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Clinical-DNA Correlates of Anxiety in Patients with Ehlers-Danlos Syndrome

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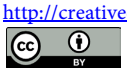

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Abstract

Introduction: Anxiety disorders have a lifetime prevalence of 34% with a similar level of heritability (31%) yet lack objective markers that could differentiate patients with underlying conditions. Up to 60%-70% of patients with Ehlers-Danlos syndrome have anxiety that meets criteria of generalized anxiety disorder, their clinical-DNA findings worth examining as biomarkers for patients with generalized anxiety. **Method:** Of the 1899 patients diagnosed with Ehlers-Danlos syndrome, 1261 were systematically evaluated for 80 history and 40 physical findings and separated into 826 who reported anxiety and 435 who did not. The most consistently reported or management-directing 60 of these clinical findings were, along with variations in genes relevant to these disorders, examined for association with anxiety. **Results:** Among the 30 anxiety-associated findings judged most predictive of Ehlers-Danlos syndrome in patients with anxiety were expected ones of adrenergic stimulation (difficulty concentrating-87% frequency and 1.26 anxiety/no anxiety ratio; chronic fatigue-84%, 1.17; sleep issues 69%, 1.52 that are criteria for generalized anxiety disorder) or of cholinergic suppression (e.g., frequent nausea 64%, 1.26). Less associated but more discriminating for underlying disease were those reflective of neuromuscular impact (e.g., chronic daily headaches 76%, 1.12); hypermobility (e.g., awareness of flexibility 72%, 1.03), or skin changes (e.g., elasticity around jaw 71%, 1.06). Anxiety-associated DNA variants included 54 of 88 in collagen type I/V/VII/IX genes, 14 of 16 in sodium channel SCN9A/10A/11A genes, 59 of 85 in POLG/MT-DNA genes, and 21 of 28 in profilaggrin-FLG genes that respectively impacted tissue laxity, sensory neural, autonomic-mitochondrial, and autonomic-inflammatory functions. **Conclusion:** Analysis of pathogenetic mechanisms in Ehlers-Danlos syndrome selected some 50 clinical-DNA findings useful for its diagnosis in those with generalized an-

xiety disorders.

Keywords

Anxiety, Generalized Anxiety Disorder, Ehlers-Danlos Syndrome, Long COVID19, Joint Hypermobility, Dysautonomia, DNA Testing, Whole Exome Sequencing, DNA Variant Qualification

1. Introduction

Many patients with psychiatric disorders face barriers to diagnosis and therapy because their symptoms are subjective with variable patterns and few modalities of objective diagnosis. Such is the case with anxiety, defined as excessive, uncontrollable worry about potential events that is disproportionate to their actual risk [1] [2]. Anxiety disorders are the most common form of mental illness [2]-[4], their global prevalence (37 - 56 million prevalent cases) and health burden (20 - 39 disability-adjusted life years) [5] [6] including some 19-34% of Americans during their lifetime [2] [3]. Generalized anxiety disorder with its 3.1% lifetime prevalence is most relevant here although other disorders excluded by its DSM 5 guidelines [7] like phobias (9.1% lifetime prevalence), post-traumatic stress disorder (3% - 4%), social anxiety disorder (7% - 13%), panic disorder (2.7%), and obsessive compulsive disorder (1.5%) may also have biological predisposition [1] [2].

Genetic influence is shown by two-fold odds for children of affected parents to have anxiety [1], yet reproducible DNA correlations have been hindered by the nonspecific behavioral phenotype. Genes plausibly related to anxiety mechanisms [1] [8] [9] include those encoding the estrogen receptor *ESR1* M133430) [10], the inflammation-related mitotic arrest inhibitor *MAD1L1* (M602686), the 5-hydroxytryptamine receptor (*5-HT1A*), and the monoamine oxidase A enzyme (*MAOA*). All of these are candidates from polymorphism association that await election by concordance of mutations and relevant anxiety symptoms in multiple families [11].

