Atypical variants in *COL1A1* and *COL3A1* associated with classical and vascular Ehlers-Danlos syndrome overlap phenotypes: expanding the clinical phenotype based on additional case reports

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ABSTRACT

The vast majority of reported (likely) pathogenic missense variants in the genes coding for the fibrillar collagens leads to the substitution of one of the obligatory glycine residues in the Gly-Xaa-Yaa repeat sequence of the triple helical domain. Their phenotypic consequences and deleterious effects have been well-documented. However, with increasing access to molecular diagnostic testing based on next-generation sequencing techniques, such as sequencing of multi-gene panels and whole-exome sequencing, non-glycine substitutions are more frequently identified in individuals suspected to have a heritable collagen disorder, but their pathogenic effect is often difficult to predict. Some specific non-glycine substitutions in the proα1(I)- (p.(Arg312Cys)) and proα1(III)- (glutamic acid to lysine at different positions) collagen chain have been identified in a number of individuals presenting a phenotype showing features of both classical and vascular Ehlers-Danlos syndrome. The number of reported individuals with these defects is currently very low, and several of these non-glycine substitutions had initially been categorised as variants of unknown significance (VUS), complicating early diagnosis, accurate counselling, management guidelines, and correct classification. This collaborative study reports on the phenotype of 22 and 7 individuals harbouring these rare variants in COL1A1 and COL3A1, respectively, expanding our knowledge on clinical presentation, phenotypic variability, and natural history, and informing on the risk for potentially life-threatening events, such as vascular, gastro-intestinal, and pregnancy-related complications.

Introduction

The Ehlers-Danlos syndromes (EDS) are a clinically and genetically heterogeneous group of heritable connective tissue disorders that share several features, including generalised joint hypermobility, soft and hyperextensible skin, and various degrees of general tissue fragility affecting skin, blood vessels, ligaments, and internal organs. Disease-causing variants have been identified in multiple genes with diverse biological functions linked to fibrillar collagen biosynthesis, fibril formation and organisation, all leading to structural and functional alterations in the extracellular matrix (ECM) (1). The 1997 Villefranche classification (2), which was used for over 20 years, described six EDS types, including the classical, vascular, hypermobility, kyphoscoliotic, arthrochalasis, and dermatosparaxis types. At the time, biochemical or genetic alterations affecting fibrillar collagens (type I, III, or V), or collagen-modifying enzymes (a disintegrin and metalloproteinase type 2 [ADAMTS2] and lysyl hydroxylase 1 [LH1]) had been identified in individuals affected by these EDS types. Advances in molecular technologies identified several additional genes associated with distinct EDS phenotypes, leading to the publication of a revised EDS classification in 2017. This classification now recognises 13 distinct clinical EDS types, for which major and minor clinical diagnostic criteria were deline-

Table I. Clinical criteria for cEDS and vEDS according to the 2017 International Classification of the Ehlers-Danlos syndromes (3).

cEDS	vEDS
Major clinical criteria	
Skin hyperextensibility and atrophic scarring	Family history of vEDS with documented causative variant in *COL3A1*
Generalised joint hypermobility	Arterial rupture at a young age
	Spontaneous sigmoid colon perforation in the absence of known diverticular disease or other bowel pathology
	Uterine rupture during the third trimester in the absence of previous C-section and/or severe peripartum perineum tears
	Carotid-cavernous sinus fistula formation in the absence of trauma
Minor clinical criteria	
Easy bruising	Bruising unrelated to identified trauma and/or in unusual sites such as cheeks and back
Soft, doughy skin	Thin, translucent skin with increased venous visibility
Skin fragility (or traumatic splitting)	Characteristic facial appearance (large eyes, periorbital pigmentation, small chin, sunken cheeks, thin nose and lips and lobeless ears)
Molluscoid pseudotumours	Spontaneous pneumothorax
Subcutaneous spheroids	Acrogeria
Hernia (or history thereof)	Talipes equinovarus
Epicanthal folds	Congenital hip dislocation
Complications of joint hypermobility (*e.g.* sprains, luxation/subluxation, pain, flexible flatfoot)	Hypermobility of small joints
Family history of a first degree relative who meets clinical criteria	Tendon and muscle rupture
	Keratoconus
	Gingival recession and gingival fragility Early onset varicose veins (under age 30 and nulliparous if female)

ated (3). In view of important clinical overlap observed between the different EDS types and locus heterogeneity for some of these types, a definite diagnosis relies on identification of a disease-causing variant in one of the causative genes, except for the hypermobile type of EDS, which remains molecularly unexplained (3).

The most prevalent, molecularly defined types of EDS, the classical (cEDS; MIM #130000 and #130010) and the vascular type (vEDS; MIM #130050), are both autosomal dominant conditions, caused by alterations in fibrillar collagens, more specifically type V collagen (encoded by *COL5A1* and *COL5A2*) for cEDS, and type III collagen (encoded by *COL3A1*) for vEDS. Fibrillar collagens are trimeric proteins consisting of three left-handed intertwining genetically distinct (heterotrimeric) or identical (homotrimeric) polypeptide chains, referred to as proα-chains (4). These proα-chains contain a long uninterrupted triple helical domain, which consists of repeating Gly-Xaa-Yaa triplets, where glycine (Gly) is the only residue small enough to reside in the sterically restricted inner aspect of the helix. The Xaa-position is most frequently occupied by proline (Pro) and the Y-position by hydroxyproline (Hyp), and these Gly-Pro-Hyp triplets largely contribute to the stabilisation of the type I collagen triple helix. Arginine (Arg) is the second most common amino acid present in the Y-position of the Gly-Xaa-Yaa triplet, where it is known to also have a stabilising effect on the triple helix (5, 6). This helical domain is flanked by globular amino- (N) and carboxy-(C) terminal domains, called propeptides, which are cleaved off by ADAMTS2 and bone morphogenic protein/mammalian tolloid metalloproteinase (BMP-1/mTLD), respectively, resulting in the formation of a mature collagen molecule that can then assemble into highly ordered cross-striated fibrils and fibres (7).

cEDS is hallmarked by generalised joint hypermobility, skin hyperextensibility and fragility with delayed wound healing and formation of atrophic scars, easy bruising, and other signs of connective tissue fragility (Table I), but with extensive inter- and intrafamilial variability (8). It is mainly caused by pathogenic variants in *COL5A1* that introduce a premature termination codon and lead to nonsense-mediated decay of the mutant allele, resulting in a reduced

Table II. Overview of the phenotypic features of the 22 individuals from this report harbouring the *COL1A1* c.934c>A, p.(Arg312Cys).

