Correlating clinical findings with genetic testing results in patients with concern for connective tissue disorders - a retrospective chart review

by

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Connective tissue disorders (CTD) are a group of conditions that specifically impact proteins in the tissues that hold the body together. Some of these conditions have an established genetic etiology or identified associated gene. Of note, there is no identified molecular etiology for either hypermobile Ehlers Danlos Syndrome (hEDS) and Hypermobility Spectrum Disorders (HSD), and the diagnosis of these is based on clinical and laboratory findings. There is limited information available to determine subsets of patients who would benefit most from genetic testing. Primary care clinicians may be the first to recognize symptoms in hEDS/HSD patients, and they often play an important role in these patients' care. The UPMC Primary Care Precision Medicine Clinic (PCPM) began seeing patients in 2019, and a significant portion of their patients are referred for concern for a connective tissue disorder. A retrospective chart review of genetic testing results and clinician notes in the electronic medical record (EMR) was completed on 135 patients evaluated by PCPM between 2019 and 2022. The goal of the study was to evaluate if any clinical findings are associated with genetic testing outcomes or final diagnoses. Data show 37% (n=50) of patients were diagnosed with hEDS. One patient received a Marfan syndrome diagnosis, one patient received a Loeys-Dietz syndrome diagnosis, and one has a potential Brittle Cornea syndrome diagnosis. The gene with the most identified variants was *TNXB* (n=12). 38% (n=51) of patients were referred by primary care providers. Findings with a statistically significant relationship to hEDS included Beighton score, papyraceous or hemosideric scars, arachnodactyly,

joint dislocations and subluxations, allergies/mast cell abnormalities, poor wound healing, and Raynaud's. There were several findings related to variants in fTAAD genes in addition to findings in autosomal dominantly inherited genes in general. We conclude that performing genetic testing on any individual with concern for CTD is warranted to rule out conditions with known genetic causes such as Loeys Dietz syndrome and Marfan syndrome. This data could be used in the future to educate primary care providers on CTDs and genetic testing for these disorders which are relatively common in the general population.

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Preface

I will never forget opening a patient message sent to me in a previous job, which was cancelling a genetics appointment for concern for hEDS because the geneticist had a bad reputation for dismissing patient pain and joint problems in this condition. This culminated a set of observations in this position that frustrated me for these patients, but this made me want to understand why people would have these perceptions and what I could do to change them. I jumped at the opportunity to do this project to do something to help these patients. I hope any patients who have HSD, hEDS, a chronic illness, or chronic pain can feel validated by this study.

I would not be in the place I am today without the help of several people. Thank you to my thesis committee for your feedback and aiding me through this process which was very new to me at the inception. Your work is inspiring and motivating for me to continue to make a difference. To Natasha, for spending countless hours keeping me on track and all your help along the way as you chaired my committee. I want to thank my classmates and friends for all of our talks and your constant encouragement, and for listening as I formed this thesis project idea. Thank you to genetic counseling program leadership for always being willing to help me throughout this process. Thank you to my family: to my mother, for her unwavering support and love throughout graduate school; to my sister, this work is dedicated to your health journey; and to my father, grandmother, and aunt for cheering me on to get me where I am today. Finally, thank you to Jodie Vento and Chris Munro for taking a chance on me and believing in me for the past four years. I would not be here today without your mentorship.

1.0 Introduction

Connective tissue disorders (CTD) are a group of conditions that specifically impact proteins in the tissues that hold the body together. These include, but are not limited to, Marfan Syndrome, Loeys-Dietz Syndrome, Ehlers Danlos Syndrome, and Hypermobility Spectrum Disorders. To date, some of these conditions have an established genetic etiology or identified associated gene. There is no identified molecular etiology for either hypermobile Ehlers Danlos Syndrome (hEDS) and Hypermobility Spectrum Disorders (HSD). The diagnosis of these is based on clinical and laboratory findings, although the clinical diagnostic criteria are updated. Clinical criteria for diagnosis include: Beighton score, presence or absence of musculoskeletal problems, and features of other EDS subtypes. Additionally, the spectrum of genes involved and types of mutations linked to all forms of Ehlers Danlos syndrome is constantly expanding (Ritelli et al 2019, Yang et al 2022). Several groups are currently looking into the genetic causes of hEDS and HSD, but presently, it is important to rule out other EDS subtypes and other genetic connective tissue disorders with genetic testing because of the clinical heterogeneity of these conditions. This is especially important due to the possibility of some conditions affecting the health of the heart and eyes while hEDS is not known to affect these at this time. A recent study found that, excluding hEDS patients, a correlation with positive molecular diagnosis exists for generalized joint hypermobility, poor healing, easy bruising, atrophic scars, skin hyperextensibility, and developmental dysplasia of the hip in children who have other EDS subtype characteristics whether or not they meet the 2017 criteria (Damseh et al 2022).

Primary care clinicians may be the first to recognize symptoms in hEDS/HSD patients, and they often play an important role in patient care by providing them referrals and help manage many

of the symptoms. Atwell and colleagues point out that there are many challenges facing primary care providers who see these patients, including any other diagnoses to consider as well as triaging these patients appropriately (Atwell et al 2021). Atwell and others also discuss the other types of symptoms that are important to recognize in people with hypermobility concerns and treatment for such conditions; however, there is no mention of genetic testing offered to this patient population (Atwell et al 2021). Furthermore, McGillis and colleagues based out of an EDS clinic in Toronto found that the assessment of Beighton score was higher when performed by a primary care physician as opposed to the providers who specialize in hypermobility, and the authors suggest utilizing the detailed description of assessing the Beighton score in the current version of the diagnostic criteria and educating these providers on how to use this tool (McGillis et al 2020).

Individuals with hypermobility spectrum disorders can be impacted in multiple body systems: musculoskeletal, dermatological, gynecological, ocular, oral, immune, mandibular, cardiovascular, autonomic, gastrointestinal, neurological as well as psychological and developmental impacts; and can include several comorbidities (Gensemer et al 2021). These patients may endure years of symptoms without a diagnosis and often seek answers from a variety of different specialties corresponding to their symptoms. There are often long wait times to get appointments, particularly in busy genetics centers throughout the country. Genetic counseling services are more frequently found in large cities (Bellaiche et al. 2021). With criteria for diagnosis changing in recent years, patients face removal of diagnosis or may undergo re-diagnosis.

1.1 Specific Aims

1. Identify clinical findings associated with connective tissue disorders through retrospective chart review. Conduct a retrospective chart review via the EPIC EMR software at the UPMC Primary Care Precision Medicine clinic to identify clinical findings through intake evaluations and subsequent outcomes in patients that correlate with positive, negative, and variants of uncertain significance findings on genetic testing.
2. Determine statistically significant clinical findings linked to genetic test outcomes. Evaluate data to correlate data with testing outcomes to see if there are any statistically significant predicting factors using statistical analysis.
3. From correlating our data, design an algorithm for clinical providers to categorize genetic testing and triaging strategies for Hypermobility Spectrum Disorder patients and create recommendations for primary care providers. Use results of statistical analysis to create an algorithm to describe individuals that would most likely fall into the categories of hypermobile Ehlers Danlos Syndrome versus Hypermobility Spectrum Disorder versus a molecular diagnosis of a connective tissue disorder for use in the primary care setting. Use the algorithm to create recommendations for patients who would be identified to have a molecular diagnosis from genetic testing and who would be identified as needing more emergent triage.

2.0 Manuscript

2.1 Background

Connective tissue disorders (CTD) affect the tissues that hold the body together. When the connective tissue is not formed properly, CTDs can present with a variety of symptoms and have been classified into different syndromes based on molecular and clinical findings. Ehlers Danlos Syndrome (EDS) has thirteen different subtypes which all have a different constellation of symptoms and genetic associations, although there is substantial overlap between the subtypes. Hypermobile Ehlers Danlos Syndrome (hEDS) does not currently have an identifiable single genetic cause but a set of diagnostic criteria established in the 2017 International Classification of Ehlers-Danlos Syndromes (Malfait et al. 2017). Additional studies have asserted faults with these criteria, including differences in assessing the Beighton score among practitioners unfamiliar with EDS (McGillis et al. 2020), diagnostic rates prior to this new set of criteria compared with now (McGillis et al. 2020), additional symptoms that providers did not understand such as the decreased efficacy of pain relief medications (Pezaro et al. 2020), and that stricter criteria are not helpful in the absence of further education (Martin 2019). The new criteria may not be serving patients in the real world as they are intending to. The way we diagnose these conditions needs work in assessing the correct findings in patients and not overlooking the patient's main concerns in the process.

Hypermobility spectrum disorders (HSD) are a spectrum of conditions characterized by hypermobility, joint instability, and chronic pain. They are classified based on where and when joint hypermobility is present, as defined in Castori et al. 2017. It is classified into generalized,

peripheral, localized, and historical diagnoses. HSD may be further classified into asymptomatic and symptomatic categories. HSD does not have an identified genetic cause at this time. With the physiologic similarities to hEDS and lack of a known genetic cause at this time, it is important to distinguish a generalized HSD diagnosis from hEDS using the diagnostic criteria for each, with specific focus on the systemic involvement in hEDS and ruling out symptoms of a different form of EDS or a different CTD altogether (Atwell et al. 2021). It is possible to have joint hypermobility and not have HSD, so it is important to get a complete workup and eliminate any other potential etiologies of hypermobility (Atwell et al. 2021). It should also be noted that prior to publication of the 2017 criteria, HSD was referred to as Joint Hypermobility Syndrome (JHS).

There are additional comorbidities which are not in the 2017 criteria that have been found in high frequencies in individuals with EDS and HSD. McGillis et al. 2020 found that orthostatic intolerance, postural orthostatic tachycardia syndrome (POTS), mast cell activation syndrome (MCAS), gastrointestinal (GI) dysfunction, psychological comorbidities, and headache/migraine, were found in similar frequencies in those meeting the 2017 criteria for hEDS and those who did not. Additionally, GI dysfunction was found in 90% of those patients meeting the 2017 diagnostic criteria for hEDS (McGillis et al. 2020). Pain is a significant part of many patients' experiences with these conditions (Schubart et al. 2022). There is limited evidence on how to manage pain in these patients, as medications may not be tolerated due to gastrointestinal comorbidities, physical therapists lack awareness of the management of the condition, and pain interventions do not have established evidence on safety and efficacy in addition to the commonly recognized resistance to local anesthesia in EDS patients (Zhou et al. 2018). Cardiopulmonary manifestations have also been described, including orthostatic symptoms (Peebles et al. 2022), exercise limitations or exercise intolerance along with respiratory manifestations (Bascom et al. 2021), and pneumothorax

(Bascom et al. 2021). Kciuk et al. 2022 determined that several pelvic floor symptoms have a high prevalence in individuals with EDS: stress urinary incontinence, urgency urinary incontinence, fecal incontinence, and pelvic organ prolapse. Many patients with EDS also report symptoms of MCAS (Cheung and Vada 2015, Seneviratne et al. 2017). Higher incidence of depression and anxiety has been observed in connective tissue disorders (Saetre and Eik 2019), and it has been suggested that autism may be a significant comorbidity with EDS (Rochetti et al. 2019). Mental health conditions in these patients have been suggested to be implicated by the restrictions imposed by their condition, healthcare limitations, social stigma, fear of the unknown, and ways of coping (Bennet et al. 2021). Individuals with suspected EDS had high levels of psychological distress (Rochetti et al. 2019).

Marfan syndrome is a connective tissue disorder characterized by aortic dissections and aneurysms, arachnodactyly, pectus carinatum, characteristic facial features, high myopia, mitral valve prolapse, and pathogenic or likely pathogenic variants in the *FBN1* gene. This condition is diagnosed via the Ghent criteria (Loeys et al. 2010). Management includes yearly echocardiograms, ophthalmology evaluations, and orthopedic evaluations in addition to avoiding contact sports and vigorous exercise (Loeys et al. 2010, Dietz et al. 2001).

Loeys Dietz syndrome (LDS) is characterized by systemic features, aortic root enlargement, and pathogenic or likely pathogenic variants in the *SMAD2*, *SMAD3*, *TGFB2*, *TGFB3*, *TGFBR1*, or *TGFBR2* gene (Loeys et al. 2018). Management recommendations include echocardiograms, ophthalmology exams, and identification and management of any arterial or craniofacial concerns (Loeys et al. 2018).

The knowledge surrounding phenotypes of connective tissue disorders is expanding using genetic testing (Ritelli et al. 2019, Ritelli et al. 2020, Yang et al. 2022). The established clinical

criteria for these conditions does not capture all patients with a molecular diagnosis from genetic testing results (Damesh et al. 2022, Shalhub et al. 2020). The genetic causes of hEDS and HSD are still under investigation. There is some, yet still insufficient, evidence that variants in the *TNXB* and *LZTS1* genes play a role in the development of hEDS, and increased expression of *MMP9* and *SNAI1* in fibroblasts of patients with hEDS has been hypothesized to play a role in the inflammatory nature of hEDS and HSD (Scicluna et al. 2021). For now, multigene panel testing can be done to detect whether there is a molecular diagnosis of one of the other CTDs This is the preferred method due to the heterogeneous nature of these conditions and the efficiency of looking at many genes at once, though there are still limitations such as detection of deletions or insertions (Junkiert-Czarnecka et al. 2022).