Sharing many symptoms with generalized anxiety disorder is the Ehlers-Danlos syndrome (EDS) [12], initially recognized by its joint hypermobility [13] [14] and skin fragility [15] but shown by holistic evaluation [16] to be a connective tissue dysplasia [17] with diverse skeletal [18], neurologic [19]-[21], and gynecologic [22] findings. Both EDS and anxiety are underdiagnosed [16] [23] and undertreated, partly explained by their 2 to 4-fold predominance in females [24].

Screening for joint flexibility that is above average in 10% - 20% of people [13] [14] would increase recognition of EDS patients and lead to an explanation for the anxiety that affects 64% of them [16]. Holistic evaluation of the diverse symptoms mentioned above would then relate several to vessel distensibility and lower body blood pooling [22], others to orthostatic intolerance with cognitive dysfunction [19], and many including anxiety to the adrenergic response that acts to

restore cerebral circulation [25] [26]. Thus anxiety in EDS becomes part of postural orthostatic tachycardia syndrome (POTS) [27], a consequence of autonomic imbalance that produces parallel symptoms of mast cell activation syndrome (MCAS) [27] [28] and the bowel dysmotility (irritable bowel syndrome or IBS [29]) that reflects cholinergic suppression.

It would then seem attractive to see how many patients with undifferentiated anxiety might have EDS or related conditions. The above-mentioned neuro-autonomic symptoms that accompany even mild degrees of joint-skin laxity [25] suggest a 1% - 2% prevalence of EDS if narrow definitions based on rare [30] and transiently differentiated [31] types are avoided. The idea is strengthened by the fact that several characteristics of EDS match DSM 5 criteria for generalized anxiety disorder [7]. Most EDS patients have excessive worry about work or school performance (item A) and experience poor symptom (pain-dysautonomia) control (item B). They usually have impairment of social-occupational functions (item D) indicated by months or years-long quests for diagnosis [16] [23], matching item C DSM criteria [2] [7] with their chronic fatigue (reported by 84%), brain fog (by 81%), muscle tension (muscle aches by 59%), and sleep issues (by 61%) [16]. EDS patients rarely reported substance abuse (item E) or other mental disorders (item F) aside from pain-related depression and rare panic attacks [16] [23].

The multiple gene changes found in EDS patients [16] [32]-[34] would provide additional diagnostic markers for anxiety patients with EDS if they were qualified by impact on tissue laxity-dysautonomia mechanisms [16] [23]. This mechanistic view explains the similarities of clinical and DNA findings between EDS and long COVID19 [23] [35], the latter's affliction of 40 million globally [36] another contributor to general anxiety. With these future possibilities in mind, findings most associated with anxiety in 1261 EDS patients will be presented.

2. Methods

Methods in prior reports [16] [23] describe the evaluation of 1979 patients for EDS (1899 diagnosed) in a medical genetics private practice from July of 2011 through October of 2020. They also describe procedures for DNA testing, all but 7 patients with prior results having insurance coverage ascertained and requisitions completed by GeneDx© Company genetic counselors. The requisitions had consents for anonymous sharing of DNA results as did clinic intake forms that included consent for medical genetic evaluation/treatment [23].

Standard methods of whole exome sequencing testing [11] with separate [37] or concordant [38] microarray analysis were used by commercial companies. A novel clinical protocol supplemented consensus qualifications [39], designating DNA variants or their combinations found in 568 of 967 patients as having 1 - 4+ significance (DNA results were collaboratively interpreted and collated with co-author VST in Lubbock) [16] [23]. Only methods relevant to the current article are detailed here.

