele type, prevalence, variations, etc.

	Article	Gene(s)	Diagnostic Criteria	Variations	Type of EDS	Sample Size	Type of Study	Allele Type	Control Group?	Prevalence	Research Question
1	https://www.ncbi.nl	C 8p22-8p21.1	Prior to 2017	49C, 158SC, 749C	NEDS, BJHS (Benign Joint)	26	3 Gen. Family	Heterozygous Variants	No	N/A	Using a deep genomic dive into one family with multiple cases of EDS to explore likely candidates with a causational link to hEDS
2	https://www.ceti	TNXB	Prior to 2017 (2003)	N/A	NEDS, BJHS	27	One Family	Recessively Inherited	No	2.5% (Mutations) 7.5% (Low Serum)	Finding genetic defects associated with hEDS/BJHS through observed phenotypes in patients (heterozygous females)
3	https://www.scie	SLC39A13	Prior to 2017 (2008)	SLC39AC	SCD-EDS; A-EDS VI	59	2 Families	Autosomal Recessive	Yes (262)	Unknown (Need more subjects)	Discovery of slight variation of EDS 6 through study of pyridinolines (collagen fibers) in 6 cases with otherwise normal levels of other collagen measuring factors
4	https://www.scie	FKBP14	Prior to 2017 (2012)	Homozygous Frameshift	EDS VIA - Kyphoscoliotic	6	6 Unrelated Families	Autosomal Recessive	Yes (Relatives + 200 Euro)	N/A	Discovery of variation of EDS VIA in individuals within normal urinary LP/HP ratios
5	https://www.scie	B3GALT6	Prior to 2017 (2013)	B3GALT6, B3GAT3, CHST3, CHSY1	EDS-PP, EDSP1, and EDSP2	9	1 Family (2A/7N)	Autosomal Recessive	Only Relatives	N/A	Proving that the malfunction of B3GALT6 (key enzyme in producing connective tissue) is responsible for a multisystemic disorder that mimics symptoms of EDS
6	https://digital.cat	BPM1	Prior to 2017 (2012)	Phe249Leu Homozygous Missense	Osteogenesis Imperfecta	12	1 Family	Autosomal Recessive	Only Relatives	N/A	Discovery of additional gene leading to OI in case of 2 children with large umbilical hernia.
7	https://analyticmp	LZTS1 (Context of Tumor)	Prior to 2017 (2012)	FEZ1	Tumor Suppressor / Cancer	N/A	Mice/Chicken Embryo	Autosomal Recessive	No	N/A	Finding function/expression of LZTS1 in vitro to predict its role in cell cycle regulation, axon growth/guidance, and migration of neurons.
8	https://pubmed.n	COL3A1	Prior to 2017 (1994)	CB4-5 Peptide (Acetylated) Glycosidase 1705-2260	NEDS	8	One Family	Homozygous (Wild Type) --> Heterozygous	Other Relatives without EDS	N/A	G to A mutation occurs at nucleotide 2512, which causes glycine 637 to serine conversion. Notes: 4 year old boy; 9/9 on Beighton Score. 4 Other family members affected, no vascular fragility
9	https://www.ncbi	LZTS1 (9q22-8p21.1)	Prior to 2017 (2015)	BMP1, LOXL2, CSGALNACT1, SLC39A14	NEDS	34	3 Generation Family	Heterozygous Variants (Variant allele frequency between 25%-75%)	4 Additional Families affected by hEDS (2/0)	2% of hEDS Patients	3 Additional Variants: c.49C>G, p.(His17Asp) /// c.1585C>T, p.(Arg529Trp) /// c.749C>A, p.(Ser250*) Notes: Analysis was performed under assumption that condition is inherited as autosomal dominant (penentrance at 90% at all ages). Disease allele frequency = 0.01, equal recombination fractions in males and females assumed.
10	https://onlinelibr	TNXB	Prior to 2017 (2006)	N/A	NEDS	33	9 Healthy (No hEDS), 8 Haploinsuffici ent, 16 Normal TNX	Heterozygous	96 Unrelated Healthy Individuals	N/A	3 Missense Mutations: nt.85C-->T(Arg29Trp) and rs3583AG (Val1169Met) and rs12097CA (Leu4035IIE) Notes: Arg29Trp likely associated with hEDS, accounts for 15% of all disease-causing mutations, Val1169Met Mutation is also potentially important, alters fiber length.

After careful consideration: COL3A1, LZTS1, and TNXB were chosen as the three to further investigate. After finding specific variants of all three along with other necessary criterias (specific location, allele frequency, prevalence, etc), they were evaluated and compared to the 20 patient full genome database. After initial sample QC, an automated PCR-free library preparation was performed using the Swift 2S Protocol, and a 60X whole-genome sequencing (WGS) of 100 bp paired–end reads were carried out on a HiSeq 2000. Fast QC was used to evaluate the quality of the reads. BWA-MEM was used for mapping the reads to GRCh38 reference of human sequence. The data was then uploaded to Illumina Basespace for final analysis. Illumina's Isaac-based whole-genome sequencing pipeline in Basespace was completed using the WGS samples and VCFs were generated for small variants. The small variant VCFs were then imported into the Variant Interpreter, which performed some basic annotation and filtered for PASS variants. The final results of the WGS analysis were verified by Sanger sequencing.

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