Although primary care providers manage care of these patients, they often lack the knowledge and information about CTDs to share with these patients (Shalhub et al. 2020, Anderson and Lane 2021). Primary care providers are able to manage and coordinate much of the care for this patient population, including treating chronic pain based on symptoms, physical and occupational therapy, and joint stabilizing devices (Atwell et al. 2021). Primary care clinics have tended to over-standardize their approach to this patient population leading to poor outcomes, but equally difficult from the patient perspective is when there is no pathway for these patients through a clinic (Anderson and Lane 2021). These providers tend to refer out to genetics centers for evaluation. There are resources available through organizations such as Mountain States Regional Genetics Network and Ehlers Danlos Support UK that are comprehensive tools; however, it is unclear how widely used or known they are, and they do not include information for when and how to order genetic testing ("The Ehlers-Danlos Syndromes GP Toolkit."; "Ehlers-Danlos Syndrome (EDS) Algorithm and Resources for Primary Care.").

The UPMC Primary Care Precision Medicine (PCPM) clinic is a multidisciplinary clinic housed in the Department of Family Medicine at the University of Pittsburgh (Massart et al. 2021). The team consists of a primary care physician with additional genetics knowledge, a pharmacist specializing in pharmacogenomics, and two certified genetic counselors. The clinic sees patients on an outpatient basis for a variety of concerns and conditions, including but not limited to a family history of cancer, carrier screening, adult-onset neurodegenerative conditions, interpretation of direct-to-consumer genetic testing, pharmacogenetic testing, and CTD. The clinic has been operational since 2019 and fills a gap in adult genetics care in Pittsburgh. The clinic does not have set criteria for when to order genetic testing for CTD patients at this time.

2.2 Methods

This study was approved by the University of Pittsburgh Institutional Review Board (STUDY22100143) under exempt review category 4. This included a Waiver of HIPAA authorization. See Appendix A for a copy of the full determination.

2.2.1 PCPM Clinic Flow

Referrals to PCPM are received from within and outside of the UPMC system. The referrals are triaged by the administrative team with input from a genetic counselor to identify more immediate referrals and then scheduled for an initial visit. At the initial visit, the genetic counselor collects personal and family history and the physician goes through a list of different concerns related to CTD, which the patients self-report. The physician and genetic counselor meet

to determine what if any genetic testing is warranted and create a testing plan. Informed consent is reviewed with the patient often during a separate visit with the genetic counselor, and the order for genetic testing is placed. Genetic testing results are reviewed by the team, and the physician and genetic counselor meet with the patient to review results, determine the final diagnosis, and plan for next steps. Patients are encouraged to follow up if additional concerns arise and after one year for updates to any variants of uncertain significance (VUS) discovered with genetic testing.

2.2.2 Study Population and Data Identification

The PCPM clinic identified patients who have been evaluated by the physician and genetic counselor and had genetic testing ordered through the clinic between 2019 and 2022 prior to data collection. Patients from this list were then identified in the EPIC electronic medical record system. Patients were included if they were located in the electronic medical record, over the age of 18 at the time of the visit, had an initial visit with the clinic staff, had genetic testing ordered, and that genetic testing had resulted. With these criteria applied, the final number of patients from whom data was collected was 135.

2.2.3 Data Collection

Initial visit notes from the physician and genetic counselor were reviewed. The physician note lists out categories of symptoms where patients self-report a ranking from 0 to 5 with how much that group of symptoms impacts them. Other symptoms were self-reported as being present or absent by the patients; data included information on whether individual symptoms were present or absent as well as specifics about the degree or characteristics of certain symptoms. The

diagnostic criteria for hEDS were often included in the physician note as a baseline for whether the patient will meet criteria after genetic testing is completed. The genetic counselor note was reviewed for complete family history information, and scanned pedigrees were available in the medical record to supplement this information. Additionally, lab work was reviewed in the medical record, specifically focusing on positive autoimmune labs. Imaging results were reviewed in the medical record as well as record of other procedures completed for the patient, and data about the findings were recorded. See section 2.2.3.2 for a complete list of data points recorded.

2.2.3.1 Genetic Testing

Genetic testing results were scanned into the chart once they became available. Results were communicated to the patients during a follow-up visit, at which time the physician and genetic counselor reviewed results and the final diagnosis with the patient. Genetic testing was sent to one of three laboratories: Blueprint Genetics, Invitae, or GeneDx. The test completed for each patient was a Connective Tissue Disorders panel, with the genes and analysis varying by lab and by date the panel was completed. The team sent the Invitae panel when there were more specific heart or vascular concerns such as aneurysms, as there are additional thoracic aortic aneurysm/dissection genes on this panel, or if there was a specific reason for desiring a quick turnaround time. They sent the GeneDx panel when the concerns were mostly hypermobility-based, and this panel included the *TNXB* gene. The Blueprint panel was sent during the beginnings of the clinic because of the ease of customization of the panel but eventually fell out of use by the team. Each of the panels covered the genes most concerning for cardiovascular concerns related to CTDs. See Appendix C for current comparison of these panels. Results were classified as positive, meaning a pathogenic variant was found in one of the genes on the panel linked to the phenotype; negative, meaning no variants in the genes linked to the phenotype were found; or a

variant of uncertain significance (VUS), which is an uncertain result and may require further study to understand the nature of this variant. Variants of uncertain significance were further delineated by the inheritance pattern of the gene: dominant, recessive, or X-linked.

2.2.3.2 Data Points

The list of data points is included here and the data points from the physician note are also listed out in Appendix B. The sets of symptoms ranked from 0 to 5 include joint instability/subluxations/dislocations (specify), GI symptoms (specify), skin stretching/scarring/bruising/tearing (specify), widespread musculoskeletal pain, mental health symptoms (specify), fatigue/brain fog (specify), autonomic dysfunction (specify), urological/gynecological symptoms, and activity intolerance (specify).

The set of symptoms that were self-reported as present or absent include scoliosis (specify), fibromyalgia, chronic fatigue, autoimmune encephalitis, proprioception issues, temporomandibular joint issues, muscle stiffness/tightness, dental overcrowding or high arched/narrow palate, allergies or mast cell abnormalities, low bone density, chronic neck strain, poor wound healing, flat feet/fallen arches, cardiovascular problems (specify), history of poor response to anesthesia, hernia (specify), organ prolapse (specify), spontaneous organ rupture or pneumothorax or vascular rupture (specify), family member diagnosed with CTD (specify family member and degree of relationship), positive family history of hypermobility (specify family member and degree of relationship), critical findings in the family history (specify family member and degree of relationship), pain in more than 2 limbs daily for 3+ months, chronic widespread pain for 3+ months, POTS, Raynaud's, congenital hip dislocation, eye problems (specify), Beighton score out of 9, height in centimeters, arm span, skin texture (specify), skin hyperextensibility (specify degree), skin striae, pectus excavatum/carinatum, piezogenic papules

of the heels, atrophic scarring, papyraceous or hemosideric scars, arachnodactyly, molluscoid pseudotumors, subcutaneous spheroids, epicanthal folds, and blue sclerae.

Reports from imaging studies and procedures completed for the patients were reviewed, including echocardiograms, ophthalmology exams, CT scans, and tilt table tests. The status of these procedures (i.e. whether they were completed or not) as well as significant results were collected. Autoimmune labs that were positive were collected from the physician note and in the medical record.

The final diagnosis after genetic testing was a data point from the physician note for the follow-up visit. hEDS diagnostic criteria 1/2a/2b/2c/3 per the 2017 hEDS diagnostic guidelines were described in the physician note or on a separate paper scanned into the media tab for patients seen in the earlier days of the clinic. Genetic testing results including variant, whether it was positive or negative of uncertain, the inheritance pattern of the uncertain variant, and laboratory where the genetic testing was completed were entered. Variants of uncertain significance were deemed suspicious if the medical or family history made sense in the context of the variant.

Demographic information collected included ancestry, gender of birth record, and the department referring to the Primary Care Precision Medicine Clinic. Ancestry information was found in the demographics section of EPIC and the family history information of the genetic counselor note.

2.2.4 Data Analysis

Summary statistics were completed by adding the number of patients reporting a clinical finding, which final diagnosis the patient was found to have, and types of genetic testing results.

Findings with a total number of patients below ten were deemed to not have the power for further analysis.

Data from the findings where enough patients reported them were entered into the statistical analysis program Stata. Genetic testing results were broken into a positive or VUS result category and a negative result category. Additionally, genetic testing results were also categorized into if the patient had a *TNXB* gene finding or not, if the patient had a collagen gene finding or not, or if the patient had a familial thoracic aortic aneurysm and dissection gene finding or not. Collagen genes refers to the genes that encode collagen proteins in which variants were found in the patients in this study. These include *COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, COL9A1, COL9A3, COL11A1, COL11A2,* and *COL12A1.* Familial thoracic aortic aneurysm and dissection genes in which variants were found in the patients in this study included *ACTA2, BGN, LOX, MAT2A, MFAP5, MYH11, MYLK, NOTCH1, PRKG1, SMAD3,* and *SMAD4.* Chi-squared tests of independence were performed comparing each clinical finding with the final diagnosis of hEDS or HSD, comparing each clinical finding with genetic testing outcome categories, and comparing each clinical finding with the gene categories; final diagnosis was also compared with genetic testing outcome categories using the Chi-squared test. For the Beighton score and height findings, a t-test was used to determine if the mean value in each category differed from the mean in all patients. The t-test was performed to compare the categories of final diagnosis of hEDS and HSD, genetic testing outcome categories, and gene categories with Beighton score and height. P-values of under 0.05 were considered statistically significant, though p-values of under 0.1 are also reported here because of the small sample size of the study. Odds ratios were also calculated for the relationships with significant p-values and reported.

2.3 Results

2.3.1 Demographic and Patient Information

The majority of the patients identified as only having European ancestry (n=122, 90%) and assigned female at birth (n=119, 88%). One patient identified as Asian and one patient identified as Hispanic. Four individuals did not have a specified ancestry in the chart. The remaining seven patients identified as European and another non-European ancestry. Referral sources were varied, and percentages of referrals coming from different departments are demonstrated in Figure 1. The largest section incudes those referred by a primary care physician at 38%, which is expected based on the location in the clinic within the department of family medicine. Self-referrals and rheumatology referrals were the next largest sources at 20% and 15%, respectively.

Genetic testing samples were sent to Blueprint Genetics (n=8), GeneDx (n=119), or Invitae (n=8). The average height of patients was 167.77 cm with a standard deviation of 9.12 cm and a range of 151.1 cm to 198.1 cm. Either before or after the initial PCPM appointment, 66 patients underwent an echocardiogram, 39 patients had an ophthalmology exam, 43 had a CT scan, and 20 underwent a tilt table test.

The final diagnosis that patients received was hEDS for 50 patients, HSD for 69 patients, Loeys-Dietz syndrome in one patient, Marfan syndrome in one patient, and Marfanoid habitus in one patient. The remaining 13 patients do not have a final diagnosis for a variety of reasons. Regarding genetic testing results, patient had negative (n=55), positive (n=2), positive for a variant in an autosomal recessive (AR) gene (n=6), variant of uncertain significance (VUS) in an autosomal dominant (AD) gene (n=48), VUS in an AR gene (n=37), and VUS in an X-linked gene (n=3) (see Figure 2 for breakdown).

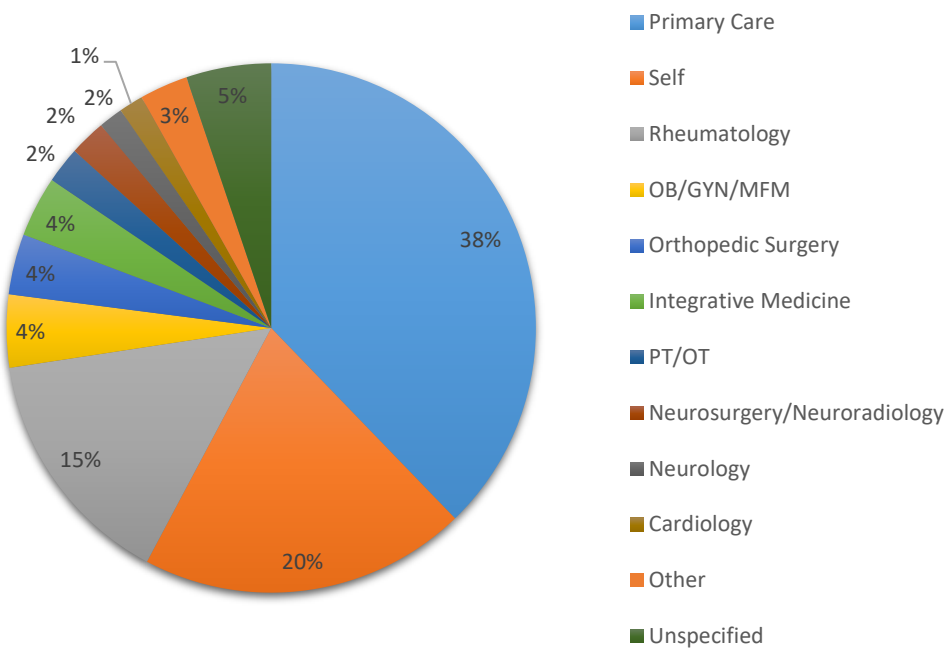


Figure 1 Referral sources

The "other" category includes referrals from thoracic surgery, hematology, physiatry, and vascular surgery.