2.1. Patient Population and the Selection of More Reliable Findings

Systematic evaluation of 120 history and 40 physical findings (see all of them in Table S1 of the Supplemental Materials for the later article [16]) found in the first 915 EDS patients was performed on three groups of EDS patients: 741 seen in clinic, 277 seen in clinic with retrospective form completion (many with salient DNA findings seen before the 120-finding forms were adopted), and 243 evaluated by online/telemedicine interaction where patients filled out the forms [16] [23]. This heterogeneity was counteracted by selecting more consistent findings for EDS group comparisons, based on the fact that women aged 21 - 40 years had very similar clinical profiles [16]. Finding frequencies, standard deviations, and coefficients of variation for their 21 - 25, 26 - 30, 31 - 35, and 36 - 40 year sub-groups were calculated [16], reasoning that intergroup variation would reflect inconsistent reporting in this homogenous population. Results are shown in columns A-C and W-Y to the left of each finding in Supplemental **Table 1**, the most consistent 50 findings among the 120 listed in rows 6-37 of that Table. Grouping by mechanism places findings reflecting hypermobility-injury [13] (10 findings), skeletal bend-deformation [18] (10), or skin fragility [15] (5) in Column D while those reflecting POTS [27] (10), IBS-MCAS [27] [28] [29] (10), and neuromuscular [19] (5) processes are in Column Z. Below the consistent findings in each of these columns are 5 selected findings listed because of implications for management. Several findings have been used as criteria for the hypermobile [13] or classical [15] types of EDS by 2017 criteria as indicated by superscripts (see the criteria in columns AU-AV of S1 Table in the previously published Supplemental Materials [16]). Anxiety was one of 11 history findings systematically assessed in the POTS category, dividing EDS patients into those reporting (826) or not reporting (435) this finding (see **Table 1** and Results-anxiety was therefore eliminated to make 10 findings in the POTS category).

2.2. Patient and DNA Databases

The 1979 EDS outpatients including 967 with DNA testing were entered into a password-protected MS Excel© GW patient database approved by the North Texas IRB (centered at Medical City Hospital, Dallas) in 2014 (exempt protocol number 2014-054). Contributing to the data in this article and available in the previously published Supplementary Materials [16] are deidentified Excel tables that 1) include the 1261 EDS patients with systematic evaluations with sex and age range demographics, type of evaluation, source of referral, detailed history-physical findings, and positive/negative but not specific DNA results (the EDS1261GW1-23 database, Sheets 5-6); 2) S2 Table summarizing the number of DNA variants by gene; 3) S3 Table listing the 917 DNA variants in 568 EDS patients, 893 qualified with 1 - 4+ relevance to EDS. The latter table, its patient numbers scrambled to protect identity, includes commercial [39] and clinical-biochemical [16] qualifications, ClinVar [40] or MitoMap [41] registration, and parental origin when determined.

Table 1. Characteristics of EDS patients with or without anxiety.

EDS patients	Units	All patients	Anxious	Not Anxious
All	No. (% of all)	1261 (100)	826 (66)*	435 (34)
Female	"	1064 (84)	734 (69)*	330 (31)
Male	"	197 (16)	92 (47)	105 (53)
EDS types				
Hypermobile EDS	No. (% of all)	892 (71)	570 (64)*	322 (36)
Classical EDS	"	332 (26)	232 (70)*	100 (30)
Dysautonomia	"	37 (3.0)	24 (65)	13 (35)
Referral source				
Primary	No. (% of all)	504 (40)	314 (62)*	190 (38)
Cardiology	"	426 (34)	298 (70)*	128 (30)
Self	"	181 (14)	119 (66)*	62 (34)
Orthopedics	"	57 (4.5)	33 (58)	24 (42)
Rheumatology	"	44 (3.5)	29 (66)*	15 (34)
Ages and total findings				
Age at evaluation	($\bar{X}$ years ± SD)	29 ± 14	30 ± 13*	27 ± 15
Onset of dysfunction	($\bar{X}$ years ± SD)	17 ± 9.2	17 ± 8.3	16 ± 11
History findings	($\bar{X}$ of 80 ± SD)	34 ± 10	37 ± 9.3*	30 ± 11
Physical findings	($\bar{X}$ of 40 ± SD)	18 ± 4.7	19 ± 4.6*	18 ± 4.6
Beighton score [14]	($\bar{X}$ of 9 ± SD)	6.7 ± 1.9	6.7 ± 1.9	6.5 ± 1.8

Note: The 1261 EDS patients with systematic evaluations are listed by characteristic (left) or DNA testing results (right). EDS hypermobile [13] or classical [15] type diagnoses were assigned by 2017 consensus criteria, 37 patients having more dysautonomia than delineating joint-skin findings. Less common referrals from allergists, gastroenterologists, etc. for ~4% of EDS patients are not listed; *significant difference in proportions or averages, $p < 0.05$ (see Methods); SD, standard deviation.

