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The Value of a Genetics Evaluation for Patients with Suspected Ehlers-Danlos Syndrome or Other Possible Connective Tissue Disorder

THESIS

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by

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DEDICATION

To

my family for their continued support through my educational journey

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ABSTRACT OF THE THESIS

The Value of a Genetics Evaluation for Patients with Suspected Ehlers-Danlos Syndrome or Other Possible Connective Tissue Disorder

By

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Master of Science in Genetic Counseling

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Ehlers-Danlos syndrome (EDS) is a group of inherited connective tissue disorders (CTDs) characterized by hyperflexibility, skin hyperextensibility, and tissue fragility. As recently as 2017, the International EDS Consortium published a new classification system with diagnostic criteria for thirteen different subtypes of EDS [Malfait et al., 2017]. The genetic etiology is known for all subtypes except hypermobile EDS (hEDS) which relies on clinical diagnostic criteria for diagnosis. Many patients are referred to the Genetics specialty for evaluation for EDS or other possible CTD. This study consisted of a retrospective chart review of referrals and Genetics consultation notes at one adult Genetics clinic to determine what types of providers are referring patients to the Genetics specialty, what types of evaluations (either imaging or evaluations by other specialties) patients are undergoing prior to seeing Genetics to further clarify if they may have a possible CTD, and what recommendations Genetics is making for these patients following evaluation. This study also compared patients who were self-referred and those who were referred by a provider and found there was no difference in the number of evaluations done prior to seeing Genetics nor the recommendations made after seeing Genetics. This study demonstrated that there is value in a Genetics evaluation for EDS or other CTDs

because patients are discussing more clinical features with Genetics providers, geneticists provide a comprehensive exam across all systems of the body, and genetic counselors are uniquely qualified to provide supportive counseling, education, and resources this patient population needs.

INTRODUCTION

I. Connective tissue disorders

Connective tissue provides the structural support for our body and organs. Connective tissue is composed of collagen and elastin. There have been 28 types of collagen described and 46 genes encoding collagen proteins have been identified [Ricard-Blum, 2011, HGNC, 2018]. Mutations, or pathogenic variants, in many of these genes have been well described to cause a variety of connective tissue disorders (CTDs). There are more than 200 CTDs and many have overlapping features. Some well described CTDs include Ehlers-Danlos syndrome, Marfan syndrome, Loeys-Dietz syndrome, and Osteogenesis Imperfecta among many others. Pathogenic variants in the collagen genes (COL) as well as several other genes that contribute to the function of connective tissue can cause CTDs. While many hereditary CTDs have been well described, there still remain some CTDs for which the genetic etiology has not yet been found or an individual may meet diagnostic criteria for a well-known CTD, but no gene mutation can be identified using the current molecular technology.

II. History of Ehlers-Danlos syndrome classifications

Ehlers-Danlos syndrome (EDS) is a group of CTDs with a wide range of phenotypes and disease classification that has varied over time. The 1986 International Nosology of Heritable Disorders of Connective Tissue Workshop held in Berlin classified eleven different types of EDS and named the subtypes with roman numerals (EDS I-XI) [Beighton et al., 1988]. With increasing clinical experience and biochemical and molecular studies, the Villefranche nosology proposed six types of EDS in 1997. These six subtypes were each given descriptive names to replace the roman numeral classification system and were each given major and minor diagnostic criteria [Beighton et al., 1998]. As recently as January 2017, the International EDS Consortium

proposed a new classification system of thirteen different subtypes of EDS [Malfait et al., 2017]. Each of the thirteen subtypes was given a descriptive name in line with the 1998 Villefranche classification (Appendix A). Currently, the molecular etiology is known for twelve of the thirteen subtypes of EDS and each subtype has been given both major and minor diagnostic criteria. While there are thirteen different subtypes, the most common subtypes of EDS are Classical EDS (cEDS), Vascular EDS (vEDS), and Hypermobile EDS (hEDS). Individuals are often referred to the Genetics specialty for suspicion of EDS or other possible CTD. Geneticists and genetic counselors provide a multisystemic assessment, give appropriate patient referrals and resources, and order genetic testing which can confirm a diagnosis of an EDS subtype with known molecular etiology. Genetic testing is unable to confirm a diagnosis of the hEDS subtype, for which the genetic cause has not yet been identified [Malfait et al., 2017].

III. Classical Ehlers-Danlos syndrome

Classical EDS (cEDS) is an autosomal dominant subtype of EDS affecting approximately 1 in 20,000 individuals [Byers, 2001]. Major diagnostic criteria for cEDS includes skin hyperextensibility and atrophic scarring and generalized joint hypermobility (GJH). Other minor features of cEDS are dislocation and/or subluxation due to increased join instability, velvety skin that can bruise easily, skin fragility, molluscoid pseudotumors, subcutaneous spheroids, hernia, and epicanthal folds (Appendix B). Muscle hypotonia and skeletal differences such as scoliosis, pectus deformities, elbow/genus/hallux valgus, and bone fragility may also be present. Vascular findings such as mitral valve prolapse have been reported in individuals with cEDS but are much less common than in other subtypes of EDS. Mitral valve prolapse occurs in about 6% of cases of cEDS and aortic root dilation has been reported but rarely progresses to aortic dissection, which can be fatal when it does occur. Individuals with cEDS are at risk for surgical

complications due to skin extensibility and fragility. Women with cEDS are also at risk for premature rupture of fetal membranes during pregnancy [Bowen et al., 2017]. Over 90% of cases of cEDS are caused by pathogenic variants in the *COL5A1* and *COL5A2* genes that disrupt the function of type V collagen [Malfait et al., 2017]. A particular variant, c.934C>T (p.Arg312Cys), in the *COL1A1* gene (a type I collagen encoding gene) has also been reported in unrelated individuals with cEDS. The majority of pathogenic variants in the *COL1A1* gene cause a different CTD called Osteogenesis Imperfecta [Nuytinck et al., 2000; Malfait et al., 2007] which can have some clinical overlap with cEDS. There is no cure for cEDS, but symptoms can be managed by avoiding trauma to the skin and joints, and treatment with anti-inflammatory drugs or other pain medications for joint pain. Surveillance includes routine echocardiograms to monitor for aortic root dilation and mitral valve prolapse, and monitoring pregnancies or surgeries carefully [Bowen et al., 2017].

IV. Vascular Ehlers-Danlos syndrome

Vascular EDS (vEDS, previously EDS type IV) is a rarer, more severe autosomal dominant form of EDS affecting 1 in 150,000 people. Major diagnostic criteria for vEDS includes arterial rupture at a young age, spontaneous sigmoid colon perforation in the absence of a known diverticular disease or other bowel pathology, and uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears. Additional minor characteristics include: bruising in the absence of trauma, thin, translucent skin, characteristic facial features, spontaneous pneumothorax, acrogeria (premature aging of skin), club foot, congenital hip dislocations, tendon or muscle rupture, gingival recession, early onset (under age 30 and nulliparous) of varicose veins (Appendix C)[Malfait et al., 2017]. Unlike other forms of EDS, patients with this subtype of EDS do not have GJH or skin hyperextensibility [Pepin et al.,

2000; Malfait et al., 2017]. As is indicated in the diagnostic criteria, individuals with vEDS are prone to ruptures of the arteries (thoracic or abdominal) or other organs such as the uterus or bowels and spontaneous pneumothorax. More than 80% of individuals with vEDS have a medical or surgical complication by age 40 [Pepin et al., 2000; Malfait et al., 2017]. Due to the risk of organ rupture, the average life expectancy of these patients is 48-51 years old [Pepin et al., 2000; Frank et al., 2019].

Although there is no cure for vEDS, a molecularly confirmed diagnosis of vEDS can influence surveillance, surgical management, pregnancy management, and reproductive counseling. Vascular EDS is caused by mutations in the *COL3A1* gene, a type III collagen encoding gene [Frank et al., 2015]. There are three rare *COL1A1* variants (c.934C>T, p.Arg312Cys; c.1720C>T, p.Arg574Cys; c.3277C>T, p.Arg1093Cys) that have been reported to cause the same vascular fragility as seen in patients with *COL3A1*-associated vEDS [Malfait et al., 2017]. A molecularly confirmed diagnosis of vEDS has been shown to decrease surgical complications and mortality following emergent surgical intervention because different surgical measures may be undertaken when the patient has a known tissue fragility condition [Shalhub et al., 2014]. There are currently no cures or preventative measures for the vascular events seen in patients with vEDS. However, patients with vEDS should establish care with a care team, including a vascular surgeon, who are well versed in the risks associated with vEDS. These individuals are also encouraged to wear a medical alert bracelet and/or carry an emergency letter alerting other health care providers of their condition. Arterial screening can be performed, but there are no guidelines surrounding elective repair of unruptured aneurysms [Pepin et al., 2000]. Surgical intervention can be lifesaving in the event of arterial or organ rupture, but minimal surgical exploration is undertaken to prevent further tissue damage [Byers, 1999]. Pregnant

women with vEDS should be considered high risk and followed at a specialized center [Pepin et al., 2000].

V. Hypermobile Ehlers-Danlos syndrome

The most common subtype of EDS is hypermobile EDS (hEDS, previously called EDS type III). It was previously thought that hEDS affects 1 in 5,000 individuals, but with recent reclassification of the diagnostic criteria for hEDS becoming more stringent, the prevalence of hEDS is unclear [Castori et al., 2010; Forghani, 2019]. Patients with hEDS typically present with joint hypermobility on the same spectrum as patients with benign joint hypermobility syndrome [Castori et al., 2010]. They may also present with recurrent dislocations, velvety skin, piezygotic papules, propensity to bruising, chronic joint pain, abdominal pain or gastrointestinal discomfort, and anxiety or depression [Castori et al., 2010]. Hypermobile EDS has several overlapping features with other types of EDS, but the features may vary in severity. For example, individuals with hEDS are described to have soft, velvety skin and they may have semi-transparent skin but these features are much more subtle than in individuals with vEDS [Tinkle et al., 2017]. Gastrointestinal complaints such as dysphagia, reflux disease with or without hiatal hernia, irritable-bowel disease-like symptoms, constipation, and diarrhea can occur in cEDS but are more common in hEDS [Bowen et al., 2017].

In one study of individuals with hEDS, more than 99% of individuals with hEDS reported experiencing pain on a regular basis [Murray et al., 2013]. Both acute and chronic pain are reported by individuals with hEDS. Some possible etiologies of the pain these individuals experience are spasms of muscles, tendons, or other connective tissues, direct trauma due to joint instability, and osteoarthritis [Tinkle et al., 2017; Syx et al., 2017]. Pain is a reported symptom in other forms of EDS, but pain frequency and intensity is higher in hEDS than cEDS [Bowen et

al., 2017]. Pain contributes to impairment of daily functioning in individuals with hEDS but treatment is poorly defined and developed [Syx et al., 2017; Rombaut et al., 2011]. Pain management typically comprises of medications such as analgesics, topical agents such as lidocaine, cannabis where allowed by law, and opioids. Lifestyle changes such as avoiding high impact, high resistance activities and physical therapy focusing on strengthening and joint stability have also been recommended to improve pain symptoms [Rombaut et al., 2011; Tinkle et al., 2017]. Surgical intervention for joint stability has been performed as well, but the results of such surgeries are limited, and in a survey of patients' relief of symptoms post-surgical intervention, only 33.9% of individuals reported a positive effect [Rombaut et al., 2011].

Cardiovascular features are not a main feature of hEDS, but studies have found that children and young adults with hEDS can have mild aortic root dilation or mitral valve prolapse [McDonnell et al., 2006; Atzinger et al., 2011]. Longitudinal studies have found that aortic root dilation is unlikely to progress and does not usually require any treatment [Atzinger et al., 2011; Ritter et al., 2017]. While individuals with hEDS may be at an increased risk for mitral valve prolapse (6% compared to 1% in the general population), Atzinger et al. [2011] found only one of 252 individuals had moderate-to-severe mitral valve prolapse. Therefore, Atzinger et al. suggest that routine echocardiogram screening may be unnecessary for individuals with hEDS in the absence of cardiac symptoms or a striking family history [Atzinger et al., 2011].

Studies have found associations with hEDS and mast cell activation disorders (MCADs), postural tachycardia syndrome (POTS), fatigue, anxiety, and depression. In recent years, there have been reports of MCAD and POTS in individuals with hEDS and joint hypermobility [Bonamichi-Santos et al., 2018]. It is well known that mast cells reside in connective tissues and play a role in tissue homeostasis and coordination of immune responses, but the exact mechanism of MCAD's contribution to hEDS is still under investigation [Seneviratne et al.,

2017a]. While there have been several studies showing an association of MCAD and POTS in individuals with hEDS, these case studies have involved small numbers of individuals and POTS and MCAD are not currently a part of the diagnostic criteria for hEDS [Wallman et al., 2014; Bonamichi-Santos et al., 2018; Seneviratne et al., 2017a].

Fatigue, anxiety, and depression are commonly reported among individuals with hEDS. In a survey of 466 individuals with a diagnosis of hEDS made in a clinic or hospital, Murray et al. [2013] found that 82% of participants reported chronic fatigue, 73% reported having anxiety, and 69% reported experiencing depression. Fatigue has been described as both mental and physical in patients with hEDS and may contribute to decreased muscle control, increased risk for injury, sleep disturbances, anxiety and depression [Tinkle et al., 2017]. The fatigue, chronic pain, and lack of effective treatment likely contribute to the psychological distress and dysfunction that many individuals with hEDS report [Syx et al., 2017; Sinibaldi et al., 2015]. These factors can contribute to resentment, distrust, and hostility towards peers, family, and health care providers [Tinkle et al., 2017] and likely contribute to the decrease in quality of life that has been reported in individuals with hEDS [Murray et al., 2013; Tinkle et al., 2017].