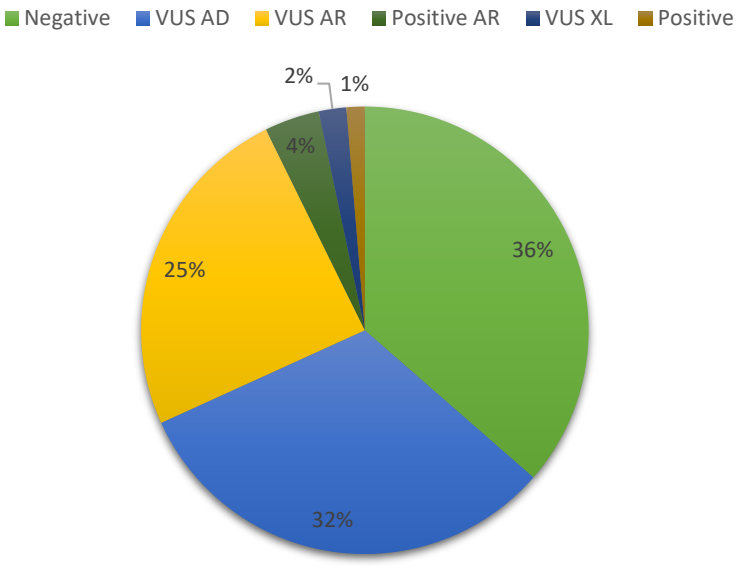


Figure 2 Breakdown of types of genetic testing results

2.3.2 Family History Information

Fifteen patients identified having a family history of a connective tissue disorder in at least one relative, including first- or second-degree relatives or both. The majority of these identified first-degree relatives (n=13), and these included mothers (n=5), daughters (n=5), sisters (n=1), and sons (n=2). Two patients identified at least one second-degree relative, which included aunts (n=2) and uncles (n=2).

Fifty-eight patients identified a positive family history of hypermobility in at least one relative, including first- or second-degree relatives or both. Again, the majority of these were in first-degree relatives (n=54), and patients identified a second-degree relative in thirteen cases. Patients identified sisters (n=14), mothers (n=18), brothers (n=11), daughters/sons/children (n=14, 5, and 2, respectively), and fathers (n=6) as first-degree relatives with hypermobility. Second-degree relatives included grandparents (n=3), nieces (n=2), half-siblings (n=5), and aunts (n=4).

Sixty-three patients identified a family history of a critical finding for CTD. The critical findings included conditions such as retinal detachments, aortic aneurysms, organ prolapses, and aortic dissections. Patients identified at least one first-degree relative (n=48) and/or at least one second degree relative (n=31). Patients identified mothers (n=24), fathers (n=15), brothers (n=6), sisters (n=10), sons (n=2), and daughters (n=2) as first-degree relatives with these findings. Second-degree relatives with these findings included grandparents (n=26), uncles (n=8), aunts (n=6), and half-siblings (n=1).

There were no statistically significant relationships between these family relationships and the diagnosis of hEDS or HSD, but a few p-values fell under 0.1. These are shown in Table 1.

Table 1 Family history criteria broken down by hEDS diagnosis status

Criteria	hEDS			
	Yes	**No**	**OR**	**p-value**
Family history of a CTD				
Yes	9	6	2.854	P=0.054
No	41	78		
First degree relative with CTD				
Yes	8	5	3.01	P=0.057
No	42	79		
Second degree relative with CTD				
Yes	1	1	1.694	P=0.709
No	49	83		
Family history of hypermobility				
Yes	24	34	1.636	P=0.312
No	25	51		
First degree relative with hypermobility				
Yes	22	31	1.369	P=0.387
No	28	54		
Second degree relative with hypermobility				
Yes	8	5	3.048	P=0.054
No	42	80		
Critical findings in family history				
Yes	22	41	0.908	P=0.790
No	26	44		
Critical findings in first degree relative				
Yes	15	33	0.716	P=0.382
No	33	52		
Critical findings in second degree relative				
Yes	11	21	0.906	P=0.817
No	37	64		

2.3.3 Clinical Findings and Diagnosis

Through statistical analysis, it was found that several clinical findings were linked to the diagnosis of hEDS and HSD. Some are currently part of the diagnostic criteria for hEDS and some are not.

2.3.3.1 Findings Currently Part of the hEDS Diagnostic Criteria

Because these findings make up the current hEDS diagnostic criteria, we predicted that these findings would be associated with the diagnosis of hEDS and not HSD; however, this was not the case for all of these findings. The findings are displayed in Table 2. The mean Beighton score was statistically significantly different between those with hEDS and those without hEDS ($p=0.0016$). The differences between Beighton scores in hEDS and HSD patients is shown in Figure 3, where the average Beighton score is higher in patients with hEDS as opposed to those with HSD (Figure 3b and 3c, respectively). While both hEDS and HSD use the Beighton score to assist with diagnosis, this suggests that the cases of HSD may be localized or historical in nature, therefore lowering the average.

Having a different skin texture such as soft, smooth, or velvety, was linked to the diagnosis of hEDS and HSD ($p<0.001$ and $p=0.011$). Only unusually soft and velvety skin is mentioned in the diagnostic criteria, but this is still to be expected. Skin striae were related to hEDS ($p=0.002$). Piezogenic papules of the heel were related to both hEDS and HSD ($p<0.001$, $p=0.008$). This suggests this criterion being non-specific to hEDS. Presence of atrophic scarring was related to both hEDS and HSD ($p=0.001$, $p=0.041$). Papyraceous or hemosideric scars were related to hEDS ($p=0.008$). This was unexpected because the diagnostic criteria are specific that any abnormal scarring must be atrophic and not papyraceous or hemosideric in order to receive a diagnosis of

hEDS, but this suggests that both types of scarring may be present in individuals anywhere on the HSD spectrum. Arachnodactyly was related to hEDS (p=0.04). Joint dislocations were found to be related to a diagnosis of hEDS (p=0.012). Pain in more than two limbs daily for 3 or more months was related to a diagnosis of hEDS (p=0.008). Chronic widespread pain for three or more months was related to a diagnosis of hEDS (p=0.028). Chronic pain is a common finding in these patients, especially those with systemic features, so this was an expected result. Findings currently on the diagnostic criteria and their p-values from this study are included in Table 2 below.

In addition to the individual findings, the overall criteria and the relationship to each diagnosis were analyzed, and this is shown in Table 3. There are significant relationships between hEDS and each of the criteria except for 2b, likely due to smaller number of these patients reporting family history of a CTD. There are also significant relationships between HSD and a few of the diagnostic criteria, indicating that these criteria are not all specific to only hEDS, even though they were designed that way.

Table 2 hEDS criteria clinical findings and final diagnosis

Total number of individuals with the criteria with final diagnosis of hEDS and HSD and p-values relating individuals with that criteria and the final diagnosis. An asterisk (*) next to p-values indicates statistical significance.

Criteria	hEDS				HSD			
	Yes	No	OR	p-value	Yes	No	OR	p-value
Beighton score meeting criteria								
With finding	48	50	--	P<0.001*	46	52	0.322	P=0.011*
Without finding	0	30			22	8		
Soft skin								
With finding	25	28	1.897	P=0.082	26	27	0.851	P=0.438
Without finding	24	51			42	33		
Velvety skin								
With finding	6	5	2.065	P=0.246	5	6	0.714	P=0.594
Without finding	43	74			63	54		

Skin hyperextensibility								
With finding	19	30	0.806	P=0.598	27	22	1.330	P=0.480
Without finding	22	28			24	26		
Skin striae								
With finding	35	37	3.468	P=0.002*	33	39	0.508	P=0.061
Without finding	12	44			35	21		
Piezogenic papules								
With finding	26	15	5.042	P<0.001*	15	26	0.359	P=0.008*
Without finding	22	64			53	33		
Hernias								
With finding	11	16	1.241	P=0.625	15	12	1.088	P=0.847
Without finding	36	65			54	47		
Atrophic scarring								
With finding	17	10	4.045	P=0.001*	10	17	0.406	P=0.041*
Without finding	29	69			58	40		
Organ prolapse								
With finding	4	10	0.667	P=0.513	10	4	2.328	P=0.164
Without finding	42	70			58	54		
Dental crowding								
With finding	23	29	1.618	P=0.193	26	26	0.767	P=0.463
Without finding	25	51			43	33		
Daily pain in 2+ limbs for 3+ months								
With finding	43	56	3.839	P=0.008*	50	49	0.591	P=0.217
Without finding	5	25			19	11		
Chronic widespread pain for 3+ months								
With finding	41	55	2.769	P=0.028*	47	49	0.480	P=0.079
Without finding	7	26			22	11		
Joint dislocations								
With finding	20	16	2.708	P=0.012*	15	21	0.542	P=0.120
Without finding	30	65			54	41		
Joint instability								
With finding	18	33	0.818	P=0.589	28	23	1.158	P=0.683
Without finding	32	48			41	39		

Table 3 hEDS diagnostic criteria categories and relationship to hEDS and HSD

Breakdown of individual hEDS diagnostic criteria and statistical significance for each diagnosis. An asterisk (*) next to p-values indicates statistical significance.

hEDS diagnostic criteria	p-value for hEDS	p-value for HSD
hEDS Criteria 1: Generalized Joint Hypermobility measured with Beighton score or two current or past signs of hypermobility	P<0.001 *	P=0.011 *
hEDS Criteria 2 Feature A: 5 or more of – • unusually soft or velvety skin; • mild skin hyperextensibility; • unexplained striae distensae or rubae in absence of weight gain/loss; • bilateral piezogenic papules of heel; • recurrent or multiple abdominal hernias; • atrophic scarring of 2+ sites without papyraceous or hemosideric scars; • pelvic floor, rectal, and/or uterine prolapse without predisposing medical condition; • dental overcrowding and high or narrow palate; • arachnodactyly (Walker/wrist sign and/or Steinberg/thumb sign bilaterally); • arm span to height ratio of 1.05 or more; • mitral valve prolapse; • aortic root dilation of Z-score of more than 2	P<0.001 *	P<0.001 *
hEDS Criteria 2 Feature B: positive family history with one or more first degree relatives independently meeting criteria for hEDS	P=0.092	P=0.436
hEDS Criteria 2 Feature C: at least one of – • musculoskeletal pain in 2+ limbs daily for at least 3 months; • chronic widespread pain for 3+ months; • recurrent joint dislocations or frank joint instability without trauma	P=0.002 *	P=0.957
hEDS Criteria 3: • absence of unusual skin fragility; • exclusion of other heritable and acquired CTDs; • exclusion of alternative diagnoses that may include joint hypermobility	P<0.001 *	P=0.001 *

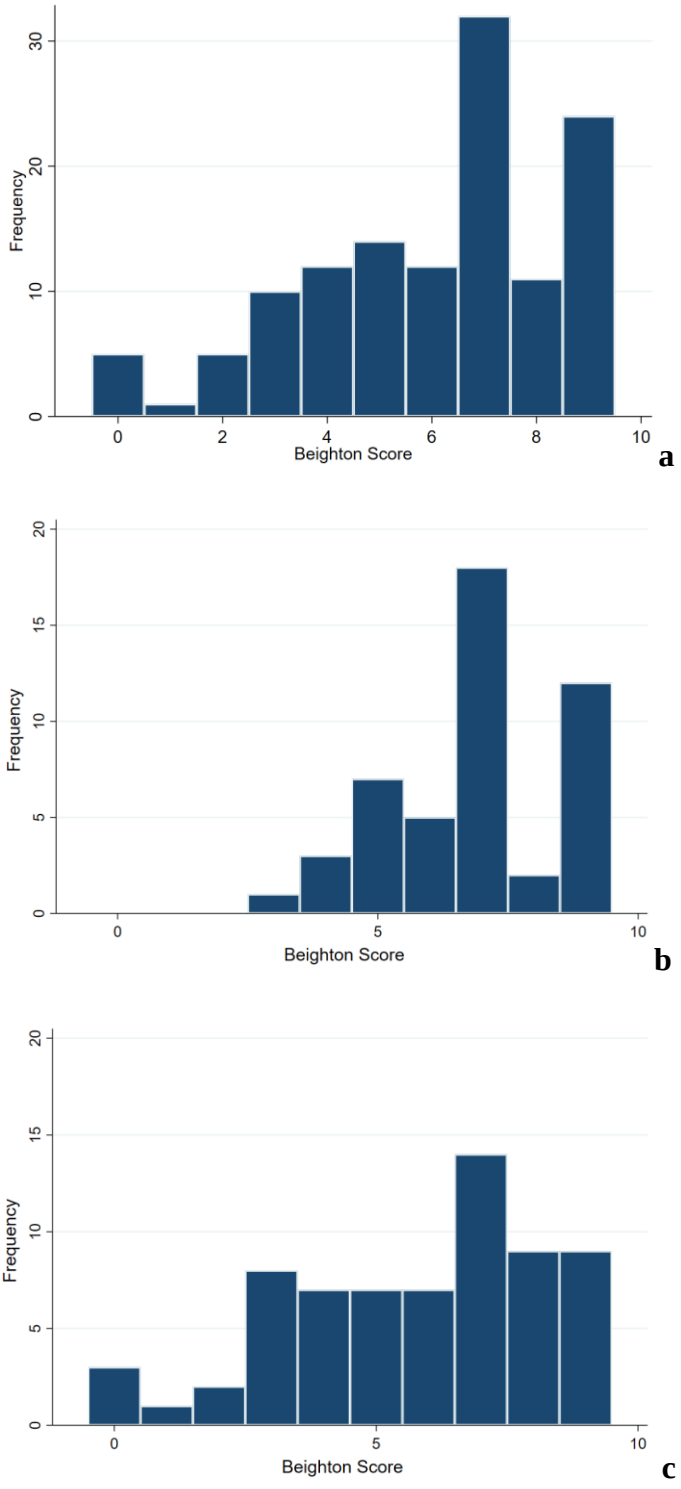


Figure 3 Histograms of patient Beighton score frequency

3a – All patients' Beighton scores. 3b – Beighton scores in hEDS patients. 3c – Beighton scores in HSD patients.