2.3. Statistics

Frequencies of the more reliable clinical findings in different EDS patient groups were tallied from the EDS1261GW1-23 database using the search, find, and sort functions of MS Excel© and are listed in Appended Table Ex1. Average frequencies, standard deviations, and significant differences at the $p < 0.05$ level were determined using Excel functions and online resources [42] [43], the latter comparing means by two-tailed t and proportions by N-1 chi-squared tests.

3. Results

Numbers of EDS patients with and without anxiety are compared in **Table 1**, females comprising 85% of all 1899 EDS patients [23] and 84% of those syste-

matically evaluated. Most patients fit consensus criteria of the hypermobile type [13], 71% matching its criteria of more joint instability with flat, white atrophic scars while 26% matched classical type [15] criteria of more joint pain-injury with raised, discolored papyraceous scars (19 and 17 hypermobile and classical criteria were among the 120 evaluated findings, 8 and 10 designated by h and c superscripts among the 60 in Supplement **Table 1**) [30]. A minority (3%) had more frequent dysautonomia findings that have unfortunately been considered inconsistent [30] rather than central to the EDS clinical profile [16] [23]. Patients with obvious vascular EDS [43] or other connective tissue dysplasias [17] like osteogenesis imperfecta (M166200) [10] or Marfan syndrome (M154700) were excluded from the EDS population.

All EDS patients but males, those with predominant dysautonomia, or those referred by orthopedics had significantly greater frequencies of anxiety (**Table 1** left), highlighted by females (69%), common types of EDS (64 and 70%), and cardiology referrals (70%). Patients with anxiety presented later (30 versus 27 years) but had significantly more total history (37 versus 30) and physical (19 and 18) findings (lower panel of **Table 1**). Note the long delays between dysfunction and diagnosis of 13 and 11 years for anxious and not anxious patients, both groups having above-average Beighton scores (6.7 and 6.5) [14] Previous data [16] showed that EDS females were more severely affected than males (36 average history findings versus 26, attributed to their lesser muscle support of joints, inherent tissue laxity necessary for parturition, and estrogen softening of bone [16].

3.1. Different Finding Frequencies in EDS Patients with or without Anxiety

Discussed in Methods was the selection of the 50 history-physical findings most consistently reported by EDS patients (per the statistics in columns A-C and W-Y of **Table 1**) and their grouping by tissue laxity (column D) or neuroautonomic (Column Z) mechanisms. In red print are findings shared by patients with long COVID19 with their average frequencies and ranges from the survey of 64 articles by Deer *et al.* [35].

Scanning the comprehensive data in **Table 1** shows 1) that ratios of standard deviation to frequency (coefficients of variation) are under 0.05 for 14 findings and under 0.10 for 35 with only 4 above 0.15 (columns C, Y of **Table 1**); 2) higher frequencies in females (columns H, AD of **Table 1**) than males (columns N, AJ) for most findings except for six of body build/skeletal deformation (e. g., height, flat feet or toeing-in), two of infancy-early childhood (colic-feeding difficulty, motor delay), and having a 30% lower weight percentile than that for height; 3) higher frequencies in all EDS patients with anxiety for 7 of 10 findings related to joint laxity-injury, 4 of 10 related skeletal deformation, 3 of 5 related to skin fragility, 10 of 10 related to POTS, 8 of 10 related to IBS/MCAS, and 5 of 5 related to neuromuscular mechanisms; and 4) that most ratios of finding frequencies in patients with/without anxiety (columns T, AP) are between 1 and

1.3, most differences deriving from finding frequencies in the predominant females over age 21.