The genetic etiology of hEDS has not yet been identified. However, based on family studies hEDS is described as an "autosomal dominant disorder 'influenced by sex,' with a predominance of symptoms in females" [Tinkle et al., 2017]. There have been case reports of hEDS due to variants in the tenascin X (*TNXB*) gene. Zweers et al. found haploinsufficiency of *TNXB* in 9 out of 14 females who had joint hypermobility, skin hyperextensibility and easy bruising [Zweers et al., 2003]. A novel missense variant, c.12172C>G (p.C4058W), in the *TNXB* gene has also been described in seven families that have a phenotype consistent with hEDS [Morissette et al., 2015]. In order to try to better understand the molecular background of hEDS, Chiarelli et al. performed immunofluorescence analysis and gene expression profiling of cultured fibroblasts in five

individuals with hEDS/joint hypermobility syndrome. Their findings suggest that several different signaling cascade pathways are required for the maintenance of the extracellular matrix (structure of connective tissue) architecture and homeostasis in patients with hEDS/joint hypermobility, and therefore, several different signaling cascades are involved in the multisystemic phenotype of these patients [Chiarelli et al., 2016].

Despite these case studies, it remains that the cause of the vast majority of hEDS cases is unknown and diagnosis relies on meeting clinic criteria, most recently defined in the 2017 International Classification of the Ehlers-Danlos Syndromes [Malfait et al., 2017]. Clinical diagnostic criteria incorporates Beighton score to assess generalized joint hypermobility. The Beighton score measures hypermobility on a 9-point scale: 1 point for each pinky finger (right and left) that extends back beyond 90 degrees when the palm and forearm are flat on a surface, 1 point for each thumb (right and left) that can passively be moved to touch the underside of the forearm when the arm is extended, 1 point for each elbow (right and left) that extends beyond 10 degrees when arms are outstretched to the sides and the palms are face up, 1 point for each knee (right and left) that extends beyond 10 degrees backward when the knees are locked, and 1 point for bending over to touch both palms flat on the floor from a standing position with straight legs. The diagnostic criteria also incorporates other clinical features previously described, chronic widespread pain, a positive family history, as well as exclusion of other connective tissue disorders [Malfait et al., 2017]. The Ehlers Danlos Society has created a diagnostic checklist for doctors across all disciplines to use to diagnose hEDS (Appendix D).

VI. Other subtypes of Ehlers-Danlos syndrome

There are three subtypes of EDS with autosomal recessive inheritance (Classic-like EDS, Cardiac-valvular EDS, and Kyphoscoliotic EDS) that share some features with the more

common subtypes of EDS previously discussed. Classical-like EDS (clEDS) is caused by biallelic mutations or deletions of the *TNXB* gene. Individuals with clEDS may exhibit generalized joint hypermobility, skin hyperextensibility, velvety skin, easy bruising skin, mild proximal and distal muscle weakness, atrophy of muscles in the hands and feet, acrogeric hands, mallet fingers, clinodactyly, brachydactyly, vaginal/uterus/rectal prolapse, or foot deformities such as pes planus, hallux valgus, or piezygotic papules [Malfait et al., 2017].

Cardiac-valvular (cvEDS) is caused by biallelic mutations in the *COL1A2* gene leading to defects in type I collagen. Individuals with cvEDS have severe progressive cardiac-valvular problems, most often involving the aortic or mitral valves. Individuals with cvEDS may also have skin hyperextensibility, atrophic scarring, thin skin, easy bruising, joint hypermobility, inguinal hernias, pectus deformity, joint dislocations, and foot deformities such as pes planus or hallux valgus [Malfait et al., 2017].

Kyphoscoliotic EDS (kEDS) is caused by biallelic mutations in the *PLOD1* and *FKBP14* genes. Major diagnostic criteria for kEDS includes congenital muscle hypotonia, congenital or early onset kyphoscoliosis, and joint hypermobility. Individuals with kEDS due to mutations in the *FKBP14* gene can have congenital hearing loss, follicular hyperkeratosis, muscle atrophy, and bladder diverticula. Individuals with kEDS more commonly have *PLOD1* mutations and they often have skin fragility, scleral and ocular fragility or rupture, microcornea, and facial dysmorphism. These individuals also have an increased deoxypyridinoline to pyridinoline (Dypr/Pyr) ratio on urinalysis and therefore, laboratory confirmation of kEDS should start with Dypr/Pyr ratio urinalysis followed by molecular confirmation [Malfait et al., 2017].

There are several other subtypes of EDS, many of which have overlapping features with the subtypes of EDS previously described such as joint hypermobility and skin and tissue fragility. Other types of EDS include: Arthrochalasia EDS, Dermatosparaxis EDS, Brittle Cornea

syndrome, Spondylodysplastic EDS, Musculocontractural EDS, Myopathic EDS, and Periodontal EDS. Some of these more rare types of EDS have distinguishable features, for example there are unique skin findings and facial features associated with Dermatosparaxis EDS and early onset periodontitis and lack of attached gingiva associated with Periodontal EDS. [Malfait et al., 2017]. For the sake of this research, these other types of EDS will not be discussed in further detail, but it is important to note that all of these other forms of EDS also have a known molecular etiology such that identification of a pathogenic variant can confirm a diagnosis [Malfait et al., 2017].

VII. Hypermobility spectrum disorders

Joint hypermobility is defined as the capability to move a joint beyond normal limits. Generalized joint hypermobility (GJH) is typically assessed by using the Beighton scoring system; individuals having a Beighton score of 4 or more out of 9 are said to have GJH [Beighton et al., 1988]. However, the scoring is somewhat subjective because it is influenced by the examiner [Castori et al., 2017]. GJH is a descriptor, not a diagnosis, and recently efforts have been made to classify individuals with joint hypermobility that lack other musculoskeletal features on a spectrum of Hypermobility Spectrum Disorders (HSDs). Castori et al. [2017] classified seven different categories of joint hypermobility based on which joints are hypermobile, Beighton score, and musculoskeletal features (Appendix E). The aim of classifying HSDs is to distinguish those individuals with joint hypermobility lacking other features from individuals who have hEDS or other CTDs [Castori et al., 2017]. It can be difficult to distinguish other CTDs, especially hEDS, because joint hypermobility is the main feature of many genetic disorders and joint hypermobility can also run in families as an isolated trait. Individuals may be found to have GJH based on Beighton score and a positive family history of joint hypermobility,

but lack the musculoskeletal criteria outlined in the new hEDS diagnostic criteria [Castori et al., 2017; Malfait et al., 2017].

VIII. Other connective tissue disorders with overlapping features of Ehlers-Danlos syndrome

Joint laxity is a common feature of many CTDs, skeletal dysplasias, and even mitochondrial and neuromuscular conditions [Castori et al., 2017]. There are several CTDs such as Marfan syndrome, Loeys-Dietz syndrome, and arterial tortuosity syndrome that have some overlapping features of EDS.

Marfan syndrome is an autosomal dominant condition caused by heterozygous pathogenic variants in the *FBN1* gene. The main features involve the cardiovascular, skeletal, and ocular systems. Individuals with Marfan syndrome can have mitral valve prolapse and aortic root dilation, similar to a subset of the hEDS and cEDS populations [Colombi et al., 2015]. However, individuals with hEDS do not show progression of aortic dilation in adulthood, unlike individuals with Marfan syndrome [Colombi et al., 2015; Atzinger et al., 2011]. Other features of Marfan that can overlap with EDS are the Marfanoid body habitus, pectus deformity and arachnodactyly, but a distinguishing feature of Marfan syndrome is ectopia lentis, which affects approximately 60% of individuals with Marfan syndrome [Colombi et al., 2015; Pyeritz, 2013].

Loeys-Dietz syndrome (LDS) is another autosomal dominant CTD that shares features such as risk for thoracic and abdominal arterial aneurysms or dissections with Marfan syndrome and vEDS [MacCarrick et al., 2014]. Individuals with LDS can present with velvety, thin, translucent skin, easy bruising and atrophic scarring similar to that seen in individuals with vEDS and cEDS [Bowen et al., 2017; Malfait et al., 2017; MacCarrick et al., 2014]. Some characteristics that may distinguish Loeys-Dietz are palatal abnormalities, craniosynostosis, and bifid uvula. There are no diagnostic criteria for LDS, but diagnosis can be confirmed in individuals with suggestive

features by identification of a pathogenic variant in the *TGFBR1, TGFBR2, SMAD3,* or *TGFB2* genes [MacCarrick et al., 2014].

Similar to vEDS, Marfan syndrome, and LDS, arterial tortuosity syndrome (ATS) is characterized by risk for vascular events such as aneurysm and dissections. ATS is characterized by severe arterial tortuosity of the aorta and other arteries [Callewaert et al., 2014]. Individuals with LDS can also experience generalized arterial tortuosity [MacCarrick et al., 2014]. Other findings in individuals with ATS are similar to vEDS and cEDS such as soft skin, pectus deformity, joint laxity, inguinal or abdominal hernias, and arachnodactyly [Malfait et al., 2017, Callewaert et al., 2014]. Diagnosis of ATS is established in individuals with arterial tortuosity and biallelic pathogenic variants in the *SLC2A10* gene [Callewaert et al., 2014]. Marfan syndrome, LDS, and ATS are just a few of the CTDs that have overlapping features with some of the subtypes of EDS. These and many other CTDs have particular features that distinguish them from EDS, but individuals with a CTD do not have every feature of the condition, so it is important to be aware of other possible differential diagnoses.

IX. Role of genetics specialty in evaluating patients and diagnosing connective tissue disorders

The Genetics specialty receives many referrals for the various types of EDS and to evaluate patients for a possible CTD. Medical geneticists and genetic counselors can provide a comprehensive evaluation for possible CTDs by conducting a physical exam, taking a three generation family pedigree, and discussing appropriate genetic testing. Medical geneticists and genetic counselors can also provide referrals for follow-up imaging or exams when appropriate and can provide patients with support group information or other patient resources.

Family history is an important tool when assessing an individual's risk for inherited conditions. A three generation pedigree is collected during each initial Genetics evaluation. A pedigree indicating which relatives have a history of features associated with CTDs can help distinguish who is at risk of inheriting a disorder, which may be especially important in cases of disorders with life-threatening risks. In cases of vEDS, patients are often not diagnosed until after an organ rupture event or even postmortem [Pepin et al., 2000] thus highlighting the importance of capturing history of an event like this when taking a pedigree. Pedigrees can also help determine which relatives may be at risk of inheriting the same condition and can indicate familial testing if a pathogenic variant is found.

Diagnostic criteria are helpful when conducting a physical examination to evaluate for a particular disorder or group of disorders. Physicians from any specialty can use the Ghent nosology to aid in diagnosing Marfan syndrome [Loeys et al., 2010] and the new 2017 diagnostic criteria for the various subtypes of EDS as outlined by Malfait et al. [2017]. When appropriate, genetic testing can be ordered to confirm a suspected diagnosis, with the exception of hEDS for which the genetic cause has not get been identified. Different types of genetic testing include gene panel tests that sequence multiple genes associated with different CTDs, familial variant testing when a pathogenic variant has been identified in a family member, single gene sequencing, and whole exome sequencing. While there are many benefits to genetic testing such as confirmation of a suspected diagnosis (which can allow for appropriate disease management) when a pathogenic or likely pathogenic variant is found, there are still costs and risks associated with genetic testing. One risk, and frustration, with genetic testing is when a patient's testing identifies a variant of uncertain significance (VUS). A VUS is a variant for which there is not enough data to classify it as pathogenic, likely pathogenic, likely benign, or benign. Medical management and surgical decisions should not be made on the basis of an identified VUS, and

familial and prenatal testing are not offered for VUSs [Richards et al., 2015]. There is a risk that patients and providers may interpret a VUS as a "positive" result or pathogenic possibly resulting in inappropriate surgical management or possibly unnecessary screening. A VUS is an uninformative result that can cause frustration and anxiety for both provider and patient. Geneticists and genetic counselors can offer appropriate counseling and education to try to alleviate some of this anxiety.

While there is great value in a Genetics evaluation for a possible CTD, it has been noted by the Genetics group at the University of California, Irvine Medical Center (UCIMC) that some Genetics groups are no longer seeing patients for this indication. Anecdotally, there has been an increasing number of referrals to the UCIMC for patients to be evaluated for EDS. Whether this is due to an increasing awareness of the condition, due to other Genetics groups not accepting referrals for CTDs, or due to other causes is unknown. Genetic counselors have written discussion board postings about how to triage an increasing number of referrals for EDS in Genetics clinics and where to find EDS specialists across the United States on the National Society of Genetic Counselors (NSGC) webpage, further supporting that more patients are being referred to Genetics clinics for this indication.

X. Gaps in current knowledge and research

As described, past studies have examined the clinical features of each subtype of EDS in detail and found some overlapping features of hEDS with other subtypes [Castori et al., 2010; Bowen et al., 2017; Frank et al., 2015]. Further research needs to be done to examine some of the unique associations specific to hEDS such as MCAD [Seneviratne et al., 2017b] and POTS [Wallman et al., 2014]. There have not been studies that examine reported phenotypes of EDS patients over time as diagnostic criteria has changed or since the new criteria for hEDS was

published in March 2017 by the International EDS Consortium [Malfait et al., 2017]. One study surveyed individuals with hEDS about their lived experience with the syndrome. Although it was not the specific aim of this study, researchers asked what type of physician gave patients their diagnosis and what type of provider is managing their clinical care [Murray et al., 2013]. There remains to be research specific to diagnosing and treating these patients within the Genetics specialty. As previously mentioned, patients are referred to the Genetics specialty for an evaluation of EDS, yet there is little research specific to which types of providers are referring patients to the Genetics specialty and what types of other evaluations patients are undergoing, if any, prior to being referred to Genetics. With some Genetics groups not accepting referrals for CTDs it is important to understand the utility of a Genetics evaluation for these indications and try to create a strategy for an effective and efficient Genetics evaluation for EDS and CTDs.