2.3.3.2 Findings Not Currently Part of the hEDS Diagnostic Criteria

Because these findings are not part of the current hEDS diagnostic criteria, we predicted that these findings would not be statistically associated with the diagnosis of hEDS; however, this was not the case for all of these findings. These findings are displayed in Table 4. Joint subluxations by themselves were associated with an hEDS diagnosis ($p=0.029$). This is importantly separate from the relationships of joint instability and dislocations to the hEDS diagnosis. While these are less severe than joint dislocations, they may still cause pain and discomfort, and it makes sense why these may be related to a disorder of the connective tissue based on their mechanism. Skin tearing had a statistically significant relationship with a diagnosis of hEDS and HSD ($p=0.01$ and $p=0.015$, respectively), though fewer patients reported this skin finding than others (see Table 4). Allergies or mast cell abnormalities such as MCAS were found to be related to the diagnosis of hEDS ($p=0.043$). MCAS is a recognized comorbidity of hEDS, but allergies themselves have not been found to be related to hEDS in the past. Poor wound healing had a statistically significant relationship with a diagnosis of hEDS ($p=0.006$). This makes sense if there was for example a collagen gene defect in these patients and makes the case for a broader definition of scarring/wound healing in the diagnostic criteria. Reporting a poor response to anesthesia was related to both hEDS and HSD diagnoses ($p<0.001$ and $p=0.003$, respectively). Again, this has been reported in the past as an association to these conditions but is not specific to an hEDS diagnosis. It therefore warrants further investigation into reasons why and if there is a way to determine which patients may have these poor responses. Raynaud's phenomenon was statistically significantly related to a diagnosis of hEDS ($p=0.003$). This vasculature issue being related to hEDS suggests a link to these sorts of vascular problems in this condition. The mean height was statistically significantly different between those with hEDS and those without hEDS

(p=0.0001). Mean height for those with HSD was 168.94 cm with a standard deviation of 8.27 cm and a range of 152.4 to 190.5 cm. Mean height for those with hEDS was 163.83 cm with a standard deviation of 7.86 cm and a range of 151.1 to 182.9 cm. These are demonstrated in the histograms in Figure 4. Height is indirectly in the diagnostic criteria as the arm span-to-height ratio. Patient height was shorter in hEDS, indicating that height is not an important marker of this condition and signals the need for more data to get a better sense of this element of the diagnostic criteria. Self-referrals were associated with the diagnosis of hEDS (p=0.026), which is not completely unexpected but interesting finding because of the lengths these patients are willing to go to get a diagnosis.

Table 4 Additional clinical findings related to hEDS and HSD diagnoses

Total number of individuals with the findings with final diagnosis of hEDS and HSD and p-values relating individuals with that criteria and the final diagnosis. An asterisk (*) next to p-values indicates statistical significance.

Criteria	hEDS				HSD			
	Yes	No	OR	p-value	Yes	No	OR	p-value
Joint instability, subluxations, dislocations								
With finding	48	70	3.771	P=0.075	62	56	0.949	P=0.929
Without finding	2	11			7	6		
Joint subluxations								
With finding	20	18	2.333	P=0.029*	17	21	0.638	P=0.245
Without finding	30	63			52	41		
GI symptoms								
With finding	47	71	3.641	P=0.084	61	57	0.669	P=0.5
Without finding	2	11			8	5		
Easy bruising								
With finding	35	46	2.107	P=0.056	40	41	0.673	P=0.278
Without finding	13	36			29	20		
Skin tearing								
With finding	8	3	5.267	P=0.01*	2	9	0.172	P=0.015*
Without finding	40	79			67	52		

Musculoskeletal pain								
With finding	47	74	5.081	P=0.096	65	56	1.451	P=0.591
Without finding	1	8			4	5		
Anxiety								
With finding	24	54	0.0541	P=0.098	46	32	1.75	P=0.122
Without finding	23	28			23	28		
Autonomic dysfunction								
With finding	45	62	2.613	P=0.069	53	54	0.429	P=0.081
Without finding	5	18			16	7		
Allergies or mast cell abnormalities								
With finding	35	44	2.203	P=0.043*	39	40	0.618	P=0.191
Without finding	13	36			30	19		
Poor wound healing								
With finding	29	29	2.778	P=0.006*	26	32	0.503	P=0.057
Without finding	18	50			42	26		
Poor response to anesthesia								
With finding	22	14	4.256	P<0.001*	12	24	0.295	P=0.003*
Without finding	24	65			56	33		
POTS								
With finding	24	28	1.938	P=0.075	26	26	0.744	P=0.415
Without finding	23	52			43	32		
Raynaud's phenomenon								
With finding	24	21	3.065	P=0.003*	20	25	0.522	P=0.083
Without finding	22	59			49	32		

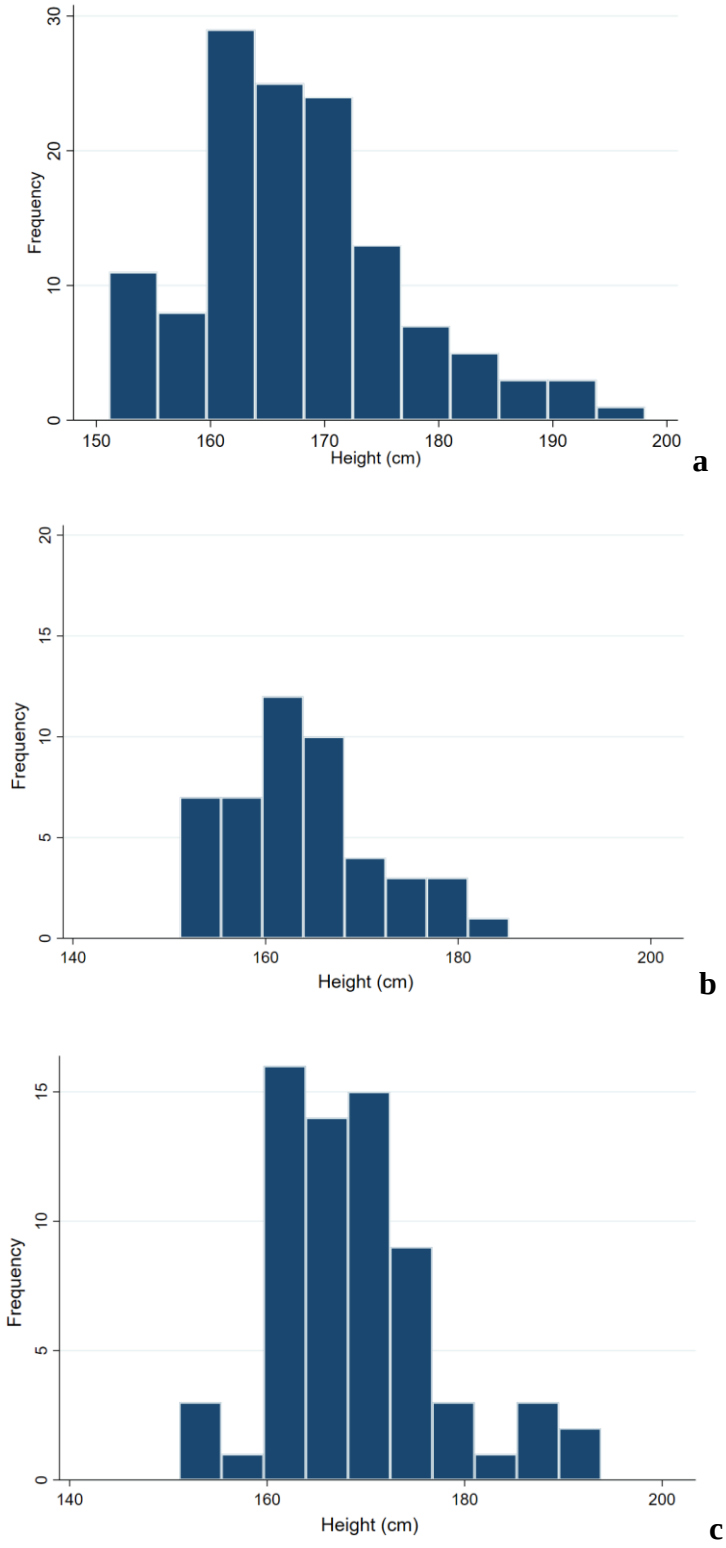
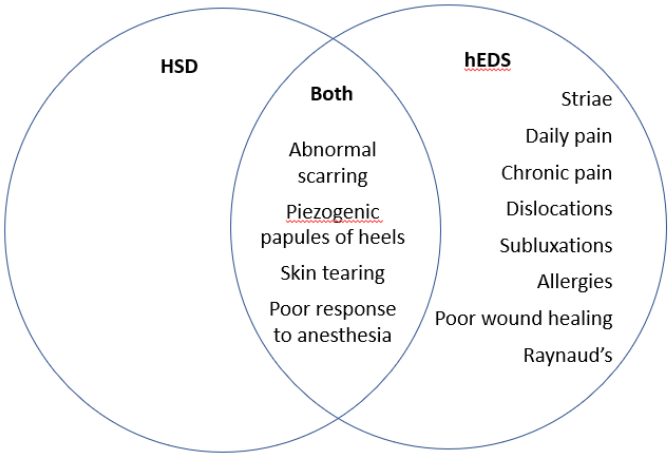


Figure 4 Histograms of heights of patients

4a – All patients' heights. 4b – Heights of hEDS patients. 4c – Heights of HSD patients.

Summaries of the findings related to the diagnosis of hEDS and HSD are demonstrated in Figure 5. Hard findings are findings that had a p-value of under 0.05, and soft findings had a p-value of under 0.1 in relationship to the diagnosis of either of these diagnoses. Of note, there are not findings that were related to only the diagnosis of HSD. This tracks with the nature of this condition being on a spectrum, with hEDS being on this spectrum. Everyone with hEDS also has HSD, but those with HSD do not also necessarily have hEDS.



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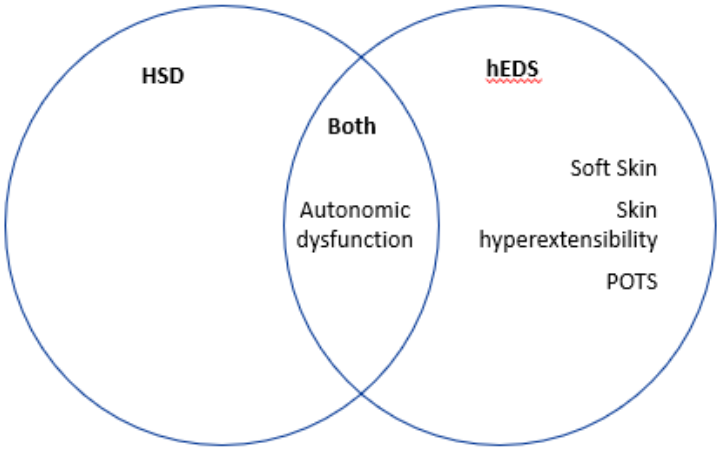

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Figure 5 Findings related to hEDS and HSD

5a – Hard findings, defined as p<0.05. 5b – Soft findings, defined as p<0.1.

2.3.4 Clinical Findings and Genetic Testing Results

There were forty-two distinct genes with one or more variants found on genetic testing. Genetic testing results were grouped into positive/VUS and negative categories and compared with the clinical findings. Skin stretching, scarring, bruising, and tearing was related to the genetic testing outcome (p=0.012). Autonomic dysfunction was related to genetic testing outcome (p=0.041). Having a first degree relative with a critical finding was related to genetic testing outcome (p=0.043). Referral from a primary care physician was related to genetic testing outcome (p=0.014).

2.3.4.1 Gene-Specific Results

Genetic testing results were further divided into finding a VUS in an autosomal dominantly inherited gene and finding a VUS in an autosomal recessively inherited gene. Finding a dominant VUS on genetic testing was associated with joint instability/subluxations/dislocations (p=0.03). Dominant VUS's were also associated with the presence of fatigue/brain fog (p=0.011). Muscle stiffness/tightness was associated with having a dominant VUS (p=0.005). Dental overcrowding was associated with having a dominant VUS (p=0.009). Neck strain was associated with having a dominant VUS (p=0.013). Organ prolapse was associated with finding a dominant VUS (p=0.045). Dominant VUSs were also associated with having chronic widespread pain for three or more months (p=0.032).

Findings in the collagen-producing genes were also grouped and compared with the clinical findings. Positive ANA lab was related to having a variant found in one of the collagen genes (p=0.012). Having a variant in one of the collagen genes was associated with having chronic widespread pain for three or more months (p=0.029). Organ prolapse was associated with having

a variant in one of the collagen genes (p=0.029). This makes sense because of the impact a collagen gene mutation could have on the structure and function of the connective tissue holding organs in place.

Findings in genes associated with familial thoracic aortic aneurysms and dissections (fTAAD) were also grouped and compared with the clinical findings. First, these fTAAD gene findings had a significant association with a final diagnosis of hEDS (p=0.01). This again seems to support potential additional cardiovascular findings in hEDS. These gene findings were also associated with joint instability/subluxations/dislocations (p=0.014) and gastrointestinal findings (p=0.014). Patient report of mental health conditions (p=0.043) and fatigue/brain fog (p=0.04) were associated with findings in the fTAAD genes. Urological and gynecological symptoms were found to be related to these gene findings (p=0.031). Muscle stiffness/tightness was associated with fTAAD gene findings (p=0.002). Allergies or mast cell abnormalities were also associated with fTAAD gene findings (p=0.007). Finally, hEDS criteria 2c was associated with fTAAD gene findings (p=0.049). These findings are in Tables 5, 6, and 7 and visualized in Figure 6.