The increase of anxiety with age is detailed in Supplement Figure 1, low proportions with anxiety under age 10 for both sexes, female proportions at 66% to 79% for older female decadal groups with a fall-off to 57% for those over 51 years. The younger presentation of males (52% between ages 10 and 20 compared to 26% of females) likely reflects their increased athletic activity since their mentioned muscle support produces milder numbers of symptoms overall (Table 1). Only

	Index of EDS predictability for finding = Percent x Ratio x 1/CoV[1]			
Finding↓ Long COVID% (range)[2] ↓	All (826)	Rank	Female (734)	Male EDS (92)
POTS Tachycardia 32(20-48)	107	1	103	98
POTS Brain fog 55 (30-70)	107	2	102	109
POTS Dizzy on standing 10 (10-50)	105	3	102	108
POTS Chronic fatigue 52 (30-60)	99	4	97	85
POTS Sleep issues 33 (20-35)	98	5	95	85
POTS Heat-cold sensitivity	89	6	90	54
IBS Bowel irregularity 22 (14-78)	88	7	86	87
Flex Reverse prayer sign	85	8	85	64
IBS Bloating-Reflux-GI pain 22 (14-78)	84	9	80	98
Nm Chronic daily headaches 8 (2-17)	83	10	86	56
Nm Muscle aches 19 (12-22)	83	11	85	55
Skin Soft, velvety[3]	82	12	83	71
MCAS Food-Medicine intolerances	79	13	77	67
POTS Salt fancy	78	14	78	60
Skin Easy-frequent bruising	76	15	75	42
IBS Frequent nausea 22 (14-78)	76	16	70	104
Flex Aware of flexibility	73	17	73	59
Flex Subluxations	72	18	75	37
Skin Jaw skin elasticity (stretch > 1 inch)	71	19	74	59
Flex Activity joint-limited	71	20	70	58
Nm Migraines	70	21	67	39
Flex Many sprains	68	22	67	60
MCAS Gum issues + Many caries	67	23	68	44
Skin Early striae	64	24	66	28
Flex Beighton 7-9	63	25	62	39
POTS Pedal pooling on stand	63	26	64	27
Nm Poor balance Hx/PE 25 (17-55)[4]	61	28	62	37
Selected Bladder issues[4]	57	31	53	40
MCAS Dyspnea-Asthma 37 (12-40)[4]	56	33	56	43
Skin Unusual scars Hx/PE[4]	51	36	50	31

Note: Findings are rated by their [1]index of EDS predictability that is mostly based on their frequency as a percentage (P) in the 1261 EDS patients with systematic evaluations, that frequency multiplied by its ratio (R) in patients with/without anxiety and by the reciprocal of its coefficient of variation (see Supplement Table 1 and Methods); [2]those EDS findings prevalent in patients with long COVID are in red print with their average percentage and range in the studies reviewed by Deer *et al.* [35]; [3]not considered reliable for assessment in anxiety patients; [4]considered more reliable for assessment than findings ranking higher (see Table 1 for all indices). POTS, postural orthostatic tachycardia syndrome; IBS, irritable bowel syndrome; Flex, joint hypermobility-injury findings; Nm, neuromuscular findings; MCAS, mast cell stimulation syndrome findings; Bladder issues were among those less consistent findings selected as important for management.

Figure 1. The 30 EDS findings considered most useful for differential diagnosis of anxiety.

the 21 - 30 age group in Figure 1 has a significant majority (63%) with anxiety.

3.2. Findings Most Predictive of EDS in Patients with Generalized Anxiety

Excerpted in Figure 1 are 30 individual EDS findings shaded by mechanistic group and ranked by indices designed to reflect their utility for suspecting EDS in the patient with general anxiety (data column 1 for all EDS patients). A high finding frequency (P) was considered most useful, multiplied by anxiety/no anxiety ratios (R) and consistency (coefficients of variation C) such that the arbitrary index $I = P \times R \times 1/C$ is similar to P (Indices for every finding are shown all EDS patients (columns U, AP), females (columns K, AG), and males (columns Q, AM) of Table 1.

Seven POTS findings in all EDS patients (yellow colors) top the list in Figure 1, five of them frequent in long COVID19 [31] [35], several of them (altered sweating, sleep issues, tachycardia, salt fancy) along with other POTS (altered sweating and syncope) and dysautonomia (gum disease plus caries, dysphagia, and bladder issues) having the highest anxiety/no anxiety ratios (1.30 to 1.64 in columns T, AP of Table 1). These findings of autonomic imbalance and their following neuromuscular findings (chronic daily headaches and muscle aches in Figure 1, also seen in long COVID19) are more predictive than traditional skin (e.g. easy bruising, elasticity around jaw) or joint (e.g. reverse prayer, subluxations) findings.