XI. Purpose and aims of this study

This study is a retrospective chart review of referrals and clinical documentation of patients referred to the Genetics specialty at UCIMC. One aim of this study is to determine what types of providers are referring patients to the Genetics specialty for an evaluation of EDS or other possible CTD and what types of evaluations (either imaging or evaluations by other specialties) these patients undergo prior to seeing Genetics to further clarify if they may have a possible CTD. Given the update of diagnostic criteria for EDS published in 2017 and increasing awareness of EDS, it is hypothesized that more patients were referred to the Genetics specialty from a wider range of specialists and that there were increasing numbers of referrals from primary care physicians in the period since the guidelines were published. A second aim of this study is to determine if there is a correlation between types of referrals (self-referred vs. referred from another specialty) and the recommendations made after the

patient is seen by the geneticist. It is hypothesized that if the patient is self-referred to the Genetics clinic, then there will be more recommendations made by the geneticist following examination and the patient will require a second visit to the Genetics clinic. By exploring referrals specific to the Genetics specialty, this study helps us to better understand why patients are referred to Genetics and what the Genetics specialty can do for these patients, particularly patients with hEDS for which a genetic cause has not yet been identified.

METHODS

I. IRB Approval

This study was approved by the University of California, Irvine (UCI) Institutional Review Board (IRB) under study HS# 2019-4925.

II. Retrospective Chart Review

Charts of patients at UCI Medical Center (UCIMC) who were referred to the Genetics specialty for an evaluation of EDS or to rule out a possible CTD were reviewed by the lead author. Charts from 2014 [when UCIMC began using an electronic medical record (EMR) system, Quest (2014-2017) and Epic (2017-2019)] through March 2019 were reviewed. Patients were identified by the referral indication entered into the secure electronic database of Genetics patients (FileMaker Pro). Patients were assigned a non-identifying patient number which corresponds to their medical record number in a secure coded key sheet. The coded key was kept on a secure UCIMC server.

A total of 151 patients were identified as referred to and attempted to be scheduled in the Adult Genetics Specialty Clinic at UCIMC for an evaluation of possible EDS or to rule out a possible CTD from January 2014 to March 2019. The patient referrals, available imaging, and

medical documentation were reviewed. A data collection sheet was generated and used for subsequent analysis. No Protected Health Information (PHI) was collected and documented on the data collection sheet.

A separate count of the total number of referrals, including those not yet attempted to be scheduled due to limited clinic availability or requests for more clinical information as part of the triage process, was completed by reviewing the referral indications listed in the UCIMC STARS referral tracking system (January 2014- October 2017) and the EPIC scheduling work queue for Genetics (November 2017-May 2019). If a patient had not been contacted in attempt to be scheduled, they were excluded from the chart review, but were counted as part of the larger referred group.

Patient demographics such as age, sex, insurance type (PPO, HMO, Medicaid/Medicare/Tricare), year referred to Genetics, and year seen by Genetics were collected. The following information was collected from patient referrals to the Genetics specialty: specialty of referring provider, whether or not the patient was self-referred, clinical features listed on referral, whether or not family history was mentioned on referral, Beighton score if available, if genetic testing was mentioned or requested, previous genetic testing results if applicable, imaging studies, biochemical testing, and if the patient had seen Cardiology, Ophthalmology, Rheumatology, Orthopedics, or Immunology/Allergy specialists. Patients were categorized into two categories: self-referred and provider-referred. If a patient was had no referring provider listed (or listed as self) or if the referral mentioned the patient was requesting the referral to Genetics, they were categorized as "self-referred."

The following information was collected from the medical documentation from the medical geneticist and genetic counselor at UCIMC: Beighton score, whether or not the patient meets the 2017 hEDS criteria 2 (see Appendix D), clinical features noted on physical exam, if a diagnosis

of hEDS was given, if other differential diagnoses were noted and if so, which other conditions were included, and if recurrence risk was discussed. To gain an assessment of family history of possible connective tissue disorders, the number of maternal and paternal first and second degree relatives reported to have a diagnosis of EDS or "features of EDS or other possible connective tissue disorders" were extracted from the Family History section of the Genetics documentation. The "features of EDS or other possible connective tissue disorders" included a reported history of: hypermobility, hyperflexibility, joint pain, dislocations, subluxations, smooth/velvety skin, easy bruising, hyperextensible skin, atrophic/abnormal scarring, GI disturbances, POTS manifestations, mast cell activation disorder, myopia, detached retina, ruptured aorta, dilated aorta, mitral valve prolapse, organ rupture, and sudden death under age 50 (not accidental).

The recommendations made by the medical geneticist and genetic counselor following the evaluation were also collected. Information was collected on whether or not imaging studies, biochemical testing, referrals to other specialists, records requests, and a return visit was recommended. Finally, information was collected on what type of genetic testing was offered to the patient: single gene testing, larger gene panel, SNP microarray, whole exome sequencing, or none. Genetic testing results were recorded if available.

Eighteen patient charts (12%) randomly selected from across the study period (January 2014-March 2019) were re-analyzed for all variables. Initially analyzed charts were compared to the re-analyzed charts to determine a data collection error rate of 3.0%.

III. Data Analysis

Data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) Statistics version 26 (IBM SPSS Statistics for Macintosh, Armonk, NY, USA, IBM Corp). Patient demographics, referral features, Genetics exam features, and Genetics recommendations

were summarized using means and standard deviations for continuous variables and counts and percentages for categorical variables. Paired t-test and chi-square values were calculated using SPSS. P-values <0.05 were considered statistically significant.

RESULTS

I. Referral information

A retrospective chart review was conducted to determine how many patients were referred to the Genetics specialty for an evaluation of EDS or other possible CTD, what types of providers are referring these patients, what types of evaluations these patients are undergoing prior to seeing Genetics, the outcome of the Genetics evaluation, and what recommendations were made by the Genetics team. The number of referrals were counted for each year from January 2014- May 2019 for a total of 255 referrals to Genetics for an indication of EDS or other possible CTD (Table 1a). A total of 93 referrals were made to UCIMC Genetics prior to the publication of the new hEDS diagnostic criteria by Malfait et al. [2017] (January 2014-December 2016) and 162 referrals were made after the publication of new hEDS diagnostic criteria (January 2017-May 10, 2019) ($p<0.001$) (Table 1b). Moreover, the number of referrals counted for 2017 and 2018 are known to be an underestimate due to technical issues regarding referral tracking in the EMR after November 2017. Referrals were counted through May 10, 2019 and therefore do not represent a full year for 2019. The referrals that were missed in the counts for these years are representative of the total number of referrals that were counted in terms of referral indication and phenotype and are not expected to represent patients that may have had a higher or lower burden of EDS.

If a patient had not been contacted in attempt to be scheduled, they were excluded from the chart review. A total of 151 patients were identified as referred to and attempted to be scheduled in the Adult Genetics Specialty Clinic at UCIMC for an evaluation of possible EDS or

to rule out a possible CTD from January 2014 to March 2019. These 151 patient referrals were reviewed. Of those 151 patients, 128 completed a visit at UCIMC and their Genetics consultation note was reviewed (Table 1). Twenty-three patients were attempted to be scheduled so their referral was analyzed but they did not complete a Genetics consultation so there was no Genetics note to review.

Table 1a. Number of patients by year

Year	Total Referrals (N=255)	No. Patient Referrals Reviewed (N=151)	No. Patient Genetics Notes Reviewed N=128)
2014	23	26[a]	21
2015	38	38	28
2016	32	30	24
2017	82[b]	26	27
2018	45[c]	30	22
2019	35[d]	1[e]	6[e]

[a]Three referrals from 2013 were included in the analysis because the patient was seen by Genetics in 2014. The remainder of patients seen in 2014 were referred in 2014.

[b]Months of November and December not accounted for due to transition to new EMR

[c]Not all referrals accounted for due to transition to new EMR

[d]January 1, 2019- May 10, 2019

[e]January 1, 2019- March 31, 2019

Table 1b. Number of referrals prior to and after the publication of new hEDS diagnostic criteria by Malfait et al. [2017]

No. referrals prior to new hEDS criteria publication	93
No. referrals after new hEDS criteria publication	162
	$p<0.001$

The demographic characteristics of the patients were analyzed (Table 2). Age of patients ranged from 18-68 years with a mean age of 36.8 years. The majority (76.2%) of patients were female and the majority of patients had private insurance (73.5%).

Table 2. Demographic information

Characteristic	(N=151) N (%)
Age (Range)	18-68
Age (Mean/SD)	36.8/13.0
Age	
18-27	42 (27.8)
28-37	45 (29.8)
38-47	28 (18.5)
48-57	24 (15.9)
58-67	11 (7.3)
68-77	1 (0.7)
Sex	
Female	115 (76.2)
Male	36 (23.8)
Insurance Type	
Private	111 (73.5)
Government	32 (21.2)
Dual Coverage	6 (4.0)
Self-pay	2 (1.3)

Fifteen different types of providers referred patients to the genetics specialty for an evaluation of EDS or to rule out another possible CTD. Thirty-six percent of the referrals were from general practice physicians (primary care, internal medicine, and family medicine physicians), 31% were from Rheumatology, 9% were from Neurology, and 4% were from Cardiology (Figure 1).

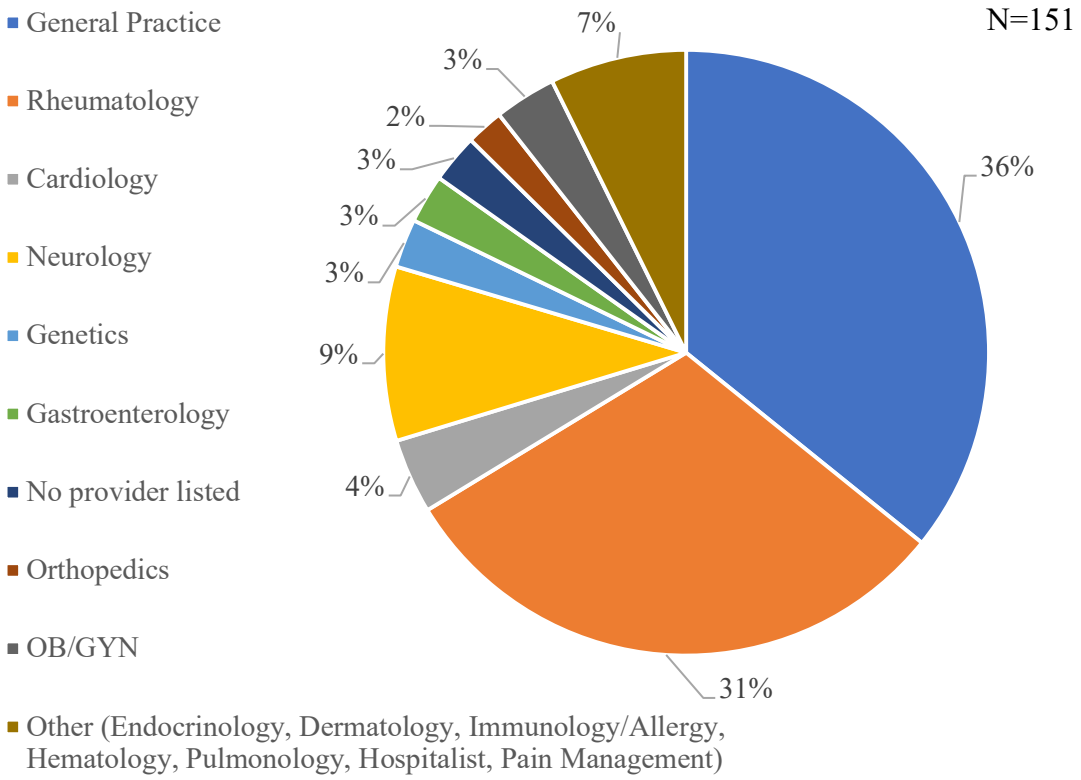

Figure 1. Referring providers by area of practice. Referrals were received from 15 different types of providers. The most common referring provider was a general practice physician (primary care/family medicine/internal medicine) (36%) followed by rheumatologist (31%), neurologist (9%), and cardiologist (4%). Smaller percentages of referrals came from geneticists, gastroenterologists, orthopedists, obstetricians/gynecologists, endocrinologists, dermatologists, immunologist/allergists, hematologists, pulmonologists, hospitalists, and pain management specialists.

Of the 151 referrals analyzed, 33 had a Beighton score documented on the referral. The Beighton score on the referral was compared to the Beighton score calculated at the Genetics exam, and 19 patients were found to have different Beighton scores once they were seen in clinic. For 6 of those 19 patients, the Beighton score given by the geneticist changed whether or not they met the Beighton score criteria based on their age for a diagnosis of GJH. Four patients' Beighton score decreased to no longer meeting the age specific cut-off for GJH (Figure 2).