Table 5 Clinical findings related to dominant VUS finding on genetic test results

Associations between finding a VUS in an autosomal dominant inherited gene and clinical findings.

Finding	Yes	No	OR	p-value
Joint instability/ subluxations/ dislocations				
With VUS	37	8	0.285	P=0.03
Without VUS	81	5		
Fatigue/ brain fog				
With VUS	37	7	0.189	P=0.011
Without VUS	84	3		
Muscle stiffness/ tightness				
With VUS	29	14	0.284	P=0.005
Without VUS	73	10		
Dental overcrowding				
With VUS	11	33	0.35	P=0.009

Without VUS	41	43		
Neck strain				
With VUS	20	22	0.385	P=0.013
Without VUS	59	25		
Organ prolapse				
With VUS	8	34	3.059	P=0.045
Without VUS	6	78		
Chronic widespread pain for 3+ months				
With VUS	27	16	0.416	P=0.032
Without VUS	69	17		

Table 6 Clinical findings related to VUS finding in a collagen gene on genetic test results

Associations between finding a VUS in a collagen gene and clinical findings.

Finding	Yes	No	OR	p-value
Positive ANA lab				
With VUS	8	18	3.556	p=0.012
Without VUS	12	96		
Chronic widespread pain for 3+ months				
With VUS	15	11	0.370	P=0.029
Without VUS	81	22		
Organ prolapse				
With VUS	6	20	3.450	P=0.029
Without VUS	8	92		

Table 7 Clinical findings related to VUS finding in a fTAAD gene on genetic test results

Associations between finding a VUS in a fTAAD gene and clinical findings.

Finding	Yes	No	OR	p-value
hEDS				
With VUS	1	14	0.103	P=0.01
Without VUS	49	71		
Joint instability/subluxations/ dislocations				
With VUS	10	4	0.208	P=0.014
Without VUS	108	9		
GI findings				
With VUS	10	4	0.208	P=0.014
Without VUS	108	9		
Mental health conditions				
With VUS	11	3	0.238	P=0.043

Without VUS	108	7		
Fatigue/brain fog				
With VUS	11	3	0.233	P=0.04
Without VUS	110	7		
Urological/Gynecological symptoms				
With VUS	6	8	0.302	P=0.031
Without VUS	82	33		
Muscle stiffness/tightness				
With VUS	7	7	0.179	P=0.002
Without VUS	95	17		
Allergies/mast cell abnormalities				
With VUS	4	10	0.208	P=0.007
Without VUS	75	39		
hEDS criteria 2c				
With VUS	11	4	0.288	P=0.049
Without VUS	105	11		

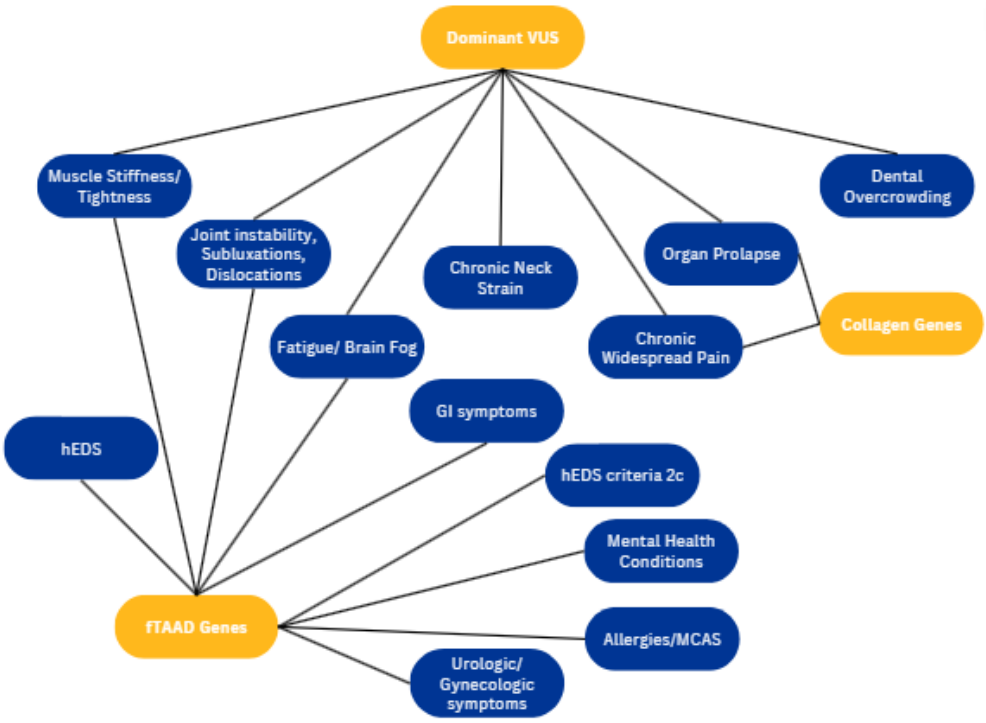

Figure 6 Genetic test VUS findings in autosomal dominant genes related to clinical findings

Yellow ovals are the specific gene findings – dominant VUS, collagen gene findings, and fTAAD gene findings. Blue ovals are the specific clinical findings, and diagnosis in the case of hEDS, related to these genetic findings. Lines linking the gene finding to a clinical finding represent relationships of $p<0.05$.

Finding a recessive VUS on genetic testing was associated with presence of autonomic dysfunction (p=0.014). Having a family history of a CTD was associated with finding a recessive VUS (p=0.009). This is interesting considering the large effect size because it suggests that these traditionally recessive genes could have a dominant inheritance pattern in the case of a condition like hEDS. The finding of piezogenic papules of the heel was associated with recessive VUS (p=0.026). Atrophic scarring as well as papyraceous or hemosideric scarring were also associated with recessive VUS (p=0.024, p=0.003). Referral to PCPM from rheumatology was related to finding a variant in the *TNXB* gene (p=0.001) as well as finding a recessive VUS in general (p=0.039). These findings are in Table 8 and visualized in Figure 7.

Table 8 Clinical findings related to VUS finding in an autosomal recessive inherited gene or a *TNXB* gene finding on genetic test results

Associations between finding a VUS in a recessive gene or a *TNXB* gene finding and clinical findings.

Finding	Yes	No	OR	p-value
Autonomic dysfunction				
With VUS	28	12	0.325	P=0.014
Without VUS	79	11		
Family history CTD				
With VUS	9	32	4.078	P=0.009
Without VUS	6	87		
Piezogenic papules of heel				
With VUS	18	21	2.422	P=0.026
Without VUS	23	65		
Atrophic scarring				
With VUS	13	25	2.711	P=0.024
Without VUS	14	73		
Papyraceous or hemosideric scarring				
With VUS	13	25	4.004	P=0.003
Without VUS	10	77		
Referral from rheumatology				
With *TNXB* finding	6	7	6.612	P=0.001
Without *TNXB* finding	14	108		

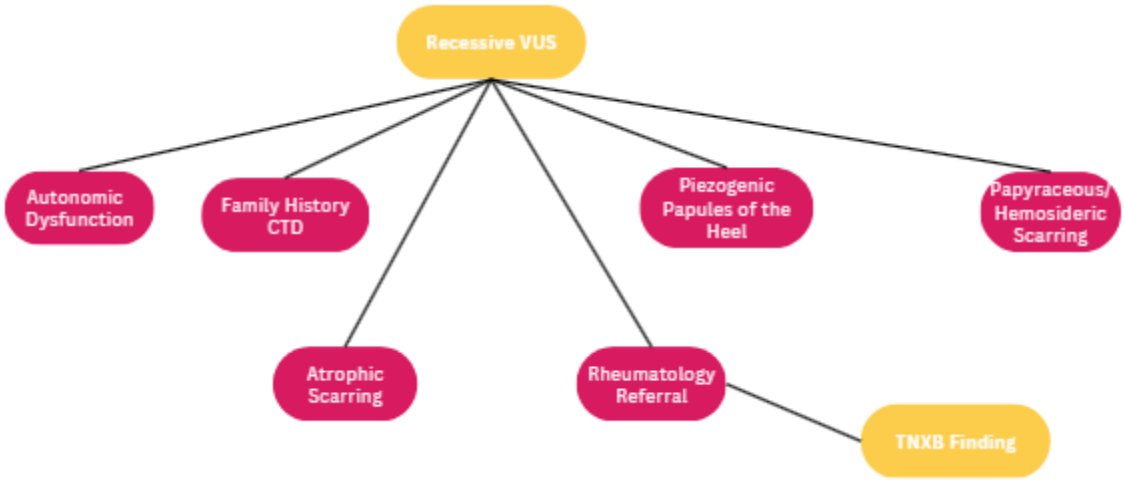

Figure 7 Genetic test VUS findings in autosomal recessive genes related to clinical findings

Yellow ovals are the specific gene findings – recessive VUS and TNXB gene findings. Pink ovals are the specific clinical and family history findings related to these genetic findings. Lines linking the gene finding to a clinical finding represent relationships of $p<0.05$.

It should be noted that several of the other associations did not quite hit the threshold of $p=0.05$ but had p-values of $p<0.1$. With the low effect sizes of these findings and uncertain nature of the variants, it is unclear at this time what many of the results could mean. As variants get reclassified and we learn more about the genetics of these conditions, these results could link clinical findings back to molecular diagnosis.

2.4 Discussion

2.4.1 Demographics

The demographic information observed in this study is consistent with other publications where this patient population is reported as majority white and female. While unfortunate, there are several possible reasons for this gap, including different views of pain in men and women

versus a true difference in diagnostic rates between these groups, mistrust in the medical system among minorities, pain syndromes in minorities being viewed as drug-seeking, and a referral gap for underprivileged individuals to genetics due to the perceived expense of genetic testing. One author discusses the possibility of gene-environment interactions in hEDS, including higher incidence in females and the idea that sex hormones may impact the development of this syndrome (Martin 2019). Females are also more likely to visit their primary care provider than men, which could explain the high prevalence of females referred by their PCP in this patient population (Thompson et al. 2016). The intersectionality of gender identity should also be noted, as patients' gender was not recorded as part of the study but several patients in PCPM are transgender or nonbinary. Inconsistency in medical record documentation made this not possible from an accuracy standpoint, but we recognize that individuals from a variety of gender identities have come through the clinic. From a cost perspective, patients seen by PCPM are able to use the available patient pay options at the laboratories used. Other providers may not be aware of self-pay options provided by labs that can help reduce costs of genetic testing for patients. Additionally, regardless of final diagnosis, the majority of these patients have reported significant amounts of pain. For example, 116 patients met criteria 2 feature c of the hEDS diagnostic criteria, which specifies that an individual has daily pain in two or more limbs for a period of three or more months, chronic widespread pain for a period of three or more months, or joint instability or recurrent joint dislocations in the absence of trauma (Malfait et al. 2017). This demonstrates that a large portion of this population is experiencing pain throughout the body and that providers should be expecting pain complaints from this population. There are several propositions for the presence of pain with hEDS, including additional pain triggers like peripheral neuropathy, muscle cramps, and tendinitis (Martin 2019). The presentation of pain in HSD and hEDS can lead to

delays in diagnosis, and the attitude that the symptoms are psychiatric rather than "physical" adds to the frustration and fear of medicine (Anderson and Lane 2021). Finally, further education for physicians is needed to recognize symptoms that are both visible and invisible in non-white individuals in order to provide the most appropriate management.

2.4.2 Referral Sources

The referral sources for these patients are varied, with the majority being from a primary care provider. This is an important distinction because these providers are seeing patients on a more regular basis and may be able to serve as the point of contact for additional referrals when a diagnosis is made. They are also able to recognize that these symptoms require further evaluation. Two of the patients who received a molecular diagnosis were referred by a family medicine provider. While this is not enough to say that there is an association between these diagnoses and a referral from a primary care provider, the additional management recommendations that come with some genetic diagnoses demonstrate the importance of getting these patients genetic testing and that primary care offices may be a great place to educate providers. The symptoms of hEDS and HSD are varied, consistently unpredictable, and include a variety of organ systems and competing comorbidities; this variety of complaints across different body systems presents a challenge for the provider to sift through and provide referrals for. Atwell et al. 2021 offer the recommendation of focusing on the most serious concerns in the provider's opinion and those causing the patient the most distress; they also suggest that providers review outside records and family history as well as "A Simple Questionnaire to Detect Hypermobility".

Self-referrals were associated with the diagnosis of hEDS based on our patient data. The large portion of self-referrals to the clinic along with this association with hEDS reinforces the

need for additional education of providers who are likely to encounter these patients and for these patients to be believed when they express their symptoms. Genetic testing should be considered in cases where CTDs in general are a concern, even when self-referred: as described in Section 2.4.3.3, one of the individuals who self-referred was diagnosed with Loeys Dietz syndrome, which only underscores these points further. This also brings up the question how many of these individuals regularly see a primary care provider and demonstrates how our current healthcare system is failing these patients.

Many of these patients also come to attention in rheumatology because of hypermobility and chronic pain. Therefore, rheumatologists have general knowledge on these conditions and when additional evaluation is warranted, which could explain the frequency of rheumatology referrals to PCPM.

