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Complement Gene Mutation and Ehlers-Danlos Syndrome

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Abstract

Background: Dental complications of Ehlers-Danlos syndrome (EDS) include periodontitis with gum fragility and inflammation, enamel hypoplasia with frequent caries, high palate with dental crowding, TMJ instability, sutural dehiscence or scarring, and insensitivity to anesthetics. **Objective:** Determine if EDS dental complications always define a specific type and genetic cause or if they can arise as a general consequence of altered inflammatory response in EDS. **Method:** We compared findings of a 58-year-old female with complement component 1R (C1R) gene mutation (c.1553A > T, p.Asp518Val) found by whole exome sequencing to 43 patients with C1R gene mutations ascertained because of periodontal disease and to 710 EDS patients conventially ascertained because of joint and skin laxity. **Result:** Female patients ascertained as periodontal EDS showed the expected higher frequency of periodontitis (96% versus 14%) but had similar frequencies of hypermobility (81% versus 90%) and some skin findings (84% versus 92% with skin fragility) as the general group and our female patient who shared their C1R gene change. Her oromandibular bone loss rather than gum disease may reflect the more carboxy-terminal position of her C1R gene mutation compared to those in the patients identified as periodontal EDS. **Conclusion:** While mutation of the C1R gene may predict more frequent periodontal, skin, and vascular complications, focus on an articulo-autonomic dysplasia process that includes mast-cell activation and altered inflammatory response rather than extreme EDS types will help dentists and other subspecialists identify all EDS patients and anticipate their frequent oral manifestations.

Keywords

Ehlers-Danlos Syndrome, Periodontitis, Complement Component 1R Gene

Mutations, Arthritis-Adrenaline Disorder, Whole Exome Sequencing

1. Introduction

The systemic impact of Ehlers-Danlos syndrome (EDS), first recognized for its joint hypermobility and skin elasticity, is exemplified by dental complications extending from tissue change (periodontitis, enamel hypoplasia, increased bleeding and caries, sutural dehiscence) to skeletal deformation (long face, high palate with dental crowding) and temporomandibular joint instability [1]. The reciprocal adrenergic stimulation that accompanies joint-tissue laxity causes the circulatory changes of postural orthostatic tachycardia syndrome, the inflammatory/allergic changes of mast cell activation disorder, and the suppressed cholinergic bowel motility of irritable bowel syndrome as well as additional oral problems. These include atypical response to many medications, frequent need for higher anesthetic doses, and decreased bone-dentin density from vitamin malabsorption and collagen alterations [2] [3].

Anticipation of EDS complications can follow visual notice of high palate, periodontitis, or enamel hypoplasia, prompting further questions about flexibility, joint pain, and poor healing with scarring and skin fragility. Unfortunately, recognition is compromised by views of EDS as a group of rare and extreme disorders, missing the 10% of men and 20% of women who are more flexible than average on the Beighton scale, those with "benign joint hypermobility" still at risk for its reciprocal dysautonomia [2].

Initial focus on an arthritis-adrenaline disorder symptom pattern rather than rare EDS types, complemented by undirected genetic testing using NextGen or massive parallel DNA sequencing [4] can enhance recognition of EDS and broaden appreciation of the pleiotropic relationships between single gene mutations, polygenic processes, and their variable clinical patterns [5]. A patient with complement C1R gene mutation and findings of general rather than periodontal EDS [pEDS, formerly EDS type VIII—[6] shows the advantage of recognizing a general EDS category and articular-autonomic process influenced by many genes rather than focusing on an extreme type determined by change in one gene.

2. Materials and Methods

The patient was evaluated using standard forms containing typical findings of EDS developed from prior [3]. She reported presence of the history and physical findings described in the case presentation and provided refining details by follow-up questioning. Similar forms contained frequent EDS findings compiled from 946 patients (2011-2016), assembled as a standard evaluation that was calibrated in 710 EDS outpatient evaluations from January 2016 to June 2018 [3]. Whole exome sequencing was coordinated as described [5] through the GeneDx

Company using standard methods, results mailed (averaging 3.1 months after sampling) with interpretative physician letters that included options for follow-up discussion and testing of relatives. Pretest counsel and GeneDx requisitions included consents for anonymous publication of DNA sequencing results. Historical, physical, and molecular findings were abbreviated and entered into a password-protected MS Excel© database with IRB approval as exempt category 4ii (secondary use of identifiable health information regulated by HIPPA).