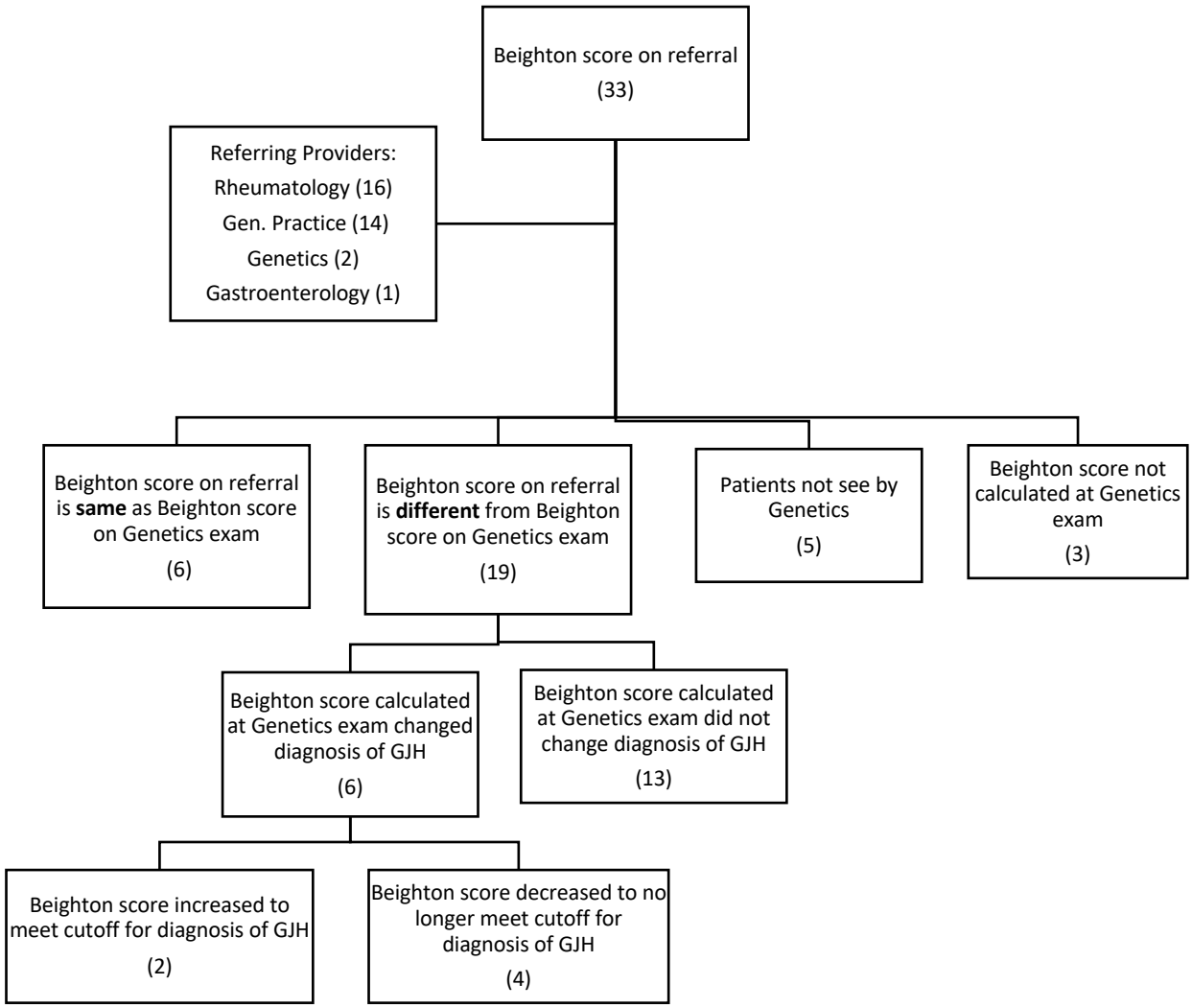

Figure 2. Differences in the Beighton score documented on referral and the Beighton score given on the Genetics exam. Thirty-three referrals had a Beighton score documented. The majority of these referrals came from rheumatologists (16) and a particular general practice physician in the community who sees many patients for concerns of EDS (14). Nineteen patients were found to have different Beighton scores once they were seen in clinic. For 6 of these 19 patients, the score given by the geneticist changed whether or not they met their age-specific criteria for a diagnosis of generalized joint hypermobility (GJH).

Comparisons were made between self-referred and provider-referred patients to determine if self-referred patients were more or less likely to have EDS-related evaluations prior to seeing Genetics and to determine if more or fewer recommendations were made after the Genetics evaluation for self-referred patients. A total of 34 (22.5%) patients were considered self-referred (defined by having no referring provider listed or if the referral mentioned the patient was requesting the referral to Genetics). Medical charts and referrals were reviewed to determine if the patient had EDS-related imaging studies (i.e. echocardiogram, CT, MRI, DEXA scan) performed or if the patient had seen Cardiology, Ophthalmology, Rheumatology, Orthopedics, or Immunology/Allergy specialists prior to seeing Genetics. The number of patients who had performed each type of evaluation listed in Table 3 were then stratified by referral status (self vs provider) and compared using Chi square; no statically significant difference was found between the two groups for any of the evaluations examined (Table 3).

Table 3. Evaluations performed prior to seeing Genetics

Evaluation	Total N=151 N (%)	Self-referred N=34 N (%)	Provider-referred N=117 N (%)	p-value
EDS-related imaging studies	115 (76.2)	22 (64.7)	93 (79.5)	0.097
Seen by Cardiology	54 (35.8)	10 (29.4)	44 (37.6)	0.380
Seen by Ophthalmology	27 (17.9)	3 (8.8)	24 (20.5)	0.117
Seen by Rheumatology	69 (45.7)	16 (47.1)	53 (45.3)	0.856
Seen by Orthopedics	24 (15.9)	7 (20.6)	17 (14.5)	0.395
Seen by Immunology/Allergy	19 (12.6)	5 (14.7)	14 (12.0)	0.672

Each clinical feature (i.e. physical features, medical diagnoses, and patient reported symptoms) listed on the patient's referral and Genetics consultation note was counted and grouped into different phenotypic categories (see Appendix F for all of the individual features that were reported and grouped into each category). The percentage of patients with at least one

feature in the given categories documented on their referral and their Genetics note is displayed in Figure 3 (see Appendix G for a table of the number of patients with at least one reported feature in each of the categories). Some patients had multiple features in some categories. Musculoskeletal findings were the most common features noted on both referrals (85.4%) and Genetics notes (97.7%). All features were more frequently reported or noted on the Genetics exam with the exception of hearing loss and metabolic findings, but one of the patients who had a metabolic finding reported on their referral was not seen by Genetics. On average referrals listed 3.8 clinical features while Genetics notes listed 7.1 clinical features ($p<0.001$) (Table 4, Figure 4).

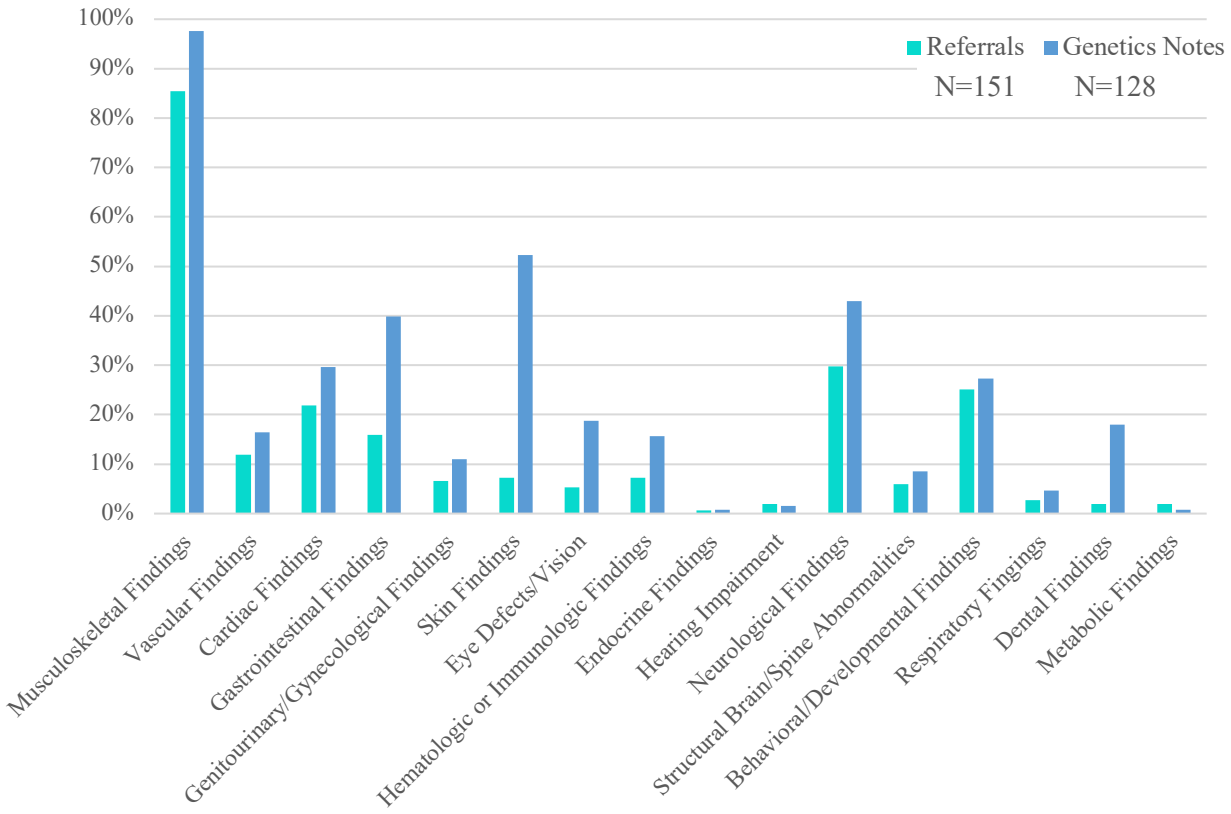

Figure 3. Clinical features listed on referrals and Genetics consultation notes. The percentage of patients with at least one clinical feature in the given phenotypic categories were compared between the referral (green) and the Genetics note (blue). Some patients had multiple features in some categories. Percentage indicates the proportion of the total number of patients for each category (referrals N=151, Genetics notes N=128).

Table 4. Average number of clinical features listed on referrals and Genetics consultation notes

	Range	Average
Number of features listed on referral	0-13	3.8
Number of features listed on Genetics consultation note	1-14	7.1
		p<0.001

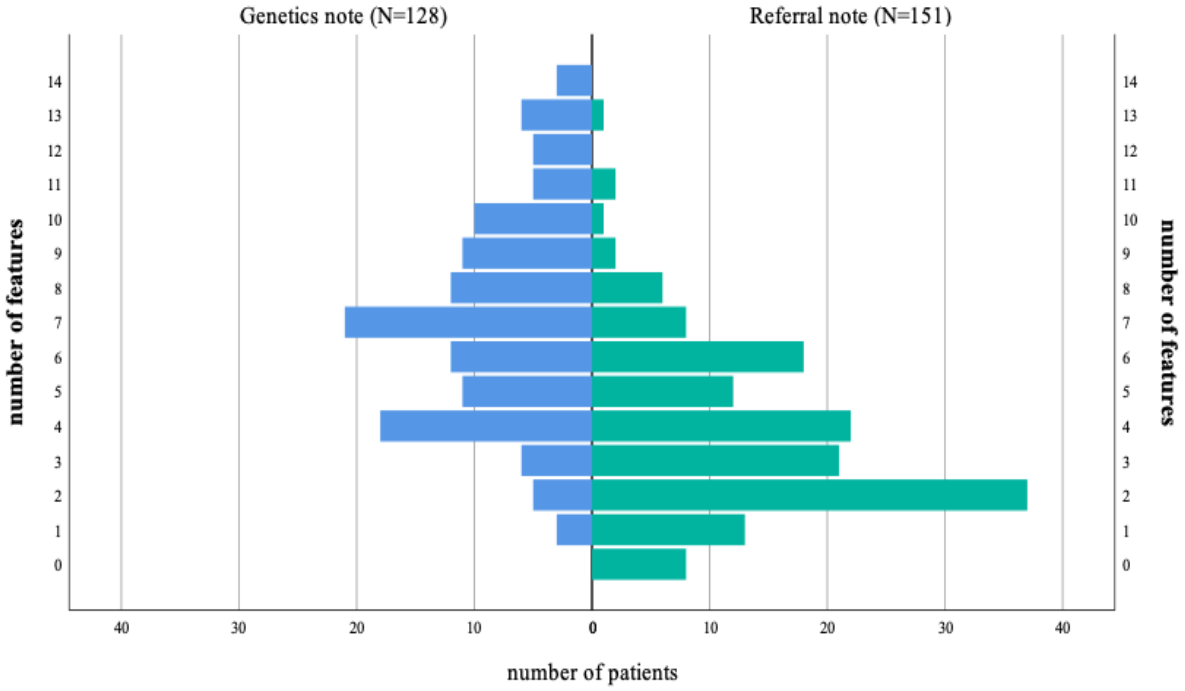

Figure 4. Distribution of the number of clinical features listed on patient referrals and Genetics notes. The number of clinical features (i.e. physical features, medical diagnoses, and patient reported symptoms) listed on each patient referral (green) and Genetics note (blue) were counted and compared. The distribution of the number of features demonstrates that more features were listed on the Genetics notes than on the referrals. The individuals with "0" features had no physical features and only "family history of EDS" reported on the referral.