An additional group of referrals came from gynecologists, obstetricians, and maternal fetal medicine physicians. This is an important group of providers to recognize the symptoms of CTDs because one estimate suggests that 6 million individuals capable of pregnancy per year are affected by hEDS or HSD worldwide (Pezaro et al. 2020). Pregnant individuals experience a lack of provider knowledge about the condition and poor treatment during pregnancy while at the same time wanting to be involved in decision making processes (Pezaro et al. 2020). They also experience physical or psychological trauma associated with the birth process, and pain relief medications did not always work (Pezaro et al. 2020). It is unclear why pregnancy is so greatly impacted, although this is a vulnerable time for these individuals.

The other specialties make up a much smaller proportion of the total number of referrals. One study of hematologists found that these physicians did not feel they received the proper training to administer tests of joint hypermobility such as the Beighton score and use them in the

evaluation (Kendel et al. 2021). Despite the previous study's focus on hematologists and that only one of our patients was referred by a hematologist, it underscores the need for training providers in any specialty where these patients may end up for their medical home.

2.4.3 Clinical Findings and hEDS/HSD

Current diagnostic criteria for hEDS are described in Malfait et al. 2017. From our data, the pain categories of criteria 2 feature c (daily pain in 2 or more limbs for 3 or more months and chronic widespread pain for three or more months) appeared to be related to only the hEDS diagnosis. Criteria 2 feature c was the only hEDS criteria that was specifically related to hEDS and not HSD diagnosis. Additionally, skin striae were related to just hEDS. Joint dislocations were related to hEDS but not HSD.

Based on patient data, some of the diagnostic criteria are not specific to hEDS and may not have a significant relationship with the final diagnosis. In addition to joint dislocations, joint subluxations have a significant relationship with hEDS and not HSD, while joint instability did not have a significant relationship with either diagnosis. Piezogenic papules of the heels had a relationship with both hEDS and HSD diagnoses as well. Criteria 1 of the hEDS diagnostic criteria, which is Beighton score or evidence of hypermobility, had a significant relationship with both hEDS and HSD diagnoses. The presence of soft skin was generally common, found in 53 patients. There was no relationship between soft or velvety skin texture and the diagnosis of hEDS or HSD. This finding in particular is important to caveat with the fact that these were telemedicine appointments. Skin hyperextensibility to some degree was found in 108 patients, but no significant relationship was found to hEDS or HSD. This was also found when accounting for degree of skin hyperextensibility: patients were divided into mild and moderate/high levels of skin

hyperextensibility and no significant relationship was found for either category and hEDS or HSD diagnosis (hEDS: p=0.598; HSD: p=0.480). Atrophic scarring was reported in 27 patients and had a significant relationship to both hEDS and HSD. Hernias were found in 27 patients, of which 13 were hiatal. No significant relationship was found between hernias and either diagnosis, although this represented a smaller proportion of patients. The same goes for organ prolapse: 14 patients had some form of prolapse, but these were not related to either diagnosis. Dental overcrowding was found in 52 patients with no significant relationship to either diagnosis. hEDS criteria 1, criteria 2 feature a, and criteria 3 had statistically significant relationships to both hEDS and HSD. Criteria 2 feature b did not have a statistically significant relationship to either diagnosis. These statistics reiterate the importance of clinically validating these criteria. One study found that 85% of the patients seen in their clinic with a diagnosis of hEDS based on pre-2017 criteria did not meet the new 2017 criteria (McGillis et al. 2020). Patients face many trials to receive a diagnosis, including the importance of delays in diagnosis, the fact that stricter diagnostic criteria are not helpful in the absence of further education, and the difficulty associated with a genetic cause not yet being identified (Martin 2019). While we recognize the importance of diagnostic criteria to make sure diagnosis is the same across individuals, we must continue to make sure these are specific, accurate, and representative of the patient experience with these symptoms.

Additionally, there are other clinical findings that are not currently accounted for in the hEDS diagnosis that may have a significant relationship with this diagnosis. Many individuals reported widespread musculoskeletal pain (n=121). While this finding was not found to be related to either diagnosis, this is still certainly the experience of the vast majority of individuals with joint hypermobility. Pain severity and pain interference have been shown to change significantly over time (Schubart et al. 2022). There are several propositions for the presence of pain with hEDS,

including additional pain triggers like peripheral neuropathy, muscle cramps, and tendinitis (Martin 2019). There need to be better ways of treating and identifying pain in these patients. Poor responses to anesthesia were reported by 36 patients, with significant relationships to both hEDS and HSD diagnoses. Some researchers have recognized a relationship between hEDS and resistance to anesthesia (Zhou et al. 2018), but there are no complete recommendations for situations requiring anesthesia hEDS or HSD patients. Some recommendations and strategies to avoid adverse events in the EDS population are described in Wiesmann et al. 2014, but they acknowledge that there is little evidence-based knowledge on this topic. While GI symptoms did not have a statistically significant relationship with hEDS diagnosis, the p-value was under 0.1. There are also a wide range of GI symptoms and disorders that have been found to be more frequent in individuals diagnosed with hEDS, including functional heartburn, functional dysphagia, functional dyspepsia, IBS (irritable bowel syndrome), functional constipation, functional diarrhea, and functional abdominal bloating (Inayet et al. 2018). Skin tearing also had a significant relationship with hEDS and HSD, though the number of patients reporting this finding was small (n=11). This finding, along with the significant findings for different types of scarring, suggests expanding the definition of poor wound healing to include skin tearing and abnormal scarring. Easy bruising was not quite related to either, as the p-value was under 0.1 for hEDS. Poor wound healing also had a significant association with hEDS, and the p-value for relationship to HSD was p=0.057. Mental health concerns, including anxiety and depression, did not have any statistically significant relationships with hEDS or HSD. However, the relationship between anxiety and hEDS had a p-value of less than 0.1. Higher incidences of anxiety and depression have been observed in CTDs, and these individuals experience restrictions imposed by their condition, healthcare limitations, social stigma, and fear of the unknown (Bennet et al. 2021). Additionally, they

experience significant worry regarding the worsening of their symptoms over time, with hopelessness and fear of the future taking over (Saetre and Eik 2019). However, incidence of anxiety and depression is likely higher and quality of life is likely lower for most individuals with a form of chronic illness. Allergies or mast cell abnormalities were related to hEDS diagnosis, which has been found to also segregate with POTS, GI disorders, and psychiatric conditions in other studies (Cheung and Vada 2015, Seneviratne et al. 2017). Autonomic dysfunction and POTS were also reported in several of our patients. While there was no statistically significant relationship, these p-values were under 0.1. One study found that their participants with HSD and hEDS reported orthostatic symptoms that were more severe than controls, and they also reported lower quality of life measures than controls because of the symptoms; however, there were no differences in overall prevalence of orthostatic symptoms between the HSD and hEDS participants (Peebles et al. 2022). Raynaud's phenomenon was significantly associated with hEDS, but the p-value for the relationship to HSD was less than 0.1. 48% of our patients with hEDS reported Raynaud's findings, which is higher than previous suggestions of 38% (Castori et al. 2010). Additionally, the distinction between Raynaud's findings and autonomic dysfunction is important due to the similarly presenting symptoms and common co-occurrence with EDS, and awareness that autonomic dysfunction could underlie Raynaud's symptoms is important for providers to be aware of (Soloway et al. 2020). We described the average height in each diagnosis group in this study. These generally line up with average heights in other studies (Rombaut et al. 2009)

Our data lines up with that of other studies. Correlation with positive molecular diagnosis in one study was found for generalized joint hypermobility, poor healing, easy bruising, atrophic scars, skin hyperextensibility, and developmental dysplasia of the hip in children who have EDS characteristics whether or not they meet the 2017 criteria (Damesh et al. 2022).

Additionally, we found some statistically significant relationships between the different symptoms. POTS was related to the GI disorders IBS and gastroparesis ($p=0.008$, $p=0.01$ respectively). POTS also was related to chronic fatigue ($p=0.08$). This is in line with other research (Tai et al. 2020). Allergies or mast cell abnormalities was also related to POTS ($p=0.021$), which is also in line with previous studies (Cheung and Vada 2015).

2.4.4 Family History

This group of patients did not have any relatives who had completed genetic testing in the past. Therefore, the family history information was also self-reported and based off clinical diagnosis. None of the familial findings had a significant association with the diagnosis of hEDS or HSD. However, there were only fifteen patients who reported a family history of a diagnosed CTD, which is a small proportion of the patients studied (15/135 or 11%). The collection of family history information remains important because there is a hereditary component to CTDs and appropriate screening for additional findings in the family history may be recommended, such as ophthalmology exams for retinal findings. There were two cases who were biologically related as mother and daughter. These individuals each qualified for their own genetic testing via a CTD panel based on their independent medical histories and were found to have the same variant of uncertain significance in *FBN1,* which was relatively unsuspicious based on their medical histories and did not give them a genetic diagnosis. Additionally, the relationship of recessive VUSs to family history of CTD suggests that there are molecular diagnoses that we do not have enough information about yet and that what were previously thought to be recessively inherited genes for these conditions could be inherited in a dominant pattern or with a haploinsufficiency model.

2.4.5 Genetic Diagnoses

Results of the chart review indicate that there was one genetic diagnosis of Marfan syndrome, one genetic diagnosis of Loeys Dietz syndrome type 3, and one genetic etiology suspicious for Brittle Cornea syndrome in addition to a clinical diagnosis of hEDS. The low number of molecular etiologies identified may be due to the more severe or apparent presentations of these conditions coming to attention in childhood, while the adult population may have been more likely to have their symptoms dismissed in the past or have symptoms worsen in adulthood. In one study, 83% of those meeting diagnostic criteria and 22% of those who did not meet diagnostic criteria had positive genetic testing; in addition, one patient was diagnosed with LDS on genetic testing after not meeting EDS diagnostic criteria and two patients were diagnosed with vascular EDS when classical EDS was suspected (Damesh et al. 2022).

It was previously mentioned that thirteen patients did not have a final diagnosis recorded. This was the case when their symptoms did not fit with a diagnosis of a CTD at this time or if the patient was undergoing additional genetic testing such as whole exome or whole genome sequencing. There are other ways to seek out molecular diagnosis in the setting of negative NGS testing and a clinical indication for testing, such as WES and WGS as well as del/dup and Sanger sequencing to account for any technical limitations of some of these technologies. An example of Sanger sequencing proving to be helpful in diagnosis is described in Ritelli et al. 2020.

2.4.5.1 Patient #93: Suspicion of Brittle Cornea Syndrome

Brittle cornea syndrome is an autosomal recessive condition associated with thin cornea with or without rupture, early onset progressive keratoconus, early onset progressive keratoglobus, and blue sclerae (Malfait et al. 2017). The minor criteria include other eye findings as well as

deafness, hypercompliant tympanic membranes, developmental dysplasia of hip, hypotonia in infancy, scoliosis, arachnodactyly, distal joint hypermobility, pes planus, mild contractures of fingers, and soft/velvety/translucent skin (Malfait et al. 2017). Criteria that are suggestive are at least thin cornea with or without rupture in addition to either one other major criterion and/or three other minor criteria (Malfait et al. 2017). The genes associated with this condition are *ZNF469* and *PRDM5* (Malfait et al. 2017).

Patient 93 is a white individual assigned female at birth referred to PCPM by their family medicine physician. They reported problems with skin bruising and tearing, anxiety, autonomic dysfunction and temperature intolerance, mild scoliosis, proprioception issues, muscle stiffness/tightness, dental overcrowding, allergies, chronic neck strain, poor wound healing, family history of hypermobility in their sister and mother, pain in more than two limbs daily for three or more months, chronic widespread pain for three or more months, myopia, Beighton score of 9, velvety skin, skin striae, piezogenic papules of the heels, papyraceous or hemosideric scars, positive Steinburg sign for arachnodactyly, and low MMA and Vitamin D. The team completed a CTD genetic testing panel through GeneDx for this patient, and they were found to have two variants in the *ZNF469* gene: one likely pathogenic (c.1171_1175del) and one VUS (c.3214_3222del). They were found to still meet the criteria for hEDS, giving them a clinical diagnosis while their parents complete testing to determine if the variants are in cis or trans and they have further exam by an ophthalmologist to see if there are any eye findings.

2.4.5.2 Patient #114: Marfan Syndrome

The diagnostic criteria for Marfan syndrome were established by Loeys et al. 2010 and include the aortic dilation size, ectopia lentis, and genetic variants in *FBN1*. There are scoring criteria for systemic features. As more individuals with *FBN1* mutations are identified, there is a

better understanding of the broad range of phenotypes of this condition. Management recommendations include yearly echocardiograms even if the individual has not developed aortic root dilation, avoiding contact sports and vigorous exercise, ophthalmology evaluation yearly to look for retinal detachment and/or ectopia lentis, and monitoring by an orthopedic specialist when skeletal manifestations are present (Loeys et al. 2010 and Dietz et al. 2001).

Patient 114 is a white assigned male at birth individual referred by his family medicine practitioner. He reported joint subluxations, IBS, GERD, dyspepsia, anxiety, autonomic dysfunction particularly with heart rate, easily fatiguing with exercise, muscle stiffness/tightness, allergies or mast cell abnormalities, poor wound healing, bilateral popliteal artery aneurysms, a history of poor response to anesthesia, a critical CTD finding in a maternal grandfather, pain in more than 2 limbs daily for three or more months, chronic widespread pain for three or more months, Raynaud's, myopia and astigmatism, a Beighton score of 2, moderate skin hyperextensibility, skin striae, molluscoid pseudotumors, and a positive ANA lab value. Upon genetic testing with a CTD panel through GeneDx, he was found to have a pathogenic variant in the *FBN1* gene (c.1837+5 G>A). Subsequent echocardiogram showed dilated aortic root and ophthalmology exam revealed cataract.