Tallies of pEDS patient findings were from the MS Excel supplement of Kapferer-Seebacher *et al.* [6], downloaded from https://doi.org/10.1016/j.ajhg.2016.08.019 that listed people from 15 families where at least one individual had a C1R gene mutation. Their data was winnowed to 27 females and 16 males who tested positive for the mutation and were living at the time of report. Significance of differences in finding frequencies were calculated (GraphPad: 2 × 2 contingency calculator at https://www.graphpad.com/quickcalcs/contingency1.cfm) using both one-tailed and two-tailed Fisher exact tests for numbers under 30 or Chi-square with Yates correction for those above, differences qualified as significant only if both tests yielded p values of less than 0.05.

3. Results

3.1. Clinical Report

A 58-year-old women requested online evaluation after becoming concerned about symptoms of connective tissue weakness. Her standard forms recorded 28 of 80 typical findings by history (more than reported by 25% of 596 females having standard outpatient EDS evaluations) and 19 of 40 on physical (more than 60% of those females). Normal growth and development followed infantile feeding difficulties as the only perinatal complication. She had minimal joint pain as a child without the clumsiness, notice of unusual flexibility, or showing off of double-jointed tricks that are common in EDS. Initial symptoms were frequent ankle and elbows sprains in yoga with slow recovery, noticing that her joints popped with movement but having no subluxations, fractures, or severe myopia (she needed glasses only at age 18). In her thirties she had a L knee meniscus injury that required surgical correction and in her forties sacral misalignment was noted by x-ray.

She had dental crowding without orthodontic treatment, TMJ locking and pain without requiring an appliance, and many cavities suggestive of poor enamel. She has never had periodontitis, gum retraction, or signs of gum fragility like bleeding. Until the past year she had had only routine dental care without tooth loss or periodontal disease. Recently she has had increased dental pain with oral x-rays showing dentin loss.

Other findings included skin fragility with striae and dry/scaly skin, menorrhagia with endometriosis, and muscle weakness. Dysautonomia symptoms included constipation/diarrhea, bloating/reflux/stomach pain as typical symptoms

of irritable bowel syndrome; fatigue, sleep problems, brain fog, cold sensitivity, tachycardia, and salt fancy as symptoms of postural orthostatic tachycardia syndrome; transient rashes, hives/reactive skin, and food-medication intolerances typical of mast cell activation disorder [1] [3]. She had the low bone density of her axial and peripheral skeleton often seen with EDS and autoimmune markers including a positive rheumatoid factor that occurred in 10% of EDS patients [3], likely reflecting the immune/inflammatory changes associated with EDS-related dysautonomia and mast-cell activation.

Family history showed no one with dental problems although a maternal aunt had some typical EDS-dysautonomia symptoms with joint pain/arthritis, joint injuries, anxiety, spinal disc issues, and thyroid disease. Her mother at age 77 has arthritis and thyroid disease and another aunt had arthritis as did her maternal grandmother.

On physical examination she reported a height of 65.5 inches (70^{th} centile for adult female), a weight of 98 lb (<3rd centile), and an arm span of 64 inches, less than her height. Findings typical for EDS included a weight less than 25% of her height (frequent with irritable bowel syndrome/mast cell issues), long fingers, and the ability to perform the thumb-little finger overlap around wrist (Walker-Murdoch) and thumb through fist (Steinberg) signs. She had a long face and high palate but reported no tooth loss or gum disease in response to follow-up questions. Her skin was soft and thin with visible veins and elastic with stretching of over 1 inch on her jaw and forearm. She was able to perform 5 out of 9 Beighton maneuvers [1]—bending back fingers beyond 90 degrees on the back of her hands bilaterally, touching thumbs to forearms bilaterally, touching palms to the floor but not able to do bilateral elbow or knee hyperextension. Skeletal findings included a kyphotic neck curve, mild levoscoliosis, and flat feet and she reported weakness on arm and leg flexion with some atrophy of that musculature. She saw an allergist with documentation of high urinary histamine levels and a diagnosis of mast cell activation syndrome.

Initial impression was of hypermobile EDS based on her soft, velvety skin and selective flexibility with no scars suggestive of classical EDS. She had a secondary diagnosis of dysautonomia in the form of irritable bowel, postural orthostatic tachycardia, and mast cell activation syndromes. Whole exome sequencing through the GeneDx Company reported one variant of uncertain significance, a heterozygous aspartic acid to valine change at amino acid position 518 of the complement component 1R gene (C1R c.1553A > T, p.Asp518Val), origin unknown because parental samples were not submitted. The considerable structural and charge difference between amino acid aspartic acid and valine, the low variant frequency in "normal" databases (5 of 246, 238 alleles, all heterozygous, in patients not necessarily evaluated for EDS), and prior EDS association [5] justified clinical interpretation as a variant having strong diagnostic utility for the arthritis-adrenaline disorder category and hypermobile EDS [3].