II. Patients who were given a diagnosis of hEDS

Thirty-four (26.5%) patients were given a diagnosis of hEDS following their Genetics evaluation. These patients ranged in age from 19-60 with an average age of 37.4, and 91.2% of these patients were female (Table 5). The clinical symptoms and physical features that were reported by these patients or were noted by the geneticist during their Genetics consultation were categorized by phenotype. Figure 5 shows the percentage of hEDS patients who reported or were found to have at least one feature in the different phenotypic categories noted on their Genetics consultation note. All of the patients who were given a diagnosis of hEDS had musculoskeletal findings, 8.8% had vascular system findings, 35.3% reported cardiac findings, 55.9% reported gastrointestinal findings, 8.8% reported genitourinary findings, 61.8% reported having or had skin findings on physical exam, 32.4% reported immunologic findings, 2.9% reported endocrine findings, 55.9% reported neurological findings, 8.8% had structural brain abnormalities, 32.4% reported behavioral findings, and 17.6% had dental findings on physical exam. On average patients who were given a diagnosis of hEDS had 8.5 clinical features noted in their Genetics consultation note, while patients who were not given a diagnosis of hEDS had an average of 6.6 features noted ($p=0.003$) (Table 6, Figure 6). Of the 34 individuals who were given a diagnosis of hEDS, 25 (73.5%) were given referrals to other specialists including: Physical Therapy, Pain Management, Ophthalmology, Cardiology, Neurology, Rheumatology, Immunology/Allergy, Orthopedics, and a provider in the community that sees many patients with EDS and 8 (23.5%) had imaging studies ordered including echocardiogram, brain MRI, electrocardiogram, and DEXA scan. Eleven (32.4%) of these patients had a Beighton score given on their referral; these account for 1/3 of the total referrals that had a Beighton score prior to seeing Genetics.

Twenty-two patients (64.7%) with hEDS had genetic testing recommended by Genetics. Of these 22 patients, 3 patients' genetic testing identified variants of uncertain significance, 1

patient (patient #300) had positive genetic testing from whole exome sequencing (WES) that explained some of his clinical features, but not his hEDS features (see description in Results section III), 7 patients had negative results, 3 patients' testing was ordered but denied by insurance, and no record of genetic testing being ordered was found for the remaining 8 patients. Eight patients who were given a diagnosis of hEDS were discharged from the Genetics clinic following their initial consultation. Twenty-six patients were recommended to arrange a return visit to Genetics, most of whom were recommended to return to discuss genetic testing results. Seven patients with hEDS returned to clinic for at least one follow-up visit, which accounts for almost half (46.7%) of the total number patients who were seen for an evaluation of EDS or other possible CTD who returned for a follow-up visit.

Table 5. Demographic information of individuals who were given a diagnosis of hEDS

Characteristic	(N=34) N (%)
Age (Range)	19-60
Age (Mean/SD)	37.4/12.2
Age	
18-27	8 (23.5)
28-37	9 (26.5)
38-47	8 (23.5)
48-57	8 (23.5)
58-67	1 (3.0)
68-77	0 (0)
Sex	
Female	31 (91.2)
Male	3 (8.8)

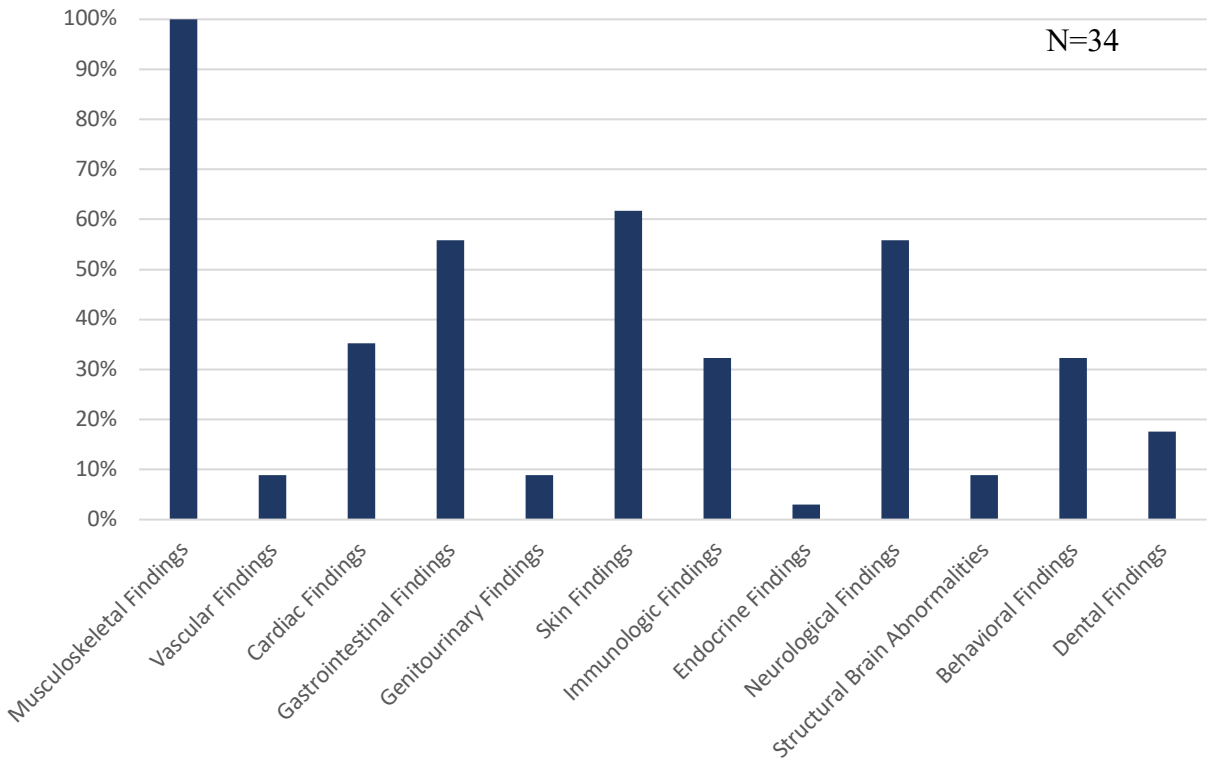

Figure 5. Clinical features of individuals who were given a diagnosis of hEDS after Genetics evaluation. The percentage of patients with at least one clinical feature in the given phenotypic categories, as reported in the Genetics note. Percentage indicates the proportion of the number of individuals who were given a diagnosis of hEDS after Genetics evaluation (N=34).

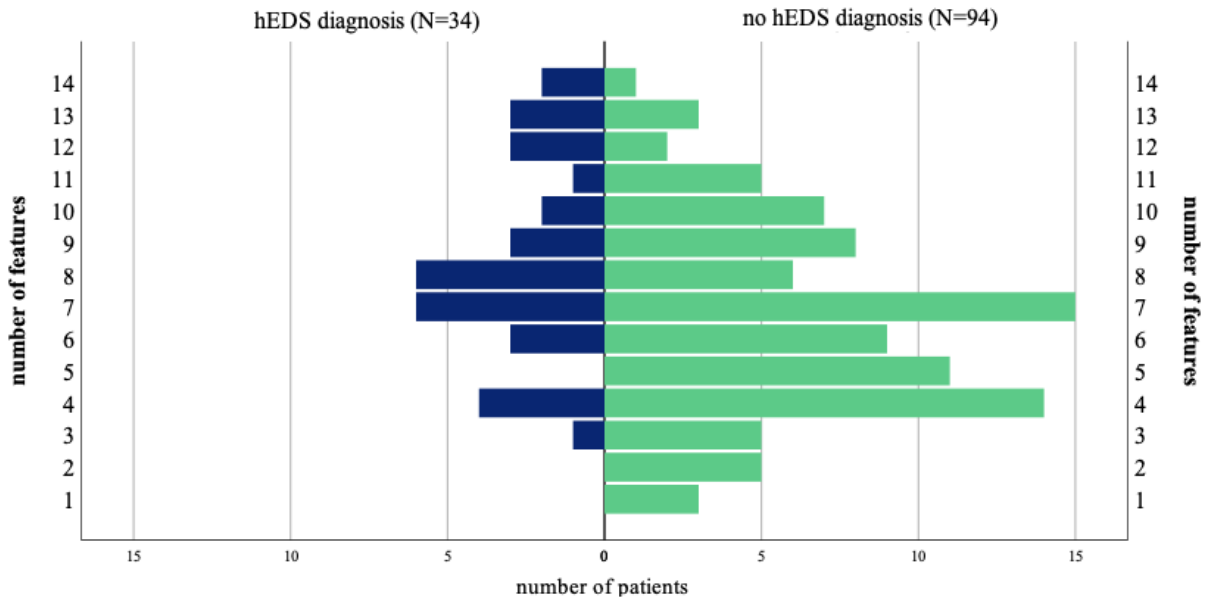

Figure 6. Distribution of the number of clinical features listed on the Genetics notes of patients who were given a diagnosis of hEDS and patients who were not given a diagnosis of hEDS. The number of clinical features listed on each patients Genetics note were counted and compared between individuals who were given a diagnosis of hEDS (blue) and individuals who were not given a diagnosis of hEDS (green). The distribution of the number of clinical features demonstrates that more features were listed in the Genetics notes of patients who were given a diagnosis of hEDS (N=34) than in the Genetics notes of patients who were not given a diagnosis of hEDS (N=94).

III. Genetics recommendations and outcomes

The recommendations of imaging studies, requests to review medical records, referrals to other specialists and return visits made following the Genetics consultation were counted and compared between the patients who were self-referred and provider-referred (Table 6). There was no statistically significant difference between the two groups. Patient charts were also examined for documentation of the recommendations made by Genetics being completed, in particular referrals to Cardiology and Ophthalmology because patients were referred to those specialists to be evaluated for other features of CTDs rather than management of their symptoms

(Table 7). See Appendix H for a complete list of the referrals that were made to other specialists. When Cardiology and Ophthalmology records were available, the number of results that were abnormal/significant were counted (Table 7). Of the imaging that was recommended, an echocardiogram was the most common (recommended for 33 patients, 26%) (Table 8). Genetics received record of the echocardiogram being performed for 14 (42%) of the patients that were recommended to have an echocardiogram and of those 14 patients, 3 had abnormal echocardiograms (Figure 7). Two of the patients who had abnormal echocardiograms (patients #161 and #216) had a mildly dilated left atrium. Their echocardiograms were otherwise within normal limits. Patient #161 was given a diagnosis of hEDS and had features of hypermobility (Beighton score 8/9), joint pain, easy bruising, poor wound healing, POTS, dislocations/subluxations, migraines/headaches, scoliosis, and arthritis. Patient #216 was not given a diagnosis of hEDS. This patient reported being hyperflexible but received a Beighton score of 1/9, and her other clinical features were velvety skin, skin striae, reported easy bruising, gastrointestinal disturbances such as diarrhea, constipation, or IBS, myopia, and cecal volvulus. The third patient who had abnormal echocardiogram results (patient #91) is the same patient with an abnormal Cardiology exam (Table 7). Patient #91 was found to have aortic root and ascending aorta dilation and mitral valve prolapse. This patient had a family history of cEDS, but he tested negative for the familial variant in *COL5A1* on a 21 gene Aortopathy panel. Whole exome sequencing was ordered but was denied by insurance. This patient's other features consisted of hypermobility (Beighton score 5/9), joint pain, dislocations/subluxations, and an arm span: height ratio >1.05.

Table 6. Recommendations made after Genetics consultation

Recommendation	Total N=128 N (%)	Self-referred N=31 N (%)	Provider-referred N=97 N (%)	p-values
Imaging studies	40 (31.3)	9 (29.0)	31 (32.0)	0.760
Records requested to review	21 (16.4)	5 (16.1)	16 (16.5)	0.962
Referrals to other specialists	63(49.2)	17 (54.8)	46 (47.4)	0.472
Return visit recommended	103 (80.5)	25 (80.6)	78 (80.4)	0.977

Table 7. Number of referrals placed to Cardiology and Ophthalmology following Genetics evaluation and the outcomes of those referrals

Specialists	N=128 N (%)	Record of Cardiology or Ophthalmology Seen N (%)	Abnormal results N (%)
Cardiology	21 (16.4)	7 (33.3)	1 (14.3)
Ophthalmology	28 (21.9)	8 (28.6)	0 (0)

Table 8. Number of referrals placed for imaging following Genetics evaluation and the outcomes of those referrals

Imaging Study	N=128 N (%)	Record of study received N (%)	Abnormal Results N (%)
Echocardiogram	33 (25.8)	14 (42.4)	3 (21.4)
EKG	12 (9.4)	7 (58.3)	0 (0)
Brain MRI	4 (3.1)	0 (0)	N/A
CT scan	2 (1.6)	1 (50.0)	1 (100.0)
X-ray	1 (0.8)	0 (0)	N/A
DEXA scan	1 (0.8)	1 (100.0)	1 (100.0)

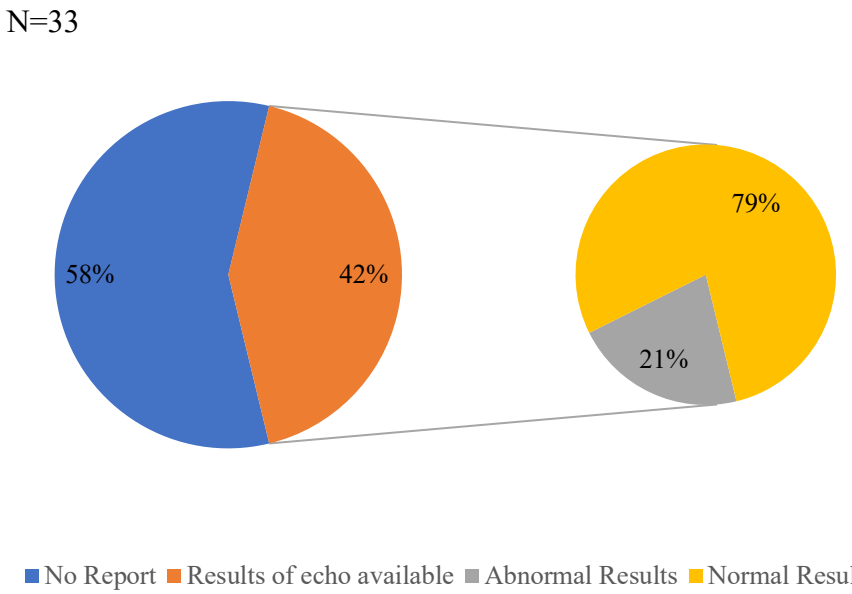

Figure 7. Outcomes of echocardiograms that were recommended. Thirty-three patients were recommended to have an echocardiogram following their Genetics consultation. Genetics received record of the echocardiogram being completed from 14 patients (42%). Of these 14 patients, 3 (21%) had abnormal echocardiogram results. Two patients (#161 and #216) had a mildly dilated left atrium and the third patient (#91) had aortic root and ascending aorta dilation and mitral valve prolapse.