2.4.5.3 Patient #81: Loeys Dietz Syndrome

The diagnosis of LDS is established if someone has a pathogenic or likely pathogenic variant in *SMAD2, SMAD3, TGFB2, TGFB3, TGFBR1,* or *TGFBR2* and either aortic root enlargement or systemic features (Loeys et al. 2018). Systemic features of this condition include aortic dilation or dissection, pectus excavatum or carinatum, scoliosis, bifid uvula or cleft palate, craniosynostosis, soft/velvety skin, dystrophic scars, blue or dusky sclera, inflammatory bowel disease, widely spaced eyes, arachnodactyly, and cervical spine malformations. Management

guidelines include echocardiograms, MRA or CT scans to identify any issues with the arteries, ophthalmologist exam, and identifying craniofacial concerns including cleft palate and craniosynostosis (Loeys et al. 2018). Loeys Dietz syndrome may be mistaken for other CTDs because of the many symptoms that overlap with other conditions, particularly vascular EDS (vEDS) (Blinc et al. 2015).

Patient 81 is a white individual assigned female at birth who self-referred to PCPM. She reported joint dislocations, GERD, depression and bipolar disorder, fatigue, scoliosis, fibromyalgia, tempromadibular joint issues, muscle stiffness/tightness, dental overcrowding, allergies or mast cell abnormalities, chronic neck strain, hiatal hernia, family history of hypermobility in a half-sister, POTS, pain in more than 2 limbs daily for three or more months, chronic widespread pain for three or more months, astigmatism, Beighton score of 9, smooth skin which was mild to moderately hyperextensible, positive Walker and Steinburg signs for arachnodactyly, positive ANA lab, and low Vitamin D. The team ordered a CTD patient for this patient which resulted with a pathogenic deletion of exon 1 in the *SMAD3* gene, giving her a diagnosis of Loeys-Dietz syndrome type 3.

2.4.6 Genetic Links to hEDS Symptoms

There is limited information available about genetic links to specific symptoms of the EDSs, likely because of the non-specific symptoms of these conditions and likely genetic overlap of the conditions. With the small data set, we were able to siphon out subsets of genes to compare to clinical findings to identify links to specific features and diagnoses. Dominantly inherited genes with VUSs in our patients were associated with joint instability, subluxations, and dislocations, fatigue and brain fog, muscle stiffness and tightness, dental overcrowding, neck strain, organ

prolapse, and chronic widespread pain. Collagen gene VUSs were associated with positive ANA labs, chronic widespread pain, and organ prolapse. The mutation spectrum and clinical picture of CTDs is constantly expanding. One study described a recessive EDS-like condition with homozygous *COL1A1* variants (Alazami et al. 2016). There also appears to be clinical overlap with these genes between the different types of EDS (Yang et al. 2022). Venable et al. 2023 describe cases with a clinical diagnosis of hEDS being found to harbor a mutation in the *COL1A1* or *COL1A2* genes. Our patient population consisted of 4 individuals having a mutation in either of these genes (n=3 for *COL1A1*, n=1 for *COL1A2*). fTAAD gene VUSs were associated with hEDS diagnosis, joint instability, subluxations, and dislocations, GI findings, mental health conditions, fatigue and brain fog, urological and gynecological symptoms, muscle stiffness or tightness, allergies or mast cell abnormalities, and hEDS criteria 2c. None of these genes have been related to hEDS, and *COL3A1* (related to vascular EDS) was included with the collagen genes. They were interestingly not related to cardiology concerns reported by patients or any vascular ruptures or organ prolapses reported, though these represented smaller numbers of the patient cohort.

Recessively inherited genes with VUSs were associated with autonomic dysfunction, family history of a CTD, piezogenic papules, atrophic scarring, papyraceous or hemosideric scarring, and referral from rheumatology. Finding a variant in the *TNXB* gene specifically was associated with referral from rheumatology. However, only 12 patients were found to have at least one variant in the *TNXB* gene. This gene encodes the protein tenascin-x, which is thought to enable collagen binding, cell-cell adhesion, and wound healing ("TNXB Tenascin XB [Homo Sapiens (Human)] - Gene - NCBI."). The *TNXB* gene has been associated with autosomal recessive classic EDS and has some evidence supporting its role in developing hEDS (Kaufman and Butler 2016,

Demirdas et al. 2017, Caliogna et al. 2021). There may also be a role for testing the levels of tenascin-x in serum to determine if they are lower given that TNXB gene mutations may be present in some individuals with hEDS and clEDS (Demirdas et al. 2017, Kaufman and Butler 2016, Scicluna et al. 2021). The *TNXB* gene is difficult to sequence, and labs may be hesitant to add it to panels due to its overlap with the pseudogene *XA* and complex gene structure in addition to only having very new information about its association with clEDS and hEDS. There was one case in our patient cohort where an individual had 2 VUSs in the *TNXB* gene, which raised suspicions for clEDS. The patient was meant to have parents undergo single site testing to see if the variants were in cis or trans.

As previously stated, the genetics underlying HSD and hEDS are still under investigation. In contrast to the potential genetic roles of the *TNXB* and *LZTS1* genes in the development of hEDS, it has been hypothesized that the collagen genes may not play a role in hEDS (Scicluna et al. 2021). There were fewer findings associated with the collagen genes in our study. There may therefore be many different genetic factors at play in the development of hEDS. The associations between fTAAD genes and the clinical findings suggests that there are some links between these genes and some of the symptoms of hEDS as well as the final diagnosis of hEDS. For each of the groups of genes analyzed, perhaps there are specific gene-to-finding relationships that were not able to be found based on the sample size obtained for this study.

2.4.7 Future Directions and Recommendations

Future research in this patient population related to interactions with genetic counselors and obtaining genetic testing is warranted. For example, even without a known genetic marker for hEDS and HSD, it would be important to know if these patients' experiences with genetic

counselors was positive because of the application of the skillset of genetic counselors for these patients. It would be helpful to compare patient experiences of receiving genetic testing through a genetics clinic in contrast to primary care clinics and other specialty clinics to understand what these patients need in terms of support and where they feel they are best serviced. Additionally, it would be interesting to know the satisfaction of these patients with the process of obtaining genetic testing and what their feelings are after they receive results because of the uncertainty of many of these results at this time in addition to the feelings of those who received a molecular diagnosis at the time of testing. Further studies of similar data in larger populations could lead to more individuals with molecular diagnoses and therefore the ability to elucidate any statistically significant relationships between molecular diagnosis and clinical findings.

Additional providers in PCPM, both genetic counselors and physicians or advanced practice providers along with related administrative support, would serve an important role for these patients and allow for this study to be updated in the near future. Collaboration with other centers could be of use as well if similar strategies for performing genetic testing on their patients is used. Ultimately, we would like to see this research inform the design of an algorithm for clinical providers to categorize genetic testing and triaging strategies for Hypermobility Spectrum Disorder patients and create recommendations for primary care providers. Using this algorithm could create recommendations for patients who would be identified to have a molecular diagnosis from genetic testing and who would be identified as needing more emergent triage. The clinic had previously designed a guide to determine when to order testing, demonstrate all the factors that contribute to HSD, and which clinical findings are of more concern than others (Figure 8). Based on this study, the clinic has updated this figure to more accurately reflect soft and intermediate findings associated with conditions on the hypermobility spectrum. Primary care providers should become

familiar with the clinical findings which are commonly found in these conditions, as they are likely to have at least a few in their practice with hypermobility concerns. Pediatricians should also learn to recognize symptoms and begin interventions sooner to reduce symptom and pain burdens on patients in the future.

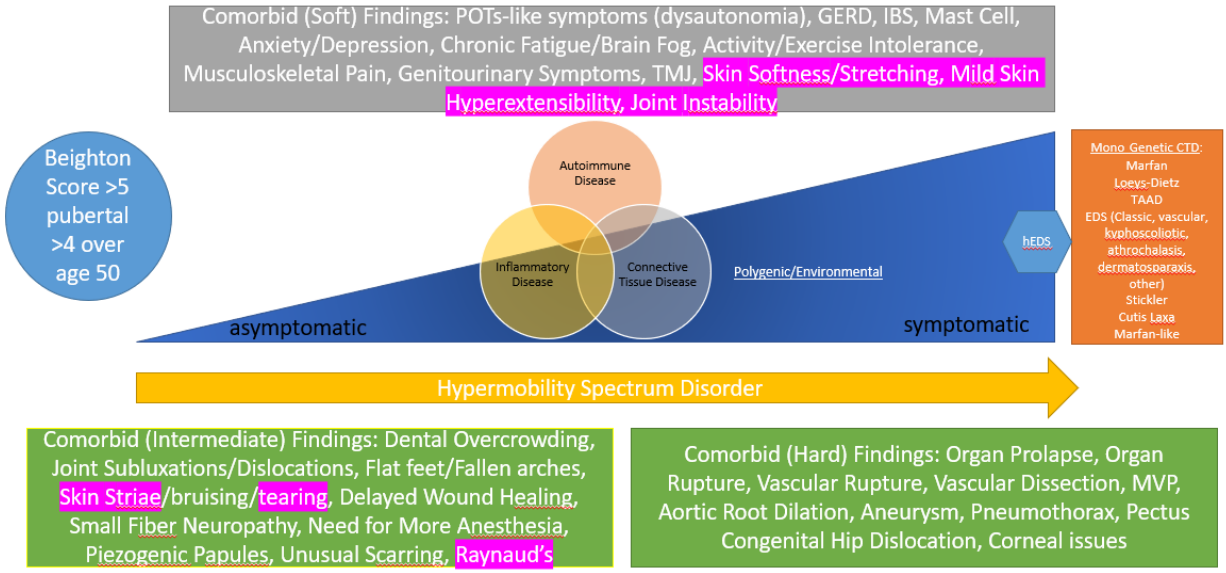

Figure 8 PCPM clinic guide to HSD and genetic CTDs

Demonstrates Beighton score necessary to be classified as HSD. Range of asymptomatic to symptomatic presentations of HSD and inflammatory/autoimmune/environmental factors that may also contribute to hypermobility. hEDS is at the highest end of this spectrum. Monogenetic CTDs are listed to the right. Comorbid soft, intermediate, and hard findings as part of these conditions are listed around this spectrum. The pink highlights are findings that were upgraded (those in intermediate) or downgraded (those in soft) on this algorithm based on the data presented here.

Another recommendation is for primary care physicians to receive genetics training to incorporate genetic testing for the patients they feel comfortable testing themselves instead of referring to genetics. The results suggest that genetic testing is warranted for all patients with hypermobility and that there should be a low threshold for offering genetic testing to these patients. We have designed a simple way to determine if genetic testing is warranted in Figure 9 below.

Screening for hypermobility with the Beighton score can determine if the patient is hypermobile. Genetic testing should be offered if the patient is hypermobile and there are vascular findings in the patient or family, there are comorbidities of HSD or hEDS, or there are other systemic findings of a connective tissue disorder. Negative genetic testing rules in hEDS and HSD, and the patient can then be managed based on clinical findings and the final diagnosis. If genetic testing reveals a molecular diagnosis, management recommendations exist to follow these patients, and they can be referred to genetics centers and specialists to help manage their care appropriately.

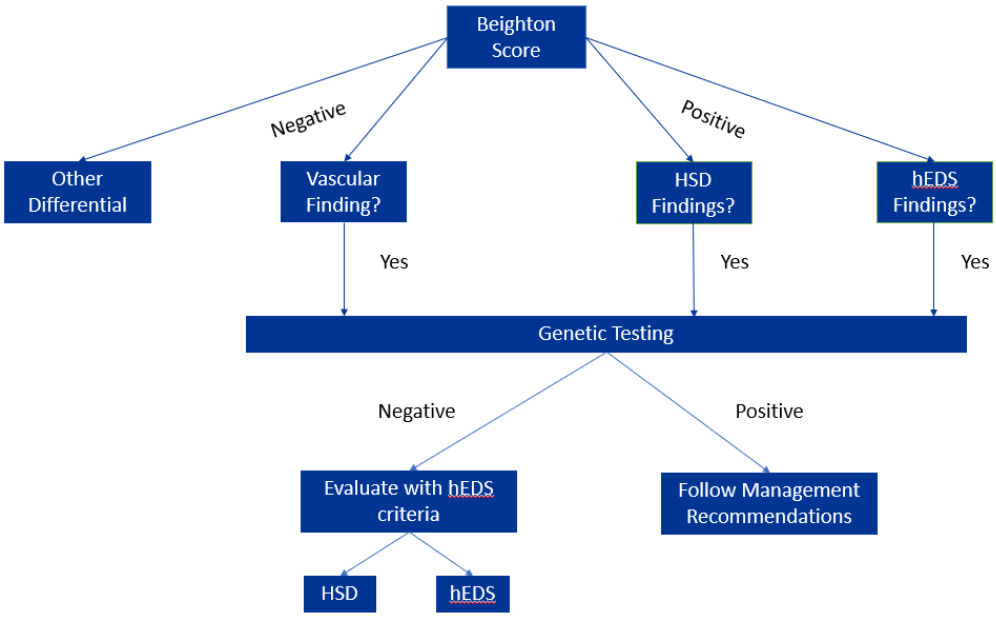

Figure 9 Decision tree for providers to order genetic testing

Further work can help these providers to choose a genetic test in the absence of genetic counselor presence, but genetic counselors are equipped with the knowledge and expertise to choose the appropriate genetic test and assist with insurance or cost issues which may arise. Incorporating additional genetic counselors into this clinic and other primary care clinics will also serve the hEDS and HSD patient population well, as they can receive psychosocial support and

genetic testing without having to wait to see a geneticist or genetics nurse practitioner in a busy genetics clinic.