3.2. Comparison of Patient Findings with Prior Reports of C1R Gene Mutations

Table 1 compares finding frequencies in 1) pEDS patients from the literature [7] [8] [9] summarized by Kapferer-Seebacher *et al.* [6] including only those demonstrated to have C1R gene mutations and alive at the time of report to ensure adequate ascertainment; 2) 710 patients referred for general EDS evaluation assessed using standardized forms [3]; and 3) the reported patient. Both groups were predominantly female (27 of 43 or 63% for pEDS, 596 of 710 or 84% for general EDS), females having slightly higher percentages of most joint or skin findings than males in the pEDS group and significantly more of these findings than males in the general EDS group. Periodontitis and findings of skin fragility were significantly more frequent in both sexes of the pEDS group while hypermobility, joint pain, subluxation/dislocation, and scoliosis were more frequent in the general EDS group. Aneurysms and bowel ruptures affected few patients in either group but were significantly higher in the pEDS versus general EDS group where no vascular EDS patients have been identified [3].

The reported patient had 13 of 16 findings that occurred in over 25% of general EDS patients and lacked 4 dental findings and 3 others (hernia, prone to infections, aneurysms) typical of the pEDS group. Note that pEDS patients have all of the general EDS findngs except those of dysautonomia [not listed in the report of Kapferer-Seebacher *et al.*, [6], their frequencies of skin findings, aneurysms, periodontitis (and probably other dental manifestations) more a difference of type rather than process.

4. Discussion

4.1. Inflammatory Change via Complement C1R Gene Mutation Can Cause Periodontitis and Other Dental Complications in EDS

Pattern recognition promoted the identification of over a thousand genetic syndromes, most over the last 60 years, an enterprise aided tremendously by dentistry as shown by prominent textbooks [10]. It is ironic that DNA delineation of these syndromes by examining every segment of all 46 chromosomes for dosage change (microarray analysis) and every nucleotide of all 23,000+ genes for sequence errors (whole exome sequencing) has reached its zenith over the same time period [4], yet the gap between genomic change and its clinical application remains wide. A major part of the problem is laboratory report of DNA change without clinical interpretation that ensures full documentation of symptoms, crucial for connective tissue dysplasias like EDS that are easier to approach as overlapping categories than as rare discrete types.

EDS headlined by one finding, as with pEDS, is a valuable diagnosis at the end of the diagnostic process, since those with complement C1R mutations clearly have more dental issues, more of certain skin findings, and likely a greater risk for aneurysms [6] [7] as shown in Table 1. Overlap of many pEDS findings with

Table 1. Comparison of patient findings with those in periodontal and general EDS.

Finding or quantity	pEDS females[a] +/total (%)	pEDS males[b] +/total (%)	EDS females[c] # (%)	EDS males[d] # (%)	Patient report[e]
Number of patients	27	16	596 (100)	114 (100)	1
Percentage female	27 (63)	--	596 (84)*	--	1 (100)
Periodontitis	25/26 (96)*	11/11 (100)*	83 (14)	13 (11)	-**
Many caries (poor enamel)	--	--	268 (45)#	29 (25)	+
Gingival recessions	24/25 (96)	10/10 (100)	--	--	-**
Gum bleeding	23/24 (92)	3/5 (60)	--	--	-**
Gum fragility	14/15 (93)	5/5 (100)	--	--	-**
Later bone resorption	--	--			+
Dental crowding	--	--	375 (63)	57 (50)	+
Marfanoid (aged) facies	--	--	9 (1.5)	1 (1.0)	-
TMJ issues	--	--	334 (56)#	36 (32)	+
Hypermobility	17/21 (81)#	3/9 (33)	536 (90)#	89 (78)*	+
Joint dislocation	2/21 (9.5)	2/27 (7.4)	429 (72)*#	59 (52)*	-
Arthralgia	8/22 (36)	9/28 (32)	530 (89)*#	72 (63)*	-
Scoliosis	4/21 (19)	4/26 (15)	238 (40)*#	33 (29)	+
Flat feet	--	--	185 (31)	34 (30)	+
Easy bruising	23/24 (96)*	31/32 (97)*	387 (65)#	51 (45)	+
Fragile skin	21/25 (84)	7/10 (70)	548 (92)#	76 (67)	+
Elastic skin	17/22 (77)*	21/28 (75)*	149 (25)#	18 (16)	+
Abnormal scars	10/22 (46)	13/30 (43)*	256 (43)#	26 (23)	-**
Prominent vasculature	7/16 (44)	2/4 (50)	185 (31)	27 (24)	+
Pretibial discoloration	19/25 (76)	27/35 (77)	--	--	?
IBS symptoms	--	--	542 (91)#	68 (60)	+
POTS symptoms	--	--	578 (97)#	81 (71)	+
MCAD symptoms	-	--	524 (88)#	67 (59)	+
Hernia	4/20 (20)	1/5 (20)	72 (12)#	3 (3.0)	-**
Prone to infections	5/20 (25)	2/4 (50)	--	--	-**
Aneurysms (5, 6, 14)	2/19 (11)*	1/6 (16)*	4 (0.67)	0 (0.0)	-**
Organ rupture (1-III-10)	0/15 (0)	1/4 (25)*	2 (0.34)	0 (0.0)	-