Family ID	**F1**	**F2**	**F3**	**F3**	**F3**	**F4**	**F4**	**F4**	**F5**
Individual ID	II-1	II-4	IV-3	III-3	IV-4	IV-2	III-5	V-6	II-1
Gender	F	M	F	M	F	F	M	F	F
Ethnicity (country)	European (Belgium)	European (Belgium)		European (Belgium)			European (Canada)		Caucasian (Italy)
Age in report	28	27	27	55	29	44	62	21	12
Relation to the proband				father	sister		paternal uncle	paternal niece (daughter of IV-4)	
					Skin				
Soft, doughy texture	+	+	+	+	-	+	+	+	+
Skin hyperextensibility	+	+	(+)	(+)	-	+	+	+	+
Skin fragility	+	+	+	+	+	+	+	+	+
Atrophic scarring	+	+	+	-	+	+	+	+	+
Translucent skin	U	+ (thoracic)	+ (thoracic)	U	-	U	-	+	-
					Musculoskeletal				
Generalised joint hypermobility	+ (Beighton 9/9)	+ (Beighton score unknown)	+ (Beighton 5/9)	+ (Beighton 4/9)	+ (Beighton 6/9)	+ (Beighton 6/9)	- (Beighton score 0/9, historical JH, distal JH)	+ (Beighton 6/9, distal JH)	+ (Beighton 9/9)
Joint (sub)luxations	(+) (patella)	-	(+) (temporomandibular subluxations)	-	-	U	-	+ (right shoulder, right elbow, both hips, right TMJ)	+ (subluxations of digits)
Scoliosis	-	+ (moderate)	-	-	-	U	-	-	-
Pectus demformity	-	+ (excavatum)	-	-	-	+ (excavatum)	(+) (excavatum)	-	-
Hand/foot deformities	pes planus	-	-	-	pes planus	U	pes planus	pes planus	pes planus
Recurrent fractures	-	-	-	-	-	-	+ (right femoral fracture identified at total knee replacement, fractures after significant trauma)	-	-
					Cardiovascular				
Structural defects	-	-	-	-	-	-	U	U	-
Valvular abnormalities	-	-	-	-	-	-	U	U	-
Vascular abnormalities/ complications	-	-	-	-	-	spontaneous intradural vertebral artery dissection with subarachnoid and intraventricular haemorrhage age 44 years	-	U	-
Easy bruising/ haematoma	+	+	+	+	-	U	+ (haematoma which split overlying skin)	+ (skin graft after haematoma)	+
Varicose veins	-	+	-	-	-	U	+	-	-
					Miscellaneous				
Motoric developmental delay	(+)	-	-	-	-	U	-	-	-
Preterm birth	-	-	-	-	-	U	-	-	-
Pneumothorax	-	-	-	-	-	+ (spontaneous age 17 years)	-	-	-
Gastro-intestinal ruptures	-	-	-	-	-	-	-	-	-
Abdominal wall herniations	-	-	-	-	-	-	+ (bilateral inguinal and umbilical)	-	+ (inguinal)
Facial features	palpebral skin redundancy	-	-	-	Retrognathia	U	infraorbital redundant skin folds	grey sclerae	epicanthal folds, palpebral skin redundancy, light blue sclerae, lingual frenulum hypoplasia
Aged appearance	-	-	-	+	-	U	-	-	-
Females: pregnancy-related complications	nulliparous	NA	nulliparous	NA	nulliparous	3 pregnancies with perineal tearing		nulliparous	nulliparous
Other clinical manifestations	-	-	back pain, functional gastro-intestinal complaints, fatigue	Achilles tendon rupture, back pain, functional gastro-intestinal complaints	muscular pain, fatigue	abnormal striae, multiple sclerosis	piezogenic papulae, left rhegmatogenous retinal detachment	piezogenic papulae, skin graft harvest site with severe fibrosis.	mild muscle hypotonia

Family ID	**F6**	**F6**	**F6**	**F6**	**F6**	**F6**	**F6**	**F6**	**F6**	**F6**	**F7**	**F7**	**F7**
Individual ID	II-3	II-4	III-6	II-6	III-7	III-8	II-1	III-1	III-2	II-2	III-1	II-1	II-2
Gender	M	M	M	F	F	M	M	F	F	F	F	F	F
Ethnicity (country)	European (Italy)												European (Italy)
Age in report	62	60	25	51	18	13	58	23	18	55	16	54	55
Relation to the proband		brother	nephew (son of II-4)	sister	niece (daughter of II-6)	nephew (son of II-6)	paternal half-brother	niece (daughter of II-1)	niece (daughter of II-1)	paternal half-sister		mother	maternal aunt