The high rankings of neuro-autonomic findings [19] [21] [25]-[28] shows why they must be recognized as part of EDS if other diseases [35] [36] and DNA findings [23] are to be correlated. Finding categories support this mandate, their average indices highest for POTS (70), skin (66), and joint-flexibility-injury (61), lowest for skeletal deformations (52-Figure 1). Indices for the majority female (red) and male (blue) findings parallel those for all patients with exceptions of male heat-cold sensitivity, neuromuscular, and IBS findings.

3.3. DNA Findings Could Also Differentiate Anxiety Patients

Adding clinical considerations [23] to consensus descriptors [38] of DNA results (see Methods) qualified DNA variants or their combinations in 566 of 967 EDS patients as significant and contributory to their symptoms [16] [23]. A key aspect of this clinical qualification was the relation of variant genes to tissue laxity or autonomic mechanisms by reference to prior disease associations, summarized for 317 EDS-relevant genes in previous publications (S2 Tables of Supplementary Materials [16] [23]) as excerpted for recurring gene variants in Table 2. Gene changes are categorized by impact on particular tissue elements in the middle of Table 2, including the 51 influencing collagen type V that, with their long EDS association [15], reinforce the relevance of these DNA results [16] [23]. The lower rows of Table 2 show genes impacting neuro-autonomic processes in EDS based on their prior disease associations, a mechanism less anticipated unless one recognizes the cycle of vessel laxity to adrenergic imbalance [25] [26] that

Table 2. DNA testing results for EDS patients with and without anxiety.

EDS patients with or without anxiety → with DNA testing and gene changes ↓	All	Anxious	Not
		No. (%)	
All	1261 (100)	826 (66)*	435 (34)
DNA test	967 (77)	621 (64)*	346 (36)
Primary DNA variants relevant to EDS[1]	566 (44)	373 (66)*	193 (34)
Primary DNA variants affecting tissue laxity mechanisms Number (%)[1]			
Impact on Bone-Joint-Skin	88 (16)	54 (61)*	34 (39)
Bone: *COL1*—15, *COL2*—2, *COL 9*—8; *COL11*—7	32 (5.7)	18 (56)	14 (44)
Joint: *COL5*—51	51 (9.0)	33 (65)*	18 (35)
Skin: *COL7*—4, *COL17*—1	5 (0.88)	3 (60)	2 (40)
Impact on vessels—clotting	54 (10)	31 (57)	23 (43)
Vessels: *COL3*—13, *FBN1*—18 *TGFB/BR*—12	43 (7.6)	26 (61)	17 (39)
Clotting: *VWF*—11	11 (1.9)	5 (46)	6 (56)
Impact on muscle: *COL6*—11, *COL12*—17	28 (4.9)	13 (46)	15 (54)
Primary DNA variants affecting neural mechanisms Number (%)[1]			
Impact on sensory nerves: *SCN9A/10A/11A*—16	16 (2.8)	14 (88)*	2 (12)
Impact on mitochondrial function:	93 (16)	61 (66)*	32 (34)
Mitochondrial respiratory chain: *MT-ATP6/8*—25, *MT-CO1/2/3*—14; *MT-CYB*—12; *ND1/2/4/5/6*—20	71 (13)	47 (66)*	24 (34)
Mitochondrial ribosomal-transfer RNA: *MT-RNA1/2*—4, *MT-tRNA*—18	22 (3.9)	14 (64)	8 (36)
Impact on dysautonomia (MNGIE-related): *POLG*—14	14 (2.5)	12 (86)*	2(14)
Impact on dysautonomia (porphyria-related): *CPOX—2*, *HFE* predisposition—3, *HMBS—5 PPOX*—1	10 (1.8)	7 (70)	3 (30)
Inflammatory impact*: FLG*—28	28 (4.9)	21 (75)*	7 (25)