As previously mentioned, genetic testing recommendations were collected for all patients. Sixty percent of the total number of patients seen by Genetics had genetic testing recommended following their evaluation and 10% of patients had genetic testing recommended if Cardiology or Ophthalmology examinations were abnormal or following the retrieval of familial genetic testing records (Figure 8). A variety of different genetic tests were recommended. Fifty-two percent of genetic testing that was recommended were multi-gene panels, 18% were single-gene tests, 8% were SNP microarray, and 8% were whole exome sequencing (WES) (Figure 9). See Appendix I for a list of the single genes and multi-gene panels that were ordered. The majority of patients' genetic testing results were negative (68%), but 3 (6%) patients had positive results (Figure 10). As previously mentioned, one patient (#300) who was diagnosed with hEDS had positive WES. This patient was described to have hemiplegic

migraines in addition to his hEDS features of joint hypermobility (Beighton score 7/9), skin elasticity, high arched palate, gastrointestinal disturbances, and dislocations/subluxations. WES found a likely pathogenic variant in the *CACNA1S* gene. Variants in this gene can cause hypokalemic periodic paralysis, so this finding may explain the patient's hemiplegic migraines but it does not explain his hEDS features. The second patient with positive genetic testing (#59) had a chromosome microarray ordered because he previously had an abnormal karyotype. This patient was referred for suspicion of Marfan syndrome and the Genetics exam noted features of arachnodactyly, myopia, spontaneous pneumothorax, kyphosis, and intellectual disability. This patient was found to carry a duplication of 9q34.11-q34.3 and a duplication of 15q11.2-q11.2. Duplication of 9q34 is a recognized duplication syndrome that can cause low birth weight, psychomotor delay, limited vocabulary acquisition, hyperactivity, joint contractures, long, thin limbs, and arachnodactyly. Marfan syndrome was a common initial diagnosis for affected individuals prior to cytogenetic and molecular studies [Allderdice et al., 1983]. Patient #59's features fit the 9q34 duplication syndrome well. The final patient with positive genetic testing results (#36) had single gene *COL3A1* sequencing ordered which found a pathogenic variant and he was given a diagnosis of vEDS. This patient was noted to have a family history of one first degree relative and two second degree relatives with a history of EDS features and on exam was reported to have an inguinal hernia, dislocations/subluxations, "EDS facial features," carotid cavernous fistula, bowel obstruction, a history of clubfoot, and gingival recession. A CT angiogram was recommended at the initial Genetics visit and found nonocclusive thrombus in the left external iliac vein and focal dissection involving the distal left common iliac artery.

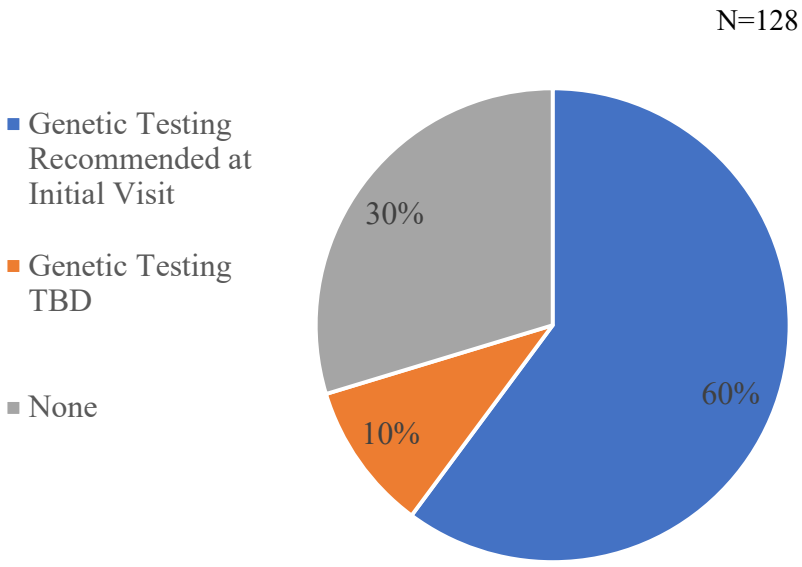

Figure 8. Genetic testing recommendations following Genetics evaluation. Sixty percent of patients had genetic testing recommended following their Genetics consultation and 10% of patients had genetic testing recommended if Cardiology or Ophthalmology examinations were abnormal or following the retrieval of familial genetic testing records (Genetic Testing TBD category). N=128

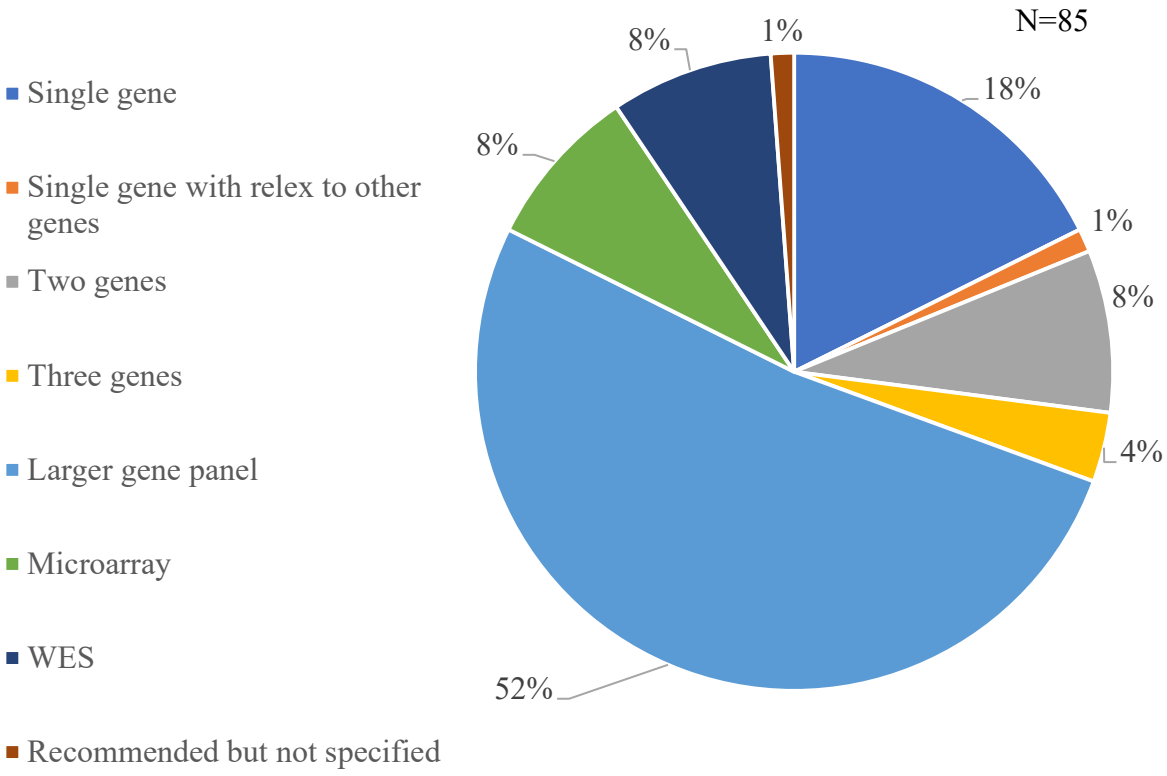

Figure 9. Types of genetic testing recommended. The most common type of genetic testing recommended was a large gene panel (52%). The large gene panels ranged in size from 4 genes to 61 genes. The most common large gene panel that was recommended was a 21-gene Aortopathy panel. See Appendix I for a list of the genes ordered and the gene panels.

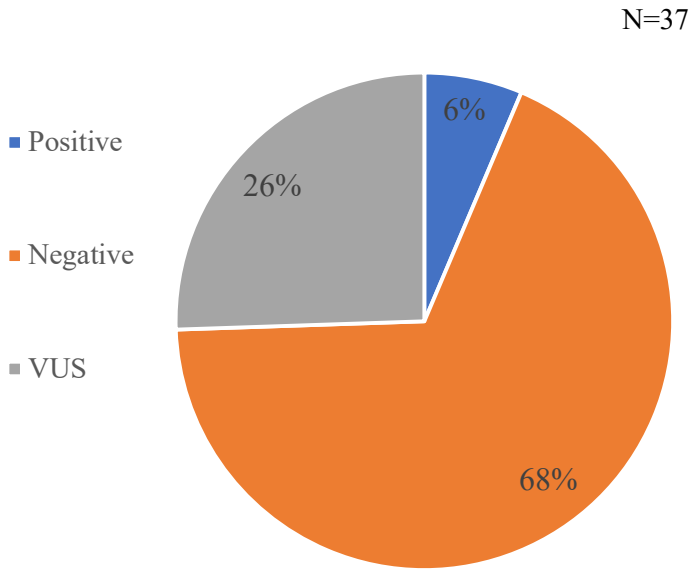

Figure 10. Genetic testing outcomes. Thirty-seven patients had record of genetic testing. Three individuals (6%) had a positive genetic testing result.

DISCUSSION

I. Referral information

This study aims to better understand which providers are referring patients to Genetics for EDS and other possible CTDs, what types of evaluations patients are having prior to seeing Genetics, and what types of recommendations Genetics is making after they assess these patients with the hopes that this information can provide guidance in evaluating these patients in the most effective and efficient way. There has been an increasing number of referrals to the Genetics specialty for an evaluation of EDS or to rule out another possible CTD. There were a total of 255 referrals for this indication from January 2014 - May 2019 at UCIMC. Referrals for this indication at UCIMC have increased since the beginning of 2017, but the clinic capacity and the number of patients seen for this indication has remained the same (Table 1). Determination of the

exact reason for the increasing number of referrals is outside of the limits of this research project, but it was noted that attached to several patient referrals were referral denial letters from other Genetics clinics saying their practice no longer sees patients for the indications of EDS or possible CTD. This may explain why UCIMC has received an increase in referrals. It should also be noted that the International EDS Consortium proposed a new classification system of the thirteen different subtypes of EDS and outlined new diagnostic criteria for hEDS in an article published at the beginning of 2017 [Malfait et al., 2017]. Perhaps with this publication came more awareness for EDS and more inclination to refer these patients to Genetics because Criteria 3 of the new hEDS diagnostic criteria states that other possible CTDs should be ruled out (Appendix D). In the two years since its publication, this article has been cited in 50 articles from a wide range of journals in the PubMed database. The article has been cited in *Gastroenterology Research*, the *Journal of Clinical Sleep Medicine*, *Pediatric Rheumatology*, *Neurogenetics*, *Obstetric Medicine*, and the *Journal of Physical Therapy Science*, just to name a few. It is evident that patients with EDS have a range of clinical features requiring multiple specialists, and that the syndrome is likely somewhat familiar to specialties other than Genetics.

Because patients with EDS can have many different phenotypes requiring multiple care specialists, this study aimed to better understand which providers are referring patients to the Genetics specialty and to determine what types of evaluations (imaging or evaluations from other specialists) these patients are undergoing prior to seeing Genetics to determine if they have a possible CTD. By better understanding this information, Genetics can more effectively and efficiently manage the increasing load of patients referred for EDS or other possible CTD. Patients were referred to the Genetics specialty from 15 different types of providers. The most common referring providers were general practice providers (primary care, internal medicine, and family medicine physicians) or rheumatologists (Figure 1). This is unsurprising because a

study surveying 466 individuals with hEDS found that most individuals with hEDS were diagnosed in Genetics or Rheumatology clinics, and the majority of patients with hEDS reported that their primary care physician is the physician that manages their diagnosis [Murray et al., 2013].

The Beighton score is an important assessment for determining if a patient has GJH. An individual must meet the age-specific cutoff Beighton score to fulfill Criteria 1 for a diagnosis of hEDS (Appendix D). It is not a requirement that a provider referring a patient to Genetics give a Beighton score on their referral, but a referring provider might do their own assessment for EDS (including a Beighton score) before referring to Genetics. Of the 151 referrals that were analyzed, 33 referrals had a Beighton score documented. Twenty-five of these individuals were seen by Genetics and given a Beighton score by the medical geneticist, and of those, 19 were given a different Beighton score by their referring provider and the geneticist. For 6 of these individuals, the change in Beighton score given by the geneticist changed whether or not they met the age-specific cut-off for a diagnosis of GJH. Four individuals were given a lower Beighton score meaning they no longer met Criteria 1 for hEDS (Figure 2). This can be challenging emotionally for some patients who attributed their constellation of symptoms to a particular disorder like hEDS and then learned they did not meet diagnostic criteria for that diagnosis upon further evaluation. The Beighton score should be a standard measurement of hypermobility, however a provider's judgment of giving points will inevitably vary. For this reason, it may not be necessary to have a referring provider give the patient a Beighton score, but if a Beighton score is provided along with other physical features it can help give the Genetics clinic an initial idea of what type of EDS the referring provider is suspicious of and this may help triage referrals.

This study found that non-hypermobility features of EDS, particularly skin features which are diagnostic criteria for various EDS subtypes, were much less frequently reported on referrals than on Genetics exams (Figure 3). More outreach and education regarding the updated criteria for EDS, especially hEDS, to the medical community may help other physicians assess patients for EDS and other possible CTDs and make appropriate referrals to Genetics with more clinical documentation specific to EDS.