Skin													
Soft, doughy texture	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin hyperextensibility	+	+	+	+	+	+	+	+	+	-	-	(+)	+
Skin fragility	+	+	+	+	+	+	+	+	+ (historical)	-	-	-	-
Atrophic scarring	+	+	+	+	+	+	+	+	+	+	+	+	+
Translucent skin	-	-	-	-	-	-	-	-	-	-	+	+	+
Musculoskeletal													
Generalised joint hypermobility	+ (Beighton 6/9, distal JH)	+ (Beighton 4/9, distal JH)	- (Beighton 4/9, distal JH)	+ (Beighton 6/9, distal JH)	+(Beighton 9/9, distal JH)	+ (Beighton 6/9, distal JH)	+ (Beighton 5/9)	+ (Beighton 6/9, distal joint hypermobility)	+ (Beighton 6/9, distal JH)	- (Beighton 0/9)	+ (Beighton 6/9)	+ (Beighton score 4/9)	+ (Beighton score 4/9)
Joint (sub)luxations	+ (digits, shoulders)	-	-	+ (shoulders, digits, knees)	+ (left shoulder subluxations)	-	+ (sporadic)	-	-	-	-	-	-
Scoliosis	+ (moderate)	+ (mild)	-	+ (treated with corset)	-	-	+	+ (surgically treated)	+ (mild)	-	lumbar hyper lordosis with mild left lumbar gibbus	-	-
Pectus demformity	+ (mild pectus excavatum)	-	-	-	-	-	-	-	-	-	-	-	-
Hand/foot deformities	left hallux valgus, pes planus	hallux valgus, pes planus	hallux valgus, pes planus	hallux valgus, pes panus	pes planus	pes planus	pes planus, hallux valgus	pes planus, sandal gap	-	ples planus	-	-	-
Recurrent fractures	- (osteopenia)	- (osteoporosis)	-	-	-	-	- (osteoporosis)	-	-	-	-	-	-
Cardiovascular													
Structural defects	left ventricular wall thickening	-	-	-	-	-	-	-	-	-	-	-	-
Valvular abnormalities	mild MV and AV, regurgitation, MV prolapse	MV regurgitation, MV prolapse	mild MV regurgitation	MV prolapse	-	-	mild MV + AV regurgitation, MV prolapse	-	-	MV prolapse	-	-	-
Vascular abnormalities/ complications	AR dilation, vertebral artery tortuosity, hepatic haemangioma	AR dilation and AA dilation	-	-	-	-	-	-	-	-	SMA and ileocolic artery aneurysm (embolisation aged 12)	SMA aneurysm	-
Easy bruising/ haematoma	+ (multiple haematomas)	+ (multiple haematomas)	+ (multiple haematomas)	+ (multiple haematomas)	+ (multiple haematomas)	+	+	+ (multiple haematomas)	+	+	+	+	+
Varicose veins	+	+	-	+	-	-	+	-	-	-	-	+	(+)
Miscellaneous													
Motoric developmental delay	- (moderate hypotonia at birth)	-	-	-	-	-	-	(+)	-	-	-	-	-
Preterm birth	-	-	-	-	-	-	-	-	-	-	-	-	-
Pneumothorax	-	-	-	-	-	-	-	-	-	-	-	-	-
Gastro-intestinal ruptures	-	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal wall herniations	+ (inguinal)	-	-	-	-	+ (bilateral inguinal)	-	-	-	-	-	-	-
Facial features	light blue sclerae, high palate, hypoplastic uvula	light blue sclerae, high palate	light blue sclerae, high palate, dental crowding	blepharochalasis, palpebral ptosis, light blue sclerae, high palate, dental crowding	light blue sclerae, downslanting palpebral fissures, elongated philtrum	deep set eyes, light blue sclerae, high palate, dental crowding	high palate, dental crowding	light blue sclerae, short philtrum, high palate	light blue sclerae	downslanting palpebral fissures, blepharoptosis	-	palpebral skin redundancy, downslanting palpebral fissures, prominent chin, high palate, lingual frenulum hypoplasia, light blue sclerae	palpebral skin redundancy, light blue sclerae
Aged appearance	-	-	-	-	-	-	-	-	-	-	-	+	(+)
Females: pregnancy-related complications	NA	NA	NA	- (2 pregnancies)	nulliparous	NA	NA	nulliparous	nulliparous	nulliparous	nulliparous	perineal tearing, threats of abortion and early membrane rupture, poor response to epidural analgesia	- (2 pregnancies)
Other clinical manifestations	molluscoid pseudotumours, subcutaneous spheroids, chronic pain and fatigue, recurrent inflammatory soft tissue lesions, hiatal hernia, constipation, lymphedema, motor dyspraxia	subcutaneous spheroids, striae distensae, gingival bleeding, chronic articular pain, chronic fatigue, inflammatory soft tissue lesions, multiple disc hernias, food intolerances	subcutaneous spheroids, sprains, inflammatory soft tissue lesions	subcutaneous spheroids, hyperkeratosis of extensor surfaces, striae distensae since young age, chronic pain, asthenia	striae distensae at young age, sporadic sprains	molluscoid pseudotumour, sporadic sprains	molluscoid pseudotumours, chronic articular pain, recurrent inflammatory soft tissue lesions, disc hernias, hiatal hernia	striae distensae at young age, recurrent gingival inflammation/ bleeding, recurrent sprains and pain, muscle cramps, easy fatigability, asthenia, impaired concentration	temporo-mandibular joint dysfunction, recurrent pain at legs and shoulders,	diffuse spondylosis along all the lumbar metameres, sacral Tarlov's cysts	keratosis pilaris	musculo-skeletal pain, lymphoedema, pelvic varicocele, Interstitial lung disease	recurrent musculo-skeletal pain

+: present; (+) mildly present; -: absent; U: unknown; F: female; M: male; JH: joint hypermobility; TMJ: temporomandibular joint; MV: mitral valve; AV: aortic valve; AR: aortic root; AA: ascending aorta; SMA: superior mesenteric artery; NA: not applicable.

Table III. Overview of the phenotypic features of the individuals from this report harbouring glutamic acid to lysine substitutions in the proα1(III)-collagen chain.