Note: [1]percentages are of 566 patients with DNA results in the first data column, the percentage of those primary variants in EDS patients with or without anxiety shown in the second and third data columns. Of the 967 EDS patients having DNA testing, 31 were tested for alleles found in relatives, 30 with gene panels, and 906 with whole exome sequencing, DNA variants relevant to EDS were found in 566 patients, 2 of them having unrelated secondary findings [38]. A clinical qualification protocol added points for synergism with EDS-dysautonomia mechanisms to single (61%) or multiple (39%) DNA variant results, converting consensus [38] descriptors of uncertain significance/likely pathogenic/pathogenic to medical diagnostic utility scores [23] of 1+ (1 result-0.18% of 566), 2+ (29% - 5.1%), 3+ (123% - 22%), or 4+ (413% - 77%). *significant $p < 0.05$; gene abbreviations defined at www.omim.org; MNGIE, a mitochondrial neurogastrointestinal encephalopathy with predominant IBS symptoms.

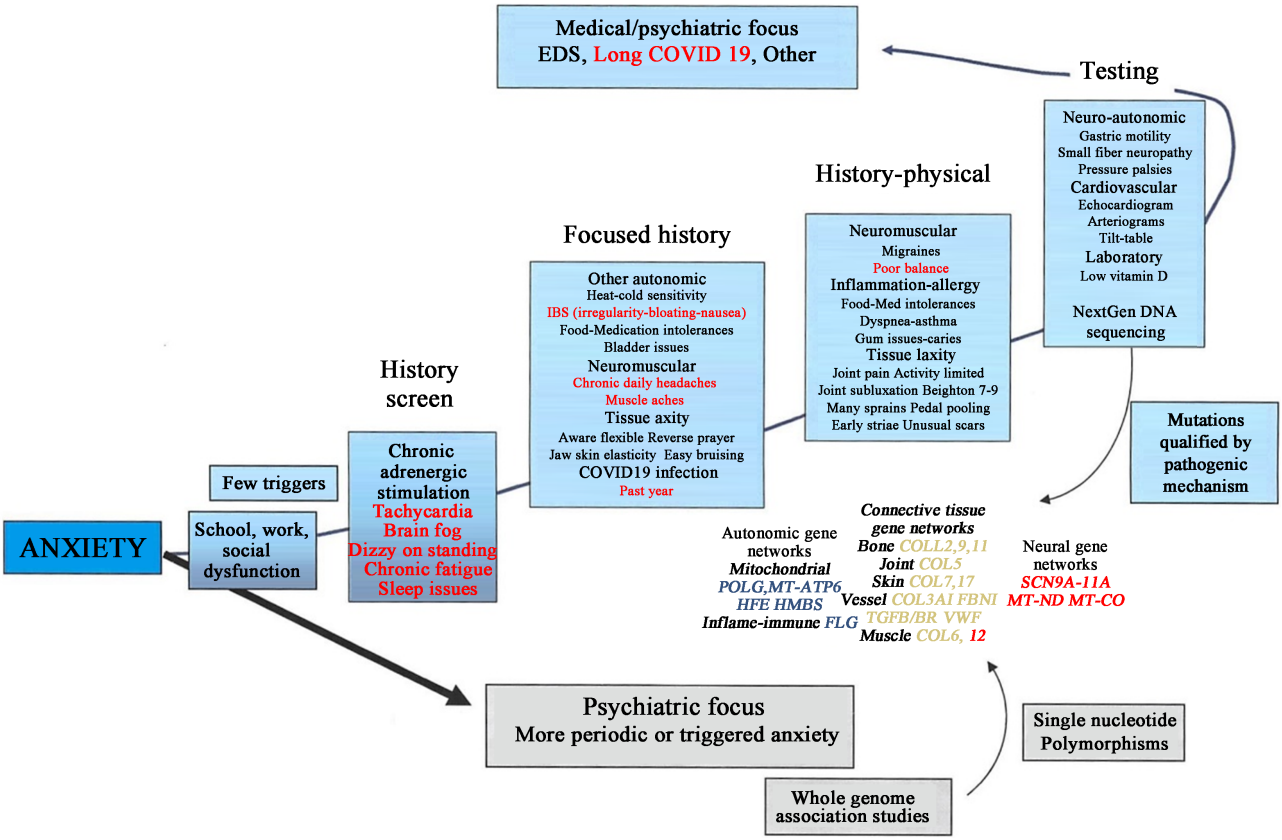
through sensory-autonomic (small fiber) neuropathy [20] reciprocally enhances tissue laxity [26] [32].