Percentages of findings for 27 females[a] and 16 males[b] with pEDS [7] [8] [9] summarized by Kapferer-Seebacher *et al.* [6] compared to 596 females[c], 114 males[d], and the reported patient[e] having systematic evaluations for general physical findings shared by all types of EDS. Like the patient, 70% of these systematically evaluated EDS patients met criteria for hypermobile hEDS, another 23% for classical cEDS, 7% for benign joint hypermobility, and 7% for primary dysautonomia without associated EDS [3]; +/total (%), number of patients with finding/total (percentage); *, significantly higher frequencies between pEDS and general EDS patients of the same sex; #, significantly higher frequencies for females versus males in the same group; **, dramatic (not significant) difference between a finding in the reported patient and those with pEDS; key differences between patient groups are highlighted in grey.

those of the more general EDS group and our patient with complement C1R gene mutation also shows the benefit of preliminary categorical diagnosis, conveniently encompassed by the term arthritis-autonomic disorder [3]. This category encompasses overlapping findings among EDS types [3] [8] [9], their shared autonomic imbalance, and relates newly discovered gene mutations to reciprocal articular and autonomic processes [11].

Focus on pathogenic process rather than disease type fosters a clinical approach to DNA result interpretation, first qualifying the impact of the mutation on protein function (charged aspartic acid to neutral valine in our patient); second qualifying relation to process (complement gene to autonomic and inflammatory alterations in EDS); third qualifying correlation with patient findings and inheritance (compatible with EDS finding profile and heterozygous dominance); fourth qualifying diagnostic utility on a traditional 1 - 4+ medical scale (3+ or strong utility for our patient)—rather than designating a laboratory change as a *molecular* diagnosis or, at the other extreme, as the unhelpful *variant of uncertain significance* [5].

4.2. Mutations Affecting Different C1R Protein Domains Produce Variable Oromandibular Manifestations within a General EDS Theme

Strengthening the relation of C1R gene mutation to EDS and particularly the dentistry-relevant pEDS is the function of its complex $C1R_2$-$C1S_2$ tetramer in the complement system to mediate innate immunity. Complement activation through formation of this complex with its calcium-dependent assembly and serine protease activity triggers phagocytosis and inflammatory responses including mast cell stimulation that can be misdirected as gingivitis and osteoarthritis in EDS. Autoimmunity does occur in EDS is shown by patients of Kapferer-Seebacher *et al.* [6] with Crohn disease, Sjogren syndrome, or vasculitis/osteolysis with antibodies against collagen type I [12] and by the 10% - 11% of our general EDS patients who have autoimmune markers [3].

It can be expected that mutations in different gene regions will produce different clinical outcomes, and it is notable that our patient's mutation but none of those in the pEDS group was in the serine protein domain, nearer the C-terminus than those of Kapferer-Seebacher *et al.* [6]. Of their 43 patients, 26 had mutations in the two CUB domains of the C1R protein, named for presence in Complement-Urchin epidermal growth factor-Bone morphogenetic proteins that are extracellular or associated with the plasma membrane proteins. These C1R protein domains have collagen-binding activity that may be impacted to produce more severe dental symptoms, very prominent in patients with osteogenesis imperfecta and collagen type I gene mutations [13]. Altered response to collagens could certainly cause diffuse connective tissue changes and provoke autoantibodies as described by Hoffman *et al.* [12]. The latter mutations also occur before the arginine 463 site where C1R protein is cleaved to yield the component that forms C1 esterase, while ours occurs after (UCSC genome browser,

https://genome.ucsc.edu/).

It is possible that our patient's mutation activates or unmasks the serine protease that will exacerbate autonomic imbalance and mast cell symptoms while those of Kapferer-Seebacher *et al.* [6] cause decreased collagen synthesis, gum integrity, and resistance to periodontitis. Interference with the bone morphogenetic protein motif in CUB domains, a protein known to be involved in dental and bone development, may also favor bone loss as occurred in our patient.

5. Conclusion

With other complications of EDS, periodontitis has a higher frequency (6-fold), earlier appearance (by 12 years in females), and correlation with specific gene changes like that in complement C1R found by genomic analysis [6]. Optimizing insights from EDS into the more common and multifactorial oromandibular diseases that affect 46% of the US population [14] requires appreciation of a common arthritis-adrenaline disorder category rather than focus on rare and extreme EDS types.

Conflicts of Interest

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

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