Family ID	F8	F9	F10	F11	F12	F13	F13	F13	F14
Individual ID	II-4	III-2	II-2	II-I	III-6	III-2	III-3	II-2	III-5
Gender	F	M	F	M	F	M	M	M	
Ethnicity (country)	European (Turkey)	European (Belgium)	European (Belgium)	Native American	European (Italy)	European (France)	European (France)	European (France)	European (France)
Age in report	56	29	49	31	75	16	18	47	48
Pathogenic variant									
c-notation	c.2110G>A	c.2791G>A	c.1387G>A	c.2791G>A	c.1342G>A	c.2791G>A	Presence of variant to be confirmed		c.1351 G>A
p-notation	p.(Glu704Lys)	p.(Glu931Lys)	p.(Glu463Lys)	p.(Glu931Lys)	p.(Glu448Lys)	p.(Glu931Lys)			p.(Glu451Lys)
Exon	30	39	20	39	19	39			20
Skin									
Soft, doughy texture	+	+	+	+	+	+	+	+	+
Skin hyperextensibility	+	+	+	+	+	+	+	+	+
Skin fragility	+	+	+	-	+	+	-	-	+
Atrophic scarring	+	+	-	+	+	+	+	+	+
Translucent skin	-	+ (thoracal)	+	-	-	U	-	-	+
Musculoskeletal									
Generalised joint hypermobility	+ (Beighton 9/9, distal JH)	+	+ (Beighton 7/9, distal JH)	+ (Beighton 5/9, distal JH)	Historical (Beighton 2/9, distal JH)	U	+ (Beighton 7/9)	U	- (Beighton 4/9, distal JH)
Joint (sub)luxations	+ (during childhood)	+	-	-	+ (small joints and shoulders)	-	(+) (maxillary subluxation)	U	+ (AC-joint (post traumatic))
Scoliosis	-	-	-	-	+ (moderate)	-	+ (mild)	U	-
Pectus deformity	-	-	-	-	-	-	-	U	-
Hand/foot deformities	Pes planus	-	-	pes planus	hallux valgus, pes cavus	pes planus	pes planus	U	-
Recurrent fractures	-	-	-	-	+ (osteopenia)	-	-	-	-
Cardiovascular									
Structural defects	-	-	-	-	-	-	-	-	-
Valvular abnormalities	-	-	-	-	mild MV prolapse	-	MV prolapse	-	-
Vascular abnormalities/ complications	-	-	-	-	-	-	-	-	+ (left internal iliac artery aneurysm, right renal artery dissection)
Easy bruising/ haematoma	+	+	+	+	+	+	-	+ (haematoma requiring drainage)	+ (multiple haematomas, 2x requiring drainage)
Varicose veins	+	U	U	-	+	U	-	-	U
Miscellaneous									
Motoric developmental delay	-	-	-	-	-	-	-	-	-
Preterm birth	-	-	-	-	-	-	-	U	-
Pneumothorax	-	-	-	-	-	-	-	-	-
Gastro-intestinal ruptures	-	-	-	-	-	-	-	-	+ (spontaneous sigmoid colon diverticula perforation age 41)
Abdominal wall herniations	-	-	-	-	-	-	-	+ (bilateral inguinal)	+ (inguinal and incisional)
Facial features	prominent eyes, palpebral skin redundancy	palpebral skin redundancy	prominent eyes	-	mild hypertelorism, light blue sclerae	-	-	-	features evocative of cEDS and vEDS
Aged appearance	+	-	-	-	+	-	-	-	-
Females: pregnancy-related complications	- (2 pregnancies)	NA	multiple miscarriages	NA	- (2 pregnancies)	NA	NA	NA	NA
Other	chronic fatigue, widespread pain, poor dental quality	poor dental quality	chronic widespread pain		chronic fatigue, widespread pain, non-parkinsonian movements disorder, gingivitis, early	-	episode of melena	diverti-culosis	gum bleeding

+: present; (+) mildly present; -: absent; U: unknown; F: Female; M: male; JH: joint hypermobility; AC-joint: acromioclavicular joint; MV: mitral valve; NA: not applicable.