A unique aspect of an evaluation in the Genetics specialty is an evaluation or notation of features or problems across all systems in the body. For example, cardiologists may be focused solely on the heart and vascular system. General practice physicians may address all systems of the body when the need arises, but geneticists are trained in dysmorphology and to examine the full body to try to tie together features from different systems. Patients with different forms of EDS have features across different body systems (musculoskeletal, cardiac, eye findings, skin findings, etc.) so a Genetics evaluation may be useful to try to put all of the patients' features together. This is evident in the number of features discussed during the Genetics evaluation compared to the number of features noted in the referrals from other specialists. The average number of features listed in the Genetics consultation note was 7.1 features, while the average number of features given on the referral notes/documentation from other specialists or general practice physicians was 3.8 features (Table 4). This was statistically significant ($p<0.001$) meaning patients are discussing more symptoms or health conditions with Genetics providers and additional physical features are noted on the Genetics physical exam . It is important for a geneticist and genetic counselor to have a thorough medical history to guide them to differential diagnoses and determine what type of genetic testing may be indicated. However, with more features/problems that are discussed this will mean more time spent with these patients. Geneticists and genetic counselors spend a considerable amount of time (arguably more time

than other specialties) with patients and doing patient-related activates such as clinical documentation, literature review, reviewing records from outside providers, and writing family letters. McPherson et al. tracked the time medical geneticists and genetic counselors spent face-to-face with patients and on patient-related activities and found that they spent an average of 7 hours for each new patient and 3.5 hours on return patients [McPherson et al., 2008]. The increased amount of time needed is a concern when there is an increasing number of referrals to Genetics for this indication.

II. Patients who were given a diagnosis of hEDS

All of the patients who were given a diagnosis of hEDS had musculoskeletal findings, 8.8% reported vascular system findings, 35.3% reported cardiac findings, 55.9% reported gastrointestinal findings, 8.8% reported genitourinary findings, 61.8% reported having or had skin findings on physical examination, 32.4% reported immunologic findings, 2.9% reported endocrine findings, 55.9% reported neurological findings, 8.8% had structural brain abnormalities, 32.4% reported behavioral findings, and 17.6% had dental findings on physical examination. These reported features are consistent with other clinical studies and a patient survey of clinical manifestations associated with hEDS [Tinkle et al., 2017; Castori et al., 2010; Murray et al., 2013]. Patients with hEDS had an average of 8.5 clinical features noted during their Genetics consultation, while patients who were not given a diagnosis of hEDS had an average of 6.6 features noted during their Genetics consultation ($p=0.003$). As previously mentioned, discussing more medical concerns and obtaining record of more medical diagnoses, takes a considerable amount of time for geneticists and genetic counselors. It may be necessary to allot more time at the initial Genetics visit or arrange return visits for patients referred for this indication to discuss medical history and provide appropriate counseling. It would also be

beneficial for referring providers to send available medical records of clinic notes and imaging with the referrals to Genetics.

Twenty-six patients who were given the diagnosis of hEDS were recommended to arrange a return visit to Genetics, most of whom were recommended to return to discuss genetic testing results. Seven patients with hEDS returned to clinic for at least one follow-up visit, which accounts for almost half (46.7%) of the total number patients in this study who returned for a follow-up visit. The exact reason for a low follow-up visit return rate is beyond the scope of this study, but perhaps some patients were given negative genetic testing results over the phone and no longer needed a follow-up visit or the patient received further care elsewhere. Genetics is often not the specialty that primarily manages hEDS patients' care long term, but Genetics can provide education and facilitate a discussion surrounding improving the patients' quality of life with both the patient and the physicians who will be managing the patient's care. Genetics can discuss precautions or activities to avoid so patients do not exacerbate pain and fatigue symptoms [Greenen, R and Lumley, 2018] and they can also provide referrals to other specialists such as physical therapists in the community who know the limitations of individuals with hEDS or an HSD and other specialists who can treat and manage pain symptoms and musculoskeletal manifestations.

III. Outcomes of recommendations made by Genetics

When evaluating a patient for hEDS, other heritable or acquired CTDs should be ruled out to fulfill Criteria 3 for a diagnosis of hEDS. Patients are often referred to other specialists such as Cardiology and Ophthalmology to rule out features that may suggest another CTD. For example, an Ophthalmology finding of a lens dislocation is suggestive of Marfan syndrome. Geneticists and genetic counselors can, and often do, refer patients to these other specialists to help evaluate

for a possible CTD, but they do not always receive record of these evaluations being completed. UCIMC Genetics received Cardiology reports from 33.3% of the patients they referred, Ophthalmology reports from 28.6% of the patients they referred (Table 7), and echocardiogram reports from 42.4% of the patients they referred (Table 8). Of note, some echocardiogram reports received did not include the Z-score for assessing aortic root dilation. One explanation for this low rate of receiving reports may be that some of the patients referred to these specialists may not have completed visits, but if a patient is suspected of having EDS or another possible CTD it is an important part of their evaluation to be assessed for serious health risks such as aortic root dilation. It is possible that patients had these evaluations done, but Genetics did not receive record of the outcome. It can be challenging and time consuming to request and review records from outside medical facilities and clinics. One study evaluated the success rate and time it took for genetic counselors to request and receive records for family members suspected of having Alzheimer disease. Genetic counselors in this study received 33.5% of the familial records they requested and spent 500 hours during the 24 month study period trying to obtain the records [Alexander et al., 2011]. It is difficult and time consuming to request and receive patient records form other institutions and if patients should have a Cardiology evaluation and/or an echocardiogram or Ophthalmology evaluations to rule out other possible CTDs, it may be beneficial for patients to have these evaluations prior to seeing Genetics and not be scheduled with Genetics until the records are reviewed. Requiring patients to have an Ophthalmology exam or Cardiology evaluation and/or an echocardiogram prior to being scheduled in Genetics may become a barrier to some patients having an evaluation with Genetics due to insurance authorization or other limitations and it would still require the time of Genetics clinic administrators or schedulers to keep track of the records that are received prior to being

scheduled. The capacity of a Genetics clinic to recommend evaluations prior to being scheduled should be considered on an individual basis.

Evaluations from Ophthalmology or findings from an echocardiogram may guide geneticists and genetic counselors to recommend genetic testing for particular CTDs. Geneticists and genetic counselors may also recommend genetic testing without those evaluations if a patient has enough clinical features on exam to make them suspicious of a CTD, or they may recommend testing to try to rule out the possibility of one of the life-threatening CTDs such as vEDS. In this study, 60% of the 128 patients seen by Genetics had genetic testing recommended following their evaluation and 10% of patients had genetic testing recommended if Cardiology/echocardiogram or Ophthalmology examinations were abnormal or following the retrieval of familial genetic testing records (Figure 8). The majority of patients' genetic testing results were negative (68%), but 3 (6%) patients had positive results (Figure 10). One patient (#300) was found to have a variant in the *CACNA1S* gene on WES. This variant described the patient's phenotype of hemiplegic migraines well but did not explain the hEDS features for which he was referred. Another patient (#59) was initially referred to Genetics for suspicion of Marfan syndrome and intellectual disability with a previously abnormal karyotype. A chromosome microarray was ordered by Genetics and the patient was found to have 9q34 duplication syndrome, which described both his intellectual disability and Marfanoid habitus well. The accurate diagnosis of a genetic condition can help guide clinical management and prognosis. In the case of patient #59, individuals with 9q34 duplication syndrome have the musculoskeletal features of Marfan syndrome, but lack the serious cardiovascular and ocular system manifestations, so they do not require the same screening as individuals with Marfan. The third patient (#36) had a diagnosis of vEDS confirmed when a pathogenic variant in *COL3A1* was found, which has great implications for his health management and possibly other family

members. More often than not, genetic testing yields negative results, but even negative results can provide information and lower the suspicion of particular genetic disorders that can have serious implications for a patient's health and management. Geneticists and genetic counselors can still treat or provide recommendations for patients' symptoms in the absence of a confirmed genetic condition. Genetic counselors are also trained to provide medical, educational, financial and psychosocial resources [Accreditation Council for Genetic Counseling, 2015], which can be of great value to patients.

IV. Self-referred patients

It is clear that patients who are referred to Genetics for EDS or other possible CTDs have many clinical features and concerns. Having documentation of their clinical features and previous evaluations prior to being seen by Genetics is helpful to be able to give a comprehensive Genetic evaluation. A concern of accepting self-referred patients is that there will be a lack of clinical information initially or that these patients will be less likely to have seen other specialists and will therefore require Genetics to make more recommendations and coordinate more follow-up. The number of patients who had imaging or evaluations by different specialists (Cardiology, Ophthalmology, Rheumatology, Orthopedics, and Immunology/Allergy) prior to seeing Genetics were compared between self-referred patients and provider-referred patients. The recommendations of imaging studies, requests to review outside medical records, referrals to other specialists and return visits made following the Genetics consultation were also counted and compared between the two groups. Whether or not someone was self-referred to Genetics did not determine how frequently or infrequently they saw other specialists or had imaging done prior to seeing Genetics (Table 3). There was also no statistically significant difference in the number of recommendations made following the Genetics consultation between

self-referred and provider-referred patients (Table 6). It is possible that this study did not have sufficient power to identify a small difference if one was there, or perhaps this may be unique to patients with EDS. One study found that 21 patients with hEDS saw 20 different providers before obtaining their diagnosis [Castori et al., 2010] and due to the number and severity of symptoms these patients have, they may be more self-motivated to seek care from different specialties. Based on the data from this study, it is not necessary to deny a patient for an evaluation of EDS if they are self-referred for lack of prior evaluations or worry of extra follow-up required for these patients. Nevertheless, it is important that self-referred patients have an established care provider if Genetics is not going to be the main provider that manages their care.

V. Conclusion

There is value in a Genetics evaluation for EDS and possible CTDs. Geneticists perform a full body exam and assess a patient's features across all systems of the body which is important for individuals with EDS and other CTDs who have clinical manifestations across many body systems. Genetic counselors are trained to provide medical, educational, financial and psychosocial resources for their patients [Accreditation Council for Genetic Counseling, 2015], so even if genetic testing is not indicated, the Genetics clinic offers education and support to patients with suspected EDS and the providers who manage their care. The number of referrals for EDS and CTDs is increasing at UCIMC and the load of referrals can be difficult to keep up with. This may be an opportunity to educate referring providers about the new EDS classifications and diagnostic criteria published by Malfait et al. [2017] and the utility of other evaluations such as an Ophthalmology exam and an echocardiogram prior to seeing Genetics so the patient can get the most out of a Genetics consultation. This study was limited by reviewing charts at a single Genetics clinic site with only five different geneticists performing clinical

evaluations throughout the study period. This study focused on the Genetics clinic perspective of evaluations for EDS and CTDs, but future studies could survey patients to try to further understand their goals for a Genetics evaluation. With a better understanding of the challenges of evaluating patients for EDS and the goals of this patient population, Genetics can provide a comprehensive evaluation for EDS and provide the support these patients need.

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APPENDIX A

Classification of the Ehlers-Danlos syndrome subtypes, inheritance pattern (IP), and genetic basis adapted from Malfait, et al., 2017

Clinical EDS Subtype	Abbreviation	IP	Genetic Basis	Protein
Classical EDS	cEDS	AD	Major: *COL5A1, COL5A2* Rare: COL1A1 c.934C>T, p.Arg312Cys	Type V collagen Type I collagen
Classical-like EDS	clEDS	AR	*TNXB*	Tenascin XB
Cardiac-valvular EDS	cvEDS	AR	*COL1A2*	Type I collagen
Vascular EDS	vEDS	AD	Major: *COL5A1* Rare: COL1A1 c.934C>T, p.Arg312Cys c.1720C>T, p.Arg574Cys c.3227C>T, p.Arg1093Cys	Type III collagen Type I collagen
Hypermobile EDS	hEDS	AD	Unknown	Unknown
Arthrochalasia EDS	aEDS	AD	*COL1A1, COL1A2*	Type I collagen
Dermatosparaxis EDS	dEDS	AR	*ADAMTS2*	ADAMTS-2
Kyphoscoliotic EDS	kEDS	AR	*PLOD1* *FKBP14*	LH1 FKBP22
Brittle Cornea syndrome	BCS	AR	*ZNF469* *PRDM5*	ZNF469 PRDM5
Spondylodysplastic EDS	spEDS	AR	*B4GALT7* *B3GALT6* *SLC39A13*	β4GalT7 β3GalT6 ZIP13
Musculocontractural EDS	mcEDS	AR	*CHST14* *DSE*	D4ST1 DSE
Myopathic EDS	mEDS	AD or AR	*COL12A1*	Type XII collagen
Periodontal EDS	pEDS	AD	*C1R* *C1S*	C1r C1s

APPENDIX B

Criteria for Classical EDS (cEDS) diagnosis as outlined by Malfait, et al. 2017

Major	Minor
1. Skin hyperextensibility and atrophic scarring	1. Easy bruising
2. Generalized joint hypermobility (GJH)	2. Soft, doughy skin
	3. Skin fragility (or traumatic splitting)
	4. Molluscoid pseudotumors
	5. Subcutaneous spheroids
	6. Hernia (or history thereof)
	7. Epicanthal folds
	8. Complications of joint hypermobility (sprains, luxations/subluxation, pain, flexible flatfoot)
	9. Family history of first degree relative who meets clinical criteria

Minimal criteria for cEDS is major criteria 1 (skin hyperextensibility and atrophic scarring) PLUS either major criteria 2 (GJH) and/or three minor criteria. Confirmatory molecular testing is obligatory to diagnose.