Note that 170% or 30.9% (16% + 10% + 4.9%) of the 566 EDS patients with relevant DNA findings have gene changes impacting tissue laxity (upper group in **Table 2**) and that 161% or 28% (2.8% + 16% + 2.5% + 1.8% + 4.9%) have changes impacting neuro-autonomic mechanisms (lower group in **Table 2**).

Neurosensory/mitochondrial [23] [44] (75 versus 34 or 89%) and autonomic-inflammatory (40 versus 12 of or 77%) gene changes are more associated with anxiety than those of tissue laxity (only those of bone-joint-skin collagen genes are significant at 54 of 88 or 61%), correlating with the clinical mechanisms of Figure 1. Genes involved in autonomic-inflammatory processes include the *POLG* mitochondrial DNA polymerase gamma [45] and the profilaggrin *FLG* gene [46].

4. Discussion

Clinical findings associated with anxiety in Ehlers-Danlos syndrome have been ranked by perceived utility, hypothesizing that their recognition in patients with generalized anxiety would mitigate the dismissal and diagnostic delays (Table 1) that especially apply to women [24]. EDS patients with anxiety symptoms in EDS often say that they appear regularly and without relation to any signature event or memory. Recognizing associated findings of adrenergic stimulation as shown in Figure 2 could be a first step toward objective medical diagnoses in



Note: The diagnosis of anxiety (left) that causes dysfunction meets DSM criteria [7], a lack of triggers consistent with generalized anxiety disorder. Chronicity of multiple adrenergic symptoms (history screen) favor a medical cause that is then pursued by the predictive history-physical findings from Figure 1 and confirmed as EDS, long COVID19, or related disorders by definitive testing (upper arrow). Medical diagnoses facilitate the identification of pathogenetic mutations rather than the association studies necessitated by undifferentiated phenotypes.

Figure 2. An approach for diagnosing medical causes of anxiety.

these patients and those with generalized anxiety. The significant but rather small increases in many anxiety patient frequencies (ratios R in **Table 1**) emphasize that anxiety is a signal but not distinctive marker for autonomic imbalance.

Once companion "fight or flight" symptoms plus significant dysfunction [7] and a lack of triggers suggest an underlying medical disorder, a history focused on past infection, neuro-autonomic, and tissue laxity symptoms (center of **Figure 2**) can begin. While cholinergic suppression (IBS, urinary retention/infection) and neural aches can occur with generalized anxiety, documenting high-index (**Figure 1**) symptoms of joint flexibility/wear-and-tear, skin elasticity, and pedal pooling would favor the upward medical path of **Figure 2**. Then definitive could confirm diagnoses of EDS or related medical disorders (a history of infectious mononucleosis [47] has been associated with chronic fatigue syndrome in EDS, low ACTH and estradiol levels in adolescents with that condition [48] intriguing for EDS and anxiety).

While the algorithm of **Figure 2** should be considered clinically, its yield and accuracy can only be established by controlled, prospective trials by physicians experienced with anxiety. Essential and especially important for interpretation of genome sequencing is the relation of clinical and DNA findings to mechanism. Just as anxiety must be related to articulo-autonomic mechanisms, so must DNA changes like those affecting the profilaggrin FLG gene be related to inflammation [49]-[51] (e.g., sensitivity to atopic dermatitis, M6058003) rather than the scaly skin (M146700) cited by commercial interpreters. Not emphasized here but evident in **Figure 1** are the overlap of many EDS and long COVID19 findings. Those anxiety patients suspected to have medical conditions like EDS or long COVID19 could have numerous proven therapies. These include dietary modifications (increased salt, protein for intravascular volume, decreased bowel irritants like gluten or dairy) [26] [29], activity enhancements (exercise [52], bracing), and medications (Corlenar, Midodrine, etc. [29]).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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