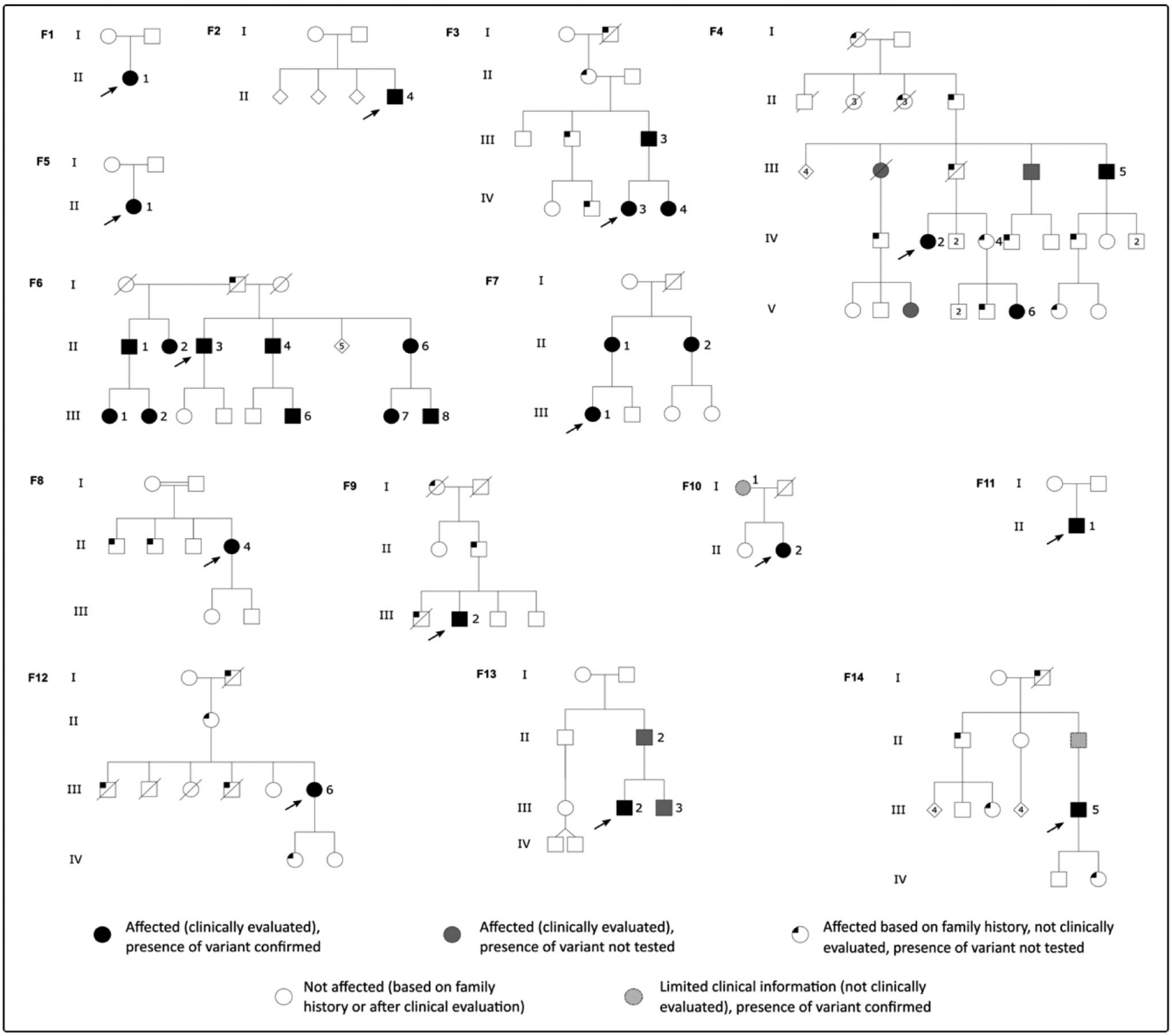

Fig. 1. Pedigrees of the families harbouring the *COL1A1* c.934C>T, p.(Arg312Cys) variant (F1-F7) and the families with glutamic acid to lysine substitutions in *COL3A1* (F8-F14).

amount of type V collagen in the ECM. Less frequently, it is caused by disease-causing variants in *COL5A1* or *COL5A2* that either lead to in-frame skipping of one or more exons or to the substitution of one of the glycine residues in the canonical helical triplet repeats (Gly-Xaa-Yaa) by a bulkier amino acid, thereby disrupting the helical structure (1). vEDS is mainly characterised by arterial fragility, with a strongly increased risk for life-threatening aneurysms, dissections and/or ruptures of medium-sized and large arteries, and ruptures of the gastro-intestinal tract, the gravid uterus, and sometimes other internal organs such as liver or spleen. The skin is thin with striking translucency, easy bruising, and formation of premature varicose veins. Additionally, individuals with vEDS often present with distal joint hypermobility and a characteristic facial appearance (Table I) (3). For type III collagen, most pathogenic alterations (about two thirds) result in the substitution of a canonical helical glycine residue by another amino acid. Other pathogenic variants include splice site variants that result in in-frame exon skipping, short in-frame deletions or insertions, and rarely variants that introduce a premature termination codon (9). Substitutions of helical glycine residues are also frequently found in the genes coding for type I procollagen (*COL1A1* and *COL1A2)*, the most abundant fibrillar collagen in the human body. These usually cause osteogenesis imperfecta (OI), another heritable connective tissue disorder that is mainly characterised by increased bone fragility and short stature; in a small number of individuals, helical *COL1A1* or *COL1A2* glycine substitutions result in an OI/EDS overlap phenotype (10, 11). Whereas the pathogenicity of glycine substitutions in the helical Gly-Xaa-Yaa repeat sequence of type I (OI and OI/EDS), type III collagen (vEDS) and type V (cEDS) is well-established, the pathogenic effect of non-glycine substi-