APPENDIX C

Criteria for Vascular EDS (vEDS) diagnosis as outlined by Malfait, F. et al. 2017

Major	**Minor**
1. Family history of vEDS with documented causative variant in *COL3A1*	1. Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
2. Arterial rupture at a young age	2. Thin, translucent skin with increased venous visibility
3. Spontaneous sigmoid colon perforation in the absence of a known diverticular disease or other bowel pathology	3. Characteristic facial appearance 4. Spontaneous pneumothorax 5. Acrogeria 6. Talipes equinovarus
4. Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears	7. Congenital hip dislocation 8. Hypermobility of the small joints 9. Tendon or muscle rupture 10. Keratoconus 11. Gingival recession and gingival fragility
5. Carotid-cavernous sinus fistula (CCSF) formation in the absence of trauma	12. Early onset varicose veins (under age 30 and nulliparous if female)

Minimal criteria suggestive of diagnostic studies for vEDS includes: a family history of vEDS, arterial rupture or dissection in individuals less than 40 years of age, unexplained sigmoid colon rupture, or spontaneous pneumothorax in the presence of other features consistent with vEDS. Testing for vEDS should also be considered in the presence of a combination of other "minor" features. Confirmatory molecular testing is obligatory to diagnose.

APPENDIX D

Criteria for a clinical diagnosis of hypermobility EDS (hEDS) as outlined by Malfait, et al., 2017

Criteria 1- Generalized Joint Hypermobility	**Criteria 2- two or more of the following features (A, B, C) must be present**	**Criteria 3- All of the following prerequisites MUST be met**
Beighton Score consistent with GJH based on age: ≥6 pre-pubertal children and adolescents ≥5 pubertal men and women to age 50 ≥4 men and women over age 50 If Beighton Score is one point below age cut-off, two or more of the following must also be present to meet criteria: 1.Can you now (or could you ever) place your hands flat on the floor without bending your knees? 2.Can you now (or could you ever) bend your thumb to touch your forearm? 3.As a child, did you amuse your friends by contorting your body into strange shapes or could you do the splits? 4.As a child or teenager, did your shoulder or kneecap dislocate on more than one occasion? 5.Do you consider yourself "double jointed"?	**Feature A (five must be present) (Musculoskeletal features)** 1. Unusually soft or velvety skin 2. Mild skin hyperextensibility 3. Unexplained striae distensae or rubae at the back, groins, thighs, breasts and/or abdomen in adolescents, men or pre-pubertal women without a significant history of significant gain or loss of weight 4. Bilateral piezogenic papules of the heel 5. Recurrent or multiple abdominal hernia(s) 6. Atrophos scarring involving at least two sites and without the formation of truly papyraceous and/or hemosideric scars as seen in cEDS 7. Pelvic floor, rectal, and/or uterine prolapse in children, men or nulliparous women without a history of morbid obesity or other known predisposing medical condition 8. Dental crowding and high or narrow palate 9. Arachnodactyly as defined by one or more of the following: i) positive wrist sign on both sides, or ii) positive thumb sign on both sides 10. Arm span-to-height ratio ≥1.05 11. Mitral valve prolapse mild or greater based on echocardiogram criteria 12. Aortic root dilation with Z-score >+2 **Feature B** Positive family history (one or more first degree relative independently meeting the current criteria for hEDS) **Feature C- Must have at least one** 1. Musculoskeletal pain in two or more limbs, recurring daily for at least 3 months 2. Chronic, widespread pain for ≥ 3 months 3. Recurrent joint dislocations or frank joint instability, in the absence of trauma	1. Absence of unusual skin fragility, which should prompt consideration of other types of EDS 2. Exclusion of other heritable and acquired connective tissue disorders (CTD), including autoimmune rheumatologic conditions. In patients with acquired CTD (e.g. arthritis), additional diagnosis of hEDS requires meeting Features A and B or Criteria 2 and Feature C of Criteria 2 cannot be counted toward diagnosis of hEDS 3. Exclusion of alternative diagnoses that may also include joint hypermobility by means of hypotonia and/or connective tissue laxity. Alternative diagnoses and diagnostic categories include, but are not limited to: neuromuscular disorders, other hereditary CTDs, and skeletal dysplasias. Exclusion of these considerations may be based on history, physical exam, and/or molecular genetic testing as indicated

Criteria 1, 2, and 3 must ALL be met for a clinical diagnosis of hEDS

APPENDIX E

Hypermobility Spectrum Disorders (adapted from Castori, et al. 2017)

HSD Classification		Beighton Score	Musculoskeletal Involvement
Asymptomatic GJH		Positive	Absent
Asymptomatic PJH		Usually Negative	Absent
Asymptomatic LJH		Negative	Absent
Generalized hypermobility spectrum disorder	G-HSD	Positive	Present
Peripheral hypermobility spectrum disorder	P-HSD	Usually Negative	Present
Localized hypermobility spectrum disorder	L-HSD	Negative	Present
Historical hypermobility spectrum disorder	H-HSD	Negative	Present

APPENDIX F

Clinical features listed on referrals or Genetics consultation note in each phenotypic category

	Noted on Referral (N=151)	Noted on Genetics Consultation Note (N=128)
Musculoskeletal Findings		
arachnodactyly	1	17
arm span: height ratio >1.05	1	2
arthritis	12	16
atrophy of muscle	1	0
chronic cervical radiculopathy	0	1
degenerative disk disease	2	1
dislocations/subluxations	35	59
dural ectasia	1	1
easy bruising	13	45
“EDS” or “EDS symptoms”	7	0
facial features	0	4
fatigue	29	26
fibromyalgia	12	13
fractures/ stress fractures	3	1
Gorlin sign	0	2
gout	0	1
joint hypermobility	82	87
joints “lock”	0	2
kyphosis	0	2
muscle cramps	0	1
osteoporosis	1	0
pain (joint, musculoskeletal, chronic)	69	83
patellar instability	0	1
pectus deformity	0	9
pes planus	2	9
possible fibromuscular dysplasia	1	1
protrosio acetabula	0	1
rectal/uterine/pelvic floor prolapse	6	6
scoliosis	6	22
small fiber neuropathy	0	1
spondylolisthesis	0	1
“suspicion of Marfan”	19	0
talipes equinovarus	1	3

temporomandibular joint dysfunction	6	15
tendon rupture	0	1
tendon tear	1	5
tissue fragility during surgery	1	1
uterine rupture	1	1
Vascular Findings		
abnormal angiography	1	0
aortic aneurysm	4	3
artery dissection	7	7
excessive bleeding	4	4
stroke	4	5
varicose veins	1	5
venous insufficiency	0	2
Cardiac Findings		
aberrant right subclavian artery	1	0
aortic root dilation	4	4
arrhythmia	0	2
atrial fibrillation	0	1
mitral valve prolapse	3	7
pericardial effusion	1	0
POTS	13	19
positional hypotension	2	2
tachycardia	4	3
thoracic outlet syndrome	1	2
tricuspid regurgitation	4	6
Gastrointestinal Findings		
abdominal pain	7	9
bowel obstruction	0	1
cecal volvus	1	1
colon perforation	1	0
delayed gastric emptying	1	0
esophagitis	0	2
GI disturbance (constipation/diarrhea/irritable bowel syndrome/disease)	20	46
intussusception	1	0
rectal duplication cysts	1	1
Genitourinary/ Gynecological Findings		
abdominal hernia	4	1
endometriosis	1	2

hiatal hernia	2	6
inguinal hernia	3	4
interstitial cystitis	0	2
overactive bladder	1	0
Skin Findings		
Acrogeria	0	1
atrophic/abnormal/wide scarring	2	21
easy tearing of the skin	0	5
heel papules	0	6
poor wound healing	2	6
skin elasticity	0	14
“skin findings”	1	0
skin striae	4	25
thin/translucent skin	2	6
velvety/soft skin	0	24
Eye Defects/Vision		
blue sclera	0	1
“cracks on retina”	0	1
dry eyes	0	1
eye pain	3	1
macular degeneration	0	1
myopia	2	18
retinal detachment	1	2
Hematologic/Immunologic Findings		
Anemia	1	0
autoimmune disorder/frequent illness or infection	4	10
Lyme disease	0	1
mast cell activation disorder	7	8
mastocytosis	1	2
Sjögrens disease	1	0
Endocrine Findings		
Cushing’s disease	1	1
panhypopituitarism	1	1
Hearing Impairment		
hearing loss	3	2
Neurological Findings		
abnormal gait	1	0
ataxia	0	1
auditory processing disorder	0	1

brain fog	5	8
dysautonomia	5	7
dysphasia	1	1
expressive aphasia	0	1
hemiplegic migraine	1	1
lightheaded	2	0
migraines/headaches	30	43
“memory problems”	1	0
multiple sclerosis	0	1
poor balance	1	0
syncope	4	0
tremors	2	4
vertigo	0	1
Structural Brain/Spine Abnormalities		
Arnold Chiari malformation	7	9
carotid cavernous fistula	1	1
tarlov cysts of the spine	1	1
Behavioral/Developmental Findings		
anxiety/ depression	33	27
insomnia/poor sleep	11	12
intellectual disability	0	1
Respiratory Findings		
pneumothorax	4	6
Dental Findings		
bifid uvula	0	2
dental crowding/ high or narrow palate	2	21
gingival recession	0	1
poor/worsening dentition	1	0
Metabolic Findings		
low alkaline phosphatase	1	1
elevated homocysteine	1	0
hyperprolactinemia	1	0

APPENDIX G

Clinical features listed on referrals and Genetics consultation notes

Feature	Referrals N=151 N(%)	Genetics Consultation N=128 N(%)
Musculoskeletal Findings	129 (85.4)	125 (97.7)
Vascular System	18 (11.9)	21 (16.4)
Cardiac Findings	33 (21.9)	38 (29.7)
Gastrointestinal Findings	24 (15.9)	51 (39.8)
Genitourinary/Gynecological Findings	10 (6.6)	14 (10.9)
Skin Findings	11 (7.3)	67 (52.3)
Eye Defects/Vision	8 (5.3)	24 (18.8)
Hematologic or Immunologic Findings	11 (7.3)	20 (15.6)
Endocrine Findings	1 (0.7)	1 (0.8)
Hearing Impairment	3 (2.0)	2 (1.6)
Neurological Findings	45 (29.8)	55 (43.0)
Structural Brain/Spine Abnormalities	9 (6.0)	11 (8.6)
Behavioral/Developmental Findings	38 (25.2)	35 (27.3)
Respiratory Findings	4 (2.6)	6 (4.7)
Dental Findings	3 (2.0)	23 (18.0)
Metabolic Findings	3 (2.0)	1 (0.8)

APPENDIX H

Number of referrals placed to other specialties following Genetics evaluation

Specialists	N=128 N (%)
Cardiology	21 (16.4)
Ophthalmology	28 (21.9)
Rheumatology	2 (1.6)
Pain Management	7 (5.5)
Physical Therapy	7 (5.5)
Neurology	4 (3.1)
Orthopedics	3 (2.3)
Immunology/Allergy	6 (4.7)
Local EDS specialist	6 (4.7)
Gastroenterology	3 (2.3)
Cancer Genetic Counseling	3 (2.3)
Dermatology	1 (0.8)
Otolaryngology	1 (0.8)
Social Work	1 (0.8)
Mental Health specialist	1 (0.8)
Nephrology	1 (0.8)

APPENDIX I

Types of genetic testing recommended/ordered

Single genes
COL3A1
FBN1
GLA
IKBKAP1
KIT
PLOD1
site specific CYP2D6
site specific familial variant
TNXB

Two genes
COL5A1, COL5A2
FBN1, FLCN
TGFB1, TGFB2

Three genes
COL3A1, COL5A1, COL5A2

Larger gene panels
5 gene "EDS Panel" *COL1A1, COL1A2, COL3A1, COL5A1, COL5A2*
10 gene "Vascular Panel" *ACTA2, COL3A1, FBN1, MYH11, MYLK, PRKG1, SMAD3,TGFB2, TGFBR1, TGFBR2*
14 gene "Marfan Syndrome and Related Aortopathies Panel" *ACTA2, COL3A1, COL5A1, COL5A2, FBN1, FBN2, MYH11, MYLK, SKI, SLC2A10, SMAD3, TGFB2, TGFBR1, TGFBR2*
15 gene "TAADNext" *ACTA2, CBS, COL3A1, COL5A1, COL5A2, FBN1, FBN2, FLNA, MED12, MYH11, SKI, SLC2A10, SMAD3, TGFBR1, TGFBR2*
18 gene "EDS Panel" *ADAMTS2, ATP7A, B3GALT6, B3GALT7, CHST14, CHST3, COL1A1, COL1A2, COL3A1, COL5A1, COL5A2, FKBP14, FLNA, FLNB, PLOD1, SLC39A13, ZNF469*
21 gene "Aortopathy Panel" *ACTA2, CBS, COL3A1, COL5A1, COL5A2, EFEMP2, FBN1, FBN2, FLNA, MYH11, MYLK, PLOD1, PLOD3, PRKG1, SKI , SLC2A10, SMAD3, SMAD4, TGFB2, TGFBR1, TGFBR2*
37 gene "Connective Tissue NGS Panel" *ABCC6, ACTA2, ACVR1, ADAMTS2, CBS, CHST14, COL11A1, COL1A1, COL2A1, COL3A1, COL4A1, COL5A1, COL5A2, ELN, FBLN5, FBN1, FBN2, FKBP14, FLNA, MED12, MYH11, MYLK, NOTCH1, PKD2, PLOD1, PRDM5, SKI, SLC2A10, SLC39A13, SMAD3, SMAD4, TGFB2, TGFBR1, TGFBR2, ZNF469*
4 gene "Neurofibromatosis Panel" *NF1, NF2, SMARCB1, SPRED1*
61 gene "Bleeding Disorders Panel"
152 gene "Otoscope Panel"