Finally, reevaluation of the hEDS diagnostic criteria is recommended. It has now been six years since the publication of the last set of criteria, and it is clear from this and other research that the clinical validation of the criteria should be critically evaluated. There needs to be a reckoning with how conditions on the hypermobility spectrum can present and how we can categorize findings in these conditions in meaningful ways to best serve patients.

2.4.8 Limitations

The study was completed in a single clinic with a relatively small sample size and was retrospective in nature. Data was only able to be collected based on chart review and therefore documentation of clinical features may be subject to interpretation or documentation errors. This is also compounded by the patient appointments in PCPM being majority telemedicine appointments. While this increases access to genetics services, some patients may benefit from addition examination and further phenotyping for clinical diagnosis. The self-reported nature of some of the clinical findings is also important to note. Since the appointments and exams were conducted via telemedicine, patients may not correctly self-report clinical features and the physician is not able to properly view features such as dental overcrowding or skin texture over a video visit. This may lead to under- or over-reporting of the clinical features in this study. While only one physician is involved in the clinic, often the questions and Beighton scores were being asked by a medical student or the resident may have seen some of these cases, so there may be differences in the way questions were asked and therefore answered.

Additionally, the sample size of the study did not yield enough genetic testing findings to elucidate any links between individual genes and clinical findings or final diagnosis. Sample size was also crucial for the number of some of the rarer findings, as the numbers were too small to get meaningful statistics. These included autoimmune encephalitis (n=1), low bone density (n=7), congenital hip dislocation (n=6), molluscoid pseudotumors (n=4), and blue sclerae (n=1). One hypothesis for the low number of patients with these findings is that individuals with findings such as blue sclerae and congenital hip dislocation often come to the attention of providers, particularly genetics departments, earlier in life in the pediatric setting and therefore would be less likely to present to an adult clinic with these symptoms. Sample size also impacted the number of genetic diagnoses that were found. Since these are rare conditions, they are less likely to be diagnosed in a group of 135 individuals. Marfan syndrome is found in approximately 1 in 5000 individuals, HSD and hEDS combined prevalence is between 1 in 600-900 individuals, and the prevalence of Loeys Dietz syndrome is unknown.

2.5 Conclusion

This study presents data on the clinical findings associated with diagnosis of different CTDs. hEDS criteria in addition to other commonly reported comorbidities were compared with the final diagnosis of hEDS and HSD. All patients in this cohort were seen in PCPM for concern for CTD and were offered genetic testing. Genetic testing findings were mostly VUSs, but there were still some significant relationships between gene finding and clinical findings. We conclude that performing genetic testing on any individual with concern for CTD is warranted to rule out conditions with known genetic causes such as Loeys Dietz syndrome and Marfan syndrome, which

aligns with current clinical criteria. We present data which clinicians without specialized training in genetics may use to understand the common findings in patients with concern for CTD, when to see patients most urgently for genetic testing, and when to offer genetic testing to these patients. Furthermore, adopting a similar clinic structure and way of ordering genetic testing for this patient population may be utilized in other institutions and clinics, particularly in primary care settings. This data will open the door to incorporating genetic counseling and genetic testing into primary care and further enhance the knowledge CTDs for primary care providers.

3.0 Research Significance to Genetic Counseling and Public Health

As defined by the National Society of Genetic Counselors, genetic counseling is "the process of helping people understand and adapt to the medical, psychological, and familial implications of the genetic contributions to disease" (Resta et al. 2006). Genetic counselors are an integral part of the healthcare team who can specialize in a variety of areas, such as pediatrics, prenatal, and cancer, and have expanded into areas such as primary care, cardiology, nephrology, immunology, and neurology (NSGC 2022b). The symptoms and presentations of CTDs span these specialties and many more, which also leads this patient population to present to clinic with unique psychosocial concerns. Some genetics centers would like to stop seeing patients with concern for hEDS or HSD altogether, and some want to build in additional time to see these patients to cover their extensive symptoms and not have providers running behind in their schedules.

Primary care providers (PCPs) serve a unique role where they likely see many patients with concerns for CTDs but are unsure what they can do for them. They tend to refer patients to other specialties to address specific concerns, refer to physical and/or occupational therapy, and help them with general pain management. In a survey of vEDS patients, a primary care provider coordinated care for about 36% of respondents but 20% had no one managing their care (Shalhub et al. 2020). Many of those same respondents found that the internet provided the most useful information, which included sites such as the Ehlers Danlos Society, NIH Genetic and Rare Diseases Information Center, and Mayo Clinic (Shalhub et al. 2020). PCPs are going to be seeing more individuals with genetic concerns and with increased awareness and knowledge about genetics more patients ask about genetics services. Primary care clinicians are therefore in a great position to inform and educate individuals about conditions such as hEDS and HSD and what they

can do to help improve symptoms. In one study, participants with hEDS were found to have a limited understanding of their disease and the symptoms they experience and a perception that the disorder threatens their health-related outcomes; however, these participants reported a moderate sense of personal control over their symptoms and were not optimistic about the benefits which treatment could provide (Hope et al. 2017). There are barriers to access genetics care in genetics specialty clinics, but primary care services may help to fill this gap and offer appropriate genetic testing and counseling services (Massart et al. 2022).

One of the ways to address some fears or worries with these conditions is to complete genetic testing to rule out any conditions with a known genetic cause that are known to have increased risk of cardiovascular concerns and other more serious complications. One study found that the group of participants with suspected EDS had the highest psychological distress overall, with the lowest mental and social-relationship quality of life scores (Rochetti et al. 2019). Genetic counselors are therefore in a unique space to be able to translate their services into the primary care setting and address concerns for CTDs.

Genetic counselors in primary care clinics could help break down barriers to accessing providers who can diagnose these patients and coordinate the necessary follow-up care. Accessibility also plays a big role in delayed diagnosis, with time, cost, and wait times to appointments playing significant parts in this delay (Anderson and Lane 2021). The fragmented healthcare system makes interdisciplinary care of CTD patients difficult: time is of the essence in short appointments to get physicians to understand their symptoms, and care for the symptoms varies between providers (Martin 2019). There is real value for genetic counselors demonstrated by the data in this study. VUSs are confusing for even some physicians to interpret, and these variants were the majority of findings in this patient cohort. Genetic counselors are trained to be

able to interpret these results and disclose them to patients in a way that will make sense to them. They provide psychosocial support to patients when receiving uncertain results or leaving without a molecular diagnosis is distressing, and they can do this in the span of 30 to 60 minutes in an appointment as opposed to 15 minutes for a typical PCP appointment. However, there are some barriers to integrating genetic counselors into these clinics. Public health crises such as the COVID-19 pandemic and the opioid crisis have proved to be barriers for adding genetic counselors into primary care settings (Slomp et al. 2022). Having genetic counselors willing to build relationships and trust with primary care providers will help integrate both genetic counselors and genetics services in general into primary care practices (Slomp et al. 2022). Therefore, genetics-focused primary care clinics such as PCPM may be able to meet patient needs by screening for common conditions and less complex cases of hereditary cancer family history, carrier screening, and conditions such as CTDs. This type of clinic could also break down barriers to genetics services by providing services via telemedicine and in clinics in underserved communities while at the same time integrating genetics into the primary care setting.

Additionally, genetics knowledge is always expanding, and this is going to continue to be the case for CTDs. EDS diagnostic criteria are constantly being revised, such as in the definition of classic-like EDS caused by *AEBP1* autosomal recessive variants in Ritelli et al. 2019. and the *TNXB* deficiency type of EDS that has also been recently described. The HEDGE study will be an important step forward to understand the molecular basis of hEDS based on the 2017 diagnostic criteria, but there will still be time for genetic testing companies to get on board with those results and more data will need to be collected to validate those results. Genetic counselors will be an important bridge during this period of time as research further describes these conditions and their genetic causes by explaining the utility of genetic testing at a particular point in time, reviewing

all types of possible test results with patients, and helping patients with feelings of uncertainty and balancing that with hope for future research to elucidate a clearer picture of these molecular etiologies.

Appendix A Copy of IRB Determination

University of Pittsburgh | Institutional Review Board
Office of Research Protections

EXEMPT DETERMINATION

Date:	December 1, 2022
IRB:	STUDY22100143
PI:	Rebecca Oberschmidt
Title:	Correlating Clinical Findings with Genetic Testing Results in Patients with Concern for Connective Tissue Disorders - A Retrospective Chart Review
Funding:	None

The Institutional Review Board reviewed and determined the above referenced study meets the regulatory requirements for exempt research under 45 CFR 46.104(d)(iii).

Determination Documentation

Determination Date:	12/1/2022
Exempt Category:	(4) Secondary research on data or specimens (no consent required)
Determinations:	• Waiver of HIPAA authorization
Approved Documents:	• Data Collection Form, Category: Data Collection;

If you have any questions, please contact the University of Pittsburgh IRB Coordinator, John Ries. **NOTE**: Modifications are only required if they will affect the exempt determination. It is important to **close your study when finished** by submitting a Continuing Review.

Please take a moment to complete our Satisfaction Survey as we appreciate your feedback.

Appendix B Physician Note Template

CTD symptoms:

Rate level of severity or significance (0-5):

1. Joint instability, subluxations, dislocations:

2. Gastrointestinal symptoms (GERD, dyspepsia, IBS):

3. Skin stretch, scaring, bruising, tearing:

4. Widespread musculoskeletal pain:

5. Anxiety, phobia, depression:

6. Fatigue, brain fog:

7. Autonomic dysfunction (temperature):

8. Uro/gyn symptoms:

9. Activity intolerance:

Do you have or been diagnosed with:

1. Scoliosis, which kind:

2. Fibromyalgia, chronic fatigue, autoimmune encephalitis:

3. Proprioception issues (trouble knowing where joints are):

4. TMJ:

5. Muscle stiffness, tightness:

6. Dental overcrowding:

7. Allergies or mast cell abnormalities:

8. Low bone density:

9. Chronic neck strain:

10. Poor wound healing:

11. Flat feet or fallen arches:

12. Any cardiovascular issues (MVP or aortic root dilation):

13. History of poor response to anesthesia:

14. Hernias:

15. Organ prolapse:

16. Spontaneous organ rupture or pneumothorax or vascular rupture:

17. Family member diagnosed with CTD:

18. Pain in more than two limbs daily for three or more months:

19. Chronic widespread pain for three or more months:

20. POTS:

21. Raynaud's:

22. Congenital hip dislocation:

23. Eye issues (myopia, astigmatism, hypermetropia, corneal issues or rupture, glaucoma):

CTD Exam:

Beighton score:

Height:

Skin texture:

Skin hyperextensibility:

Striae:

Pectus:

Scoliosis:

Piezogenic papules of the heels:

Atrophic scarring:

Papyraceous or hemosideric scars:

Arachnodactyly (Walker:wrist sign or Steinberg:thumb sign):

Molluscoid pseudotumors:

Subcutaneous spheroids:

Epicanthal folds:

Blue sclerae:

Joint hypermobility:

Suspect:

Appendix C Concert Genetics Genetic Testing Comparison

Test Name	Heritable Disorders of Connective Tissue Panel	Invitae Connective Tissue Disorders Panel
Lab Name	GeneDx	Invitae Corporation
Category	Connective Tissue Disorders Panel Tests	Connective Tissue Disorders Panel Tests
Test Code	J555	434340
Source		
Price	Call for Price	$1,500.00
TAT	28 days	10-21 days
Techniques	Deletion/Duplication, Sequencing	Deletion/Duplication, Sequencing
Mechanisms	Deletion/Duplication, Next Generation Sequencing	
Overlapping Genes	ACTA2, ADAMTS2, AEBP1, ALDH18A1, ATP6V0A2, ATP6V1E1, ATP7A, B3GALT6, B3GAT3, B4GALT7, BGN, CBS, CHST14, COL11A1, COL11A2, COL12A1, COL1A1, COL1A2, COL2A1, COL3A1, COL4A1, COL5A1, COL5A2, COL9A1, COL9A2, COL9A3, DSE, EFEMP2, ELN, FBLN5, FBN1, FBN2, FKBP14, FLNA, LOX, LTBP4, MAT2A, MED12, MFAP5, MYH11, MYLK, NOTCH1, PLOD1, PRDM5, PRKG1, PYCR1, RIN2, SKI, SLC2A10, SLC39A13, SMAD2, SMAD3, SMAD4, TGFB2, TGFB3, TGFBR1, TGFBR2, ZNF469	
Unique Genes	TAB2, TNXB	ABCC6, ABL1, ACVR1, ADAMTS10, ADAMTS17, ADAMTSL4, ARIH1, ATP6V1A, C1S, CHST3, COG7, CRTAP, DCHS1, FLCN, FLNB, FOXE3, GGCX, GORAB, HCN4, LEMD3, LOXL3, LTBP2, LTBP3, LZTS1, NOG, P3H1, PKD2, PLOD3, SLC26A2, SMAD6, SPARC, TALDO1, TGFB1, UPF3B
Coverage		

Concert Genetics Compare Genetic Tests tool (https://app.concertgenetics.com/apps/search/#/) comparing the GeneDx Heritable Disorders of Connective Tissue panel and the Invitae Connective Tissue Disorders panel (as of March 2023).

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