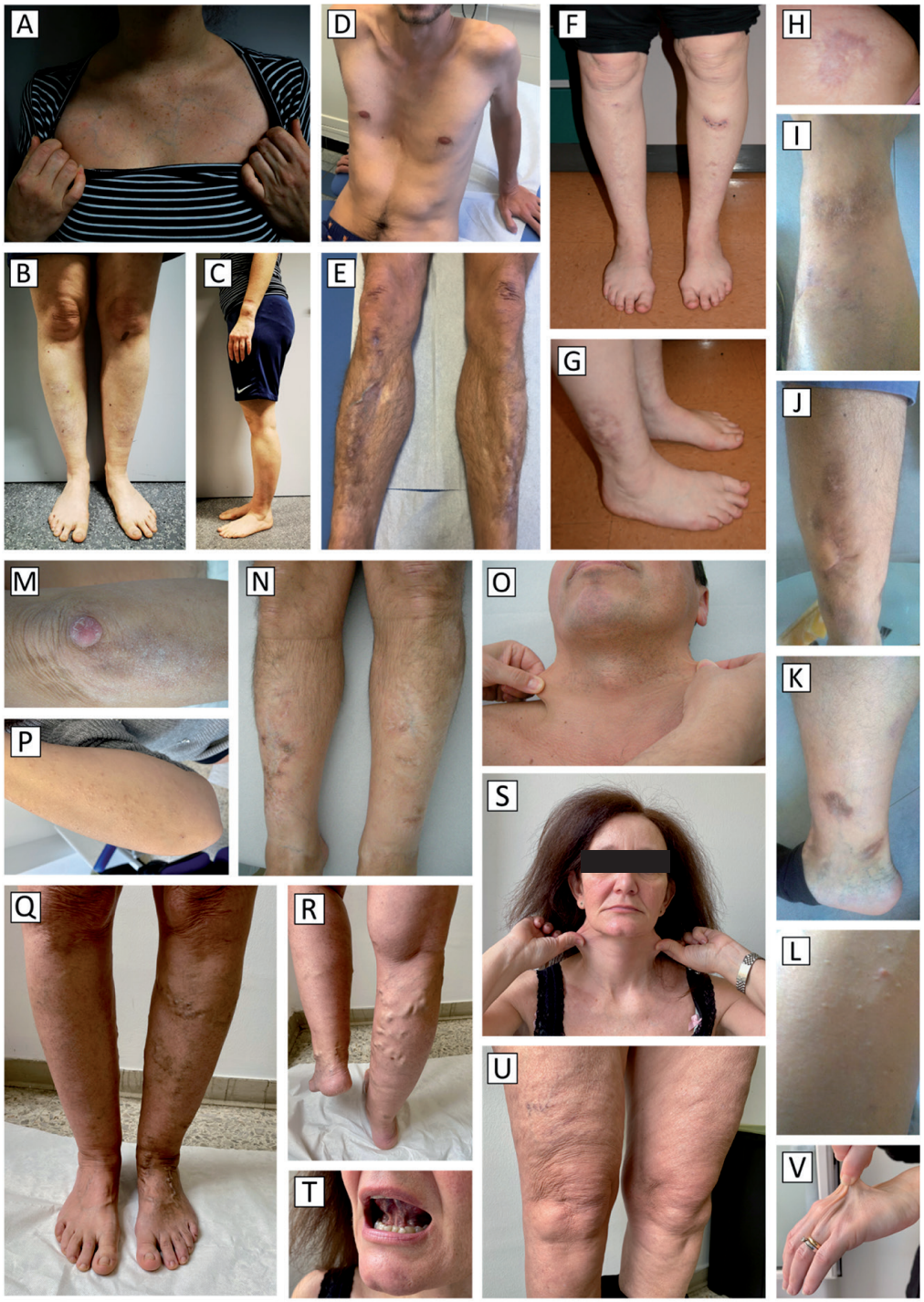

Fig. 2. F3 IV-3 presenting thoracal skin translucency (A) and few small atrophic scars on the knees and shins (B), but no manifest knee hyperextensibility nor pedes plani (C). F2 II-4 with thoracal skin translucency and pectus excavatum (D), and varicose veins, atrophic scars and haemosiderotic plaques at the lower limbs (E). F4 V-6 with multiple atrophic scars (F - G) and fibrosis at a skin graft side (H). F6 II-4 with haemosiderin depots, increased vascular visibility (I,K), atrophic scars (J) and subcutaneous spheroids (L). F6 II-3 with a molluscoid pseudotumour (M), haemosiderin depots, atrophic scars (N) and skin hyperextensibility (O). F7 II-1 with keratosis pilaris (P), severe varicose veins of the lower legs (Q,R), redundant skin at the knees (Q), mild skin hyperextensibility, downslanting palpebral fissures, light blue sclerae, aged appearance (S) and hypoplasia of the lingual frenulum (T). F7 II-2 with mild varicose veins, redundant skin at the knees (U) and mild skin hyperextensibility (V).

tutions in these molecules is often harder to predict. Through increased molecular sequencing, in combination with accurate clinical phenotyping, several non-glycine substitutions in type I and III collagens have been linked to phenotypes overlapping with classical and vascular EDS. These mainly include a specific arginine to cystine (Arg-to-Cys) substitution in the proα1(I)-collagen chain, *COL1A1* c.934C>T, p.(Arg312Cys) (12-18) and a series of glutamic acid to lysine (Glu-to-Lys) substitutions in the proα1(III)-collagen chain (at positions 241, 682 and 1171) (19). The number of individuals affected by these conditions is currently still small, hampering our knowledge about the phenotypic variability, natural history, and prognosis associated with these variants. This limited knowledge presumably also delays diagnosis for affected individuals, and the overlapping phenotypes complicate their classification, which may lead to confusion amongst affected families and their health care professionals.

The purpose of this collaborative study is to report on the clinical phenotype of novel and previously reported individuals harbouring these rare *COL1A1* and *COL3A1* variants, to expand the knowledge on these conditions. These data

will allow to better classify these rare conditions within the EDS spectrum and formulate patient-tailored management guidelines.

Case descriptions

Subjects selected for the study were evaluated in the Center for Medical Genetics Ghent or within a Medical/Clinical Genetics department in Italy, France, or Canada. Relatives were only included when they were clinically evaluated at a clinical genetics department. Informed consent was obtained from all included individuals. A summary of the clinical manifestations of the reported individuals can be found in Table II and Table III and the pedigrees of the included families are depicted in Figure 1.

Family 1

The proband (F1 II-1) was previously reported by Nuytinck *et al*. (17) at age 5 years. At the time, the girl was clinically suspected to have cEDS based on a history of easy bruising, a soft, velvety, and hyperextensible skin and presence of atrophic scars, but molecular testing revealed the presence of a *de novo COL1A1* c.934C>T, p.(Arg312Cys) variant. Follow-up clinical evaluation at age 28 years revealed multiple dilated and atrophic scars on the face, elbows, knees, and shins, pedes plani and generalised joint hypermobility (Beighton score 9/9). She suffered twice from patellar dislocation. Her facial appearance included redundant skin folds on the eyelids but no other dysmorphic features. She did not report chronic pain and no major vascular complications had occurred. Magnetic Resonance Angiography (MRA) head-to-pelvis did not show aneurysms nor arterial tortuosity, and echocardiographic investigations were also normal.

Family 2

The proband of family 2 (F2 II-4; Fig. 2 D-E) was also published by Nuytinck *et al*. (17) when he was 7 years old. Like individual F1 II-1, he was diagnosed with cEDS based on very similar features, and molecular investigations revealed the *de novo COL1A1* c.934C>T, p.(Arg312Cys) variant. Follow-up examination at 27 years revealed a soft, doughy and hyperextensible skin with multiple atrophic scars on knees and shins, and increased skin translucency. His skin remained fragile with delayed and problematic wound healing, easy bruising, and severe varicose veins at the lower limbs. There was generalised joint hypermobility (Beighton score unknown), and severe pectus excavatum. He did not report any musculoskeletal complaints nor any major cardiovascular complications. MRA head-to-pelvis at 26 years revealed no vascular abnormalities.

Family 3

A Belgian woman (F3 IV-3; Fig. 2 A-C) presented at the age of 24 years with a history of skin fragility and problematic wound healing. Clinical examination revealed a soft, doughy, and mildly hyperextensible skin with increased thoracic vascular visibility and presence of multiple small atrophic scars. Generalised joint hypermobility (Beighton score 5/9) was present and she suffered from recurrent mandibular subluxations and periodic low back pain. She also reported easy bruising.

Her father (F3 III-3) and sister (F3 IV-4) were also clinically evaluated and presented a similar phenotype. Her father (F3 III-3) reported skin fragility during childhood and skin bruising with spontaneous ecchymoses. Clinical examination at 56 years revealed a soft, doughy, and mildly hyperextensible skin without atrophic scars. His Beighton score was 4/9 and he reported no joint dislocations. He suffered from musculoskeletal pain and his medical history included a traumatic Achilles tendon rupture. Her sister (F3 IV-4), age 29 years at the last clinical evaluation, reported increased skin fragility with presence of discolored scars but her skin was not soft, doughy, nor hyperextensible, and she did not report easy bruising. She presented a mild scoliosis, severe pedes plani and generalised joint hypermobility (Beighton score 6/9) without recurrent dislocations. She also complained of muscular pain and fatigue. None of these three relatives had any major cardiovascular complications and MRA revealed no vascular anomalies. Targeted sequencing of an EDS gene panel revealed the presence of the *COL1A1* c.934C>T, p.(Arg312Cys) variant in the proband, her father and her sister.

Family 4

At the age of 44 years, the proband of family 4 (F4 IV-2) was diagnosed with a subarachnoid and intraventricular haemorrhage. Computed tomography angiography also demonstrated a growing pseudoaneurysm from a spontaneous intradural vertebral artery dissection and fibromuscular dysplasia in her vertebral and carotid arteries. (Fig. 3). She underwent an uncomplicated endovascular vertebral artery sacrifice and recovered well. On clinical exam she was noted to have generalised joint hypermobility (Beighton score 6/9), hyperextensible skin with abnormal scars, abnormal striae on her abdomen and knee, severe pectus excavatum (Fig. 3) and flat feet. Skeletal radiographies revealed protrusio acetabuli and kyphoscoliosis. Further cardiovascular imaging revealed normal diameters of the aortic root and ascending aorta but showed a dilated ovarian vein. Her medical history also included a spontaneous pneumothorax at age 17 and she was recently diagnosed with multiple sclerosis. She had three successful pregnancies with perineal tearing but no post-partum haemorrhage.

Genetic testing revealed the *COL1A1* c.934C>T, p.(Arg312Cys) variant. The family history revealed a history of EDS on her paternal side and presence of the variant was confirmed in two relatives, a paternal uncle (F4 III-5) and a niece (F4 V-6, Fig. 2 F-H). Both had a similar skin phenotype including hyperextensibility, a soft and doughy aspect, fragility, and problematic wound healing with multiple atrophic scars. In addition, both suffered from large haematomas with splitting of the skin after a haematoma in III-5 and the need for a skin graft after a large haematoma with fibrosis at graft side in V-6. Skin translucency was not present in III-5 but was in V-6. At 62 years, III-2 did not display generalised joint hypermobility (Beighton 0/9, historical joint hypermobility) and a Beighton score

of 6/9 was noted in V-6 who also suffered from recurrent dislocations. III-5 was further noted to have a mild pectus excavatum, varicose veins, infra-orbital redundant skin folds and had a history of bilateral inguinal and umbilical hernia. V-6 suffered from functional bowel disorder with constipation, presented grey sclerae and had no history of pregnancies.

Family 5

An Italian girl (F5 II-1) was clinically diagnosed with cEDS at the age of eight years because of the presence of the typical cutaneous and articular signs, and physical examination at age 12 years indeed revealed a soft, doughy, and hyperextensible skin with multiple haemosiderotic and widened atrophic scars (especially at pretibial area, knees, and elbows). She also presented generalised joint hypermobility (Beighton score 9/9) with complications of joint hypermobility including pedes plani and sporadic subluxations of the digits. Furthermore, clumsiness in infancy, mild muscle hypotonia, and a surgically treated inguinal hernia was referred. Facial features included epicanthal folds, infraorbital creases, palpebral skin redundancy, light blue sclerae and absence of lingual frenulum. There was pronounced easy bruising of the skin, but there were no major vascular complications and echocardiographic investigations were normal. Molecular analysis by a multigene panel including the EDS-associated genes revealed the *de novo COL1A1* c.934C>T, p.(Arg312Cys) variant.

Family 6

The proband (F6 II-3, Fig. 2 M-O) was previously reported by Ritelli *et al*. 2013 (18) when he was 53 years old. The man was clinically suspected to have cEDS and molecular testing by Sanger sequencing of *COL5A1*, *COL5A2,* and *COL1A1* revealed the *COL1A1* c.934C>T, p.(Arg312Cys) variant. Follow-up clinical evaluation at age 62 years confirmed the marked skin involvement with soft, doughy texture, hyperextensibility, skin fragility with delayed wound healing and multiple, widened, atrophic and haemosiderotic scars. Further mucocutaneous signs included molluscoid pseudotumours, subcutaneous spheroids, piezogenic papulae, hypoplastic uvula, light blue sclerae, and xerosis. The Beighton score remained unchanged (6/9) with only sporadic joint dislocations. Dual-Energy X-ray Absorptiometry (DEXA) and Magnetic Resonance Imaging (MRI) revealed multiple disc hernias and moderate osteopenia. Further musculoskeletal features comprised pectus excavatum, severe left hallux valgus, bilateral pedes plani, and high arched palate. No major vascular complications have been occurred and yearly cardiovascular surveillance showed mild mitral and aortic regurgitation, mitral valve prolapse, left ventricular wall thickening, slow progressive aortic root dilatation (from 38.6

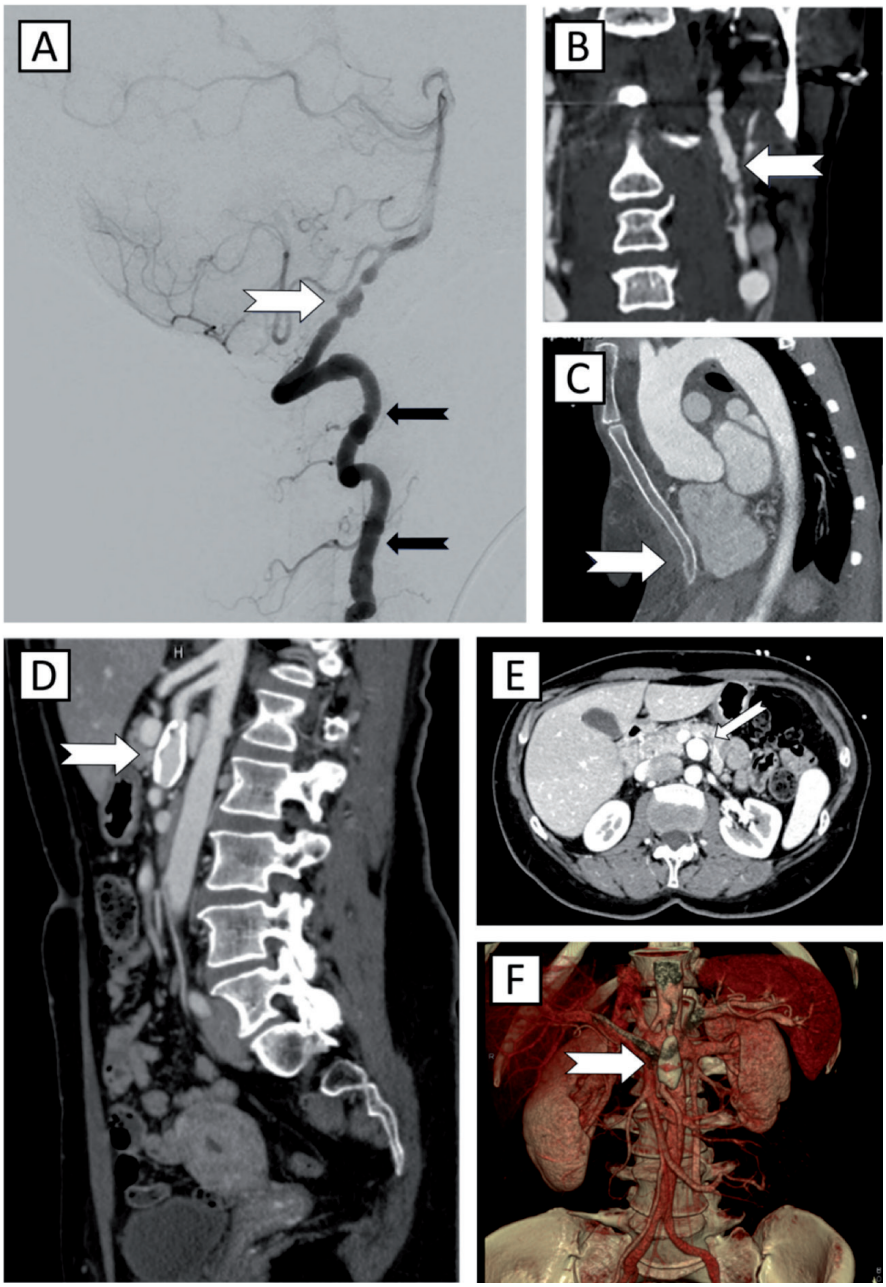

Fig. 3. F4 IV-2 with (A) a dissecting growing pseudoaneurysm on digital subtraction angiography of the left vertebral artery in sagittal (white arrow) and presence of corrugated vessel wall indicative of fibromuscular dysplasia (black arrows), coronal CT angiography (B) showing similar corrugated appearance of the left internal carotid artery and sagittal CT angiography (C) demonstrating pectus excavatum. F7 II-1 with a superior mesenteric artery aneurysm (transverse diameter 14 mm; longitudinal diameter 36 mm) on CT angiography (D,E,F).

mm to 42.8 mm), and uncomplicated vertebral artery tortuosity. Additional issues included multiple haematomas, varicose veins, gastroesophageal reflux (hiatal hernia), delayed colonic transit, and a surgically treated inguinal hernia. He also reported worsening regarding chronic articular pain, recurrent migraine, sleep disturbances, aggravated motor dyspraxia and recurrent inflammatory soft tissue lesions.

Following the characterisation of the proband, nine additional affected family members were evaluated: a 60-year-old brother (F6 II-4, Fig. 2 I-L) with his 25-year-old son (F6 III-6), a 51-year-old sister (F6 II-6) with her 18-year-old daughter (F6 III-7) and 13-year-old son (F6 III-8), a 58-year-old paternal half-brother (F6 II-1) and his 23-year-old (F6 III-1) and 18-year-old (F6 III-2) daughters, and a 55-year-old paternal half-sister (F6 II-2). All these individuals were molecularly confirmed having the *COL1A1* c.934C>T, p.(Arg312Cys) variant. Among the nine evaluated relatives, all individuals showed similar cutaneous involvement, *i.e*. skin hyperextensibility, multiple atrophic scars and/or easy bruising. Joint hypermobility and its complications were present in all patients except for individual II-2 who had a Beighton score of 0/9 with diffuse painful stiffness. Further peculiar findings in this individual were spondylosis along all lumbar metamers and multiple sacral Tarlov's cysts. Nobody experienced severe complications, but mitral valve prolapse was present in five of the evaluated relatives. The proband's brother (II-4) also showed mitral valve regurgitation with mild haemodynamic involvement, nonprogressive aortic root dilatation (41.7 mm in the last 10 years) and slow progressive ascending aorta dilatation (from 35.6 mm to 41.2 mm). For detailed clinical data of proband's relatives see Table II. In addition, the proband's father, who died at the age of 73 years due to an aortic dissection was neither clinically evaluated nor tested for the familial mutation, but was reported to display a similar phenotype.

Family 7

At 12 years of age, the proband (F7 III-1) of this family was diagnosed with an aneurysm of the distal superior mesenteric (at about 57 mm from the origin) and ileo-colic arteries (transverse diameter 14 mm; longitudinal diameter 18 mm). She presented a soft, doughy and hyperextensible skin, but did not suffer from increased skin fragility. She had some atrophic scars and she reported easy bruising and presence of keratosis pilaris. Musculoskeletal features included generalised, but uncomplicated, joint hypermobility (Beighton score 6/9) and lumbar hyperlordosis with a mild left lumbar gibbus. Targeted genetic panel testing (including *FLT4*; *GJC2*; *VEGFC*; *GLMN*; *RASA1*; *TEK*; *COL3A1*; *COL5A1*; *COL5A2*; *COL1A1*; *COL1A2*; *AEBP1*) was performed and revealed the *COL1A1* c.934C>T, p.(Arg312Cys) variant.

This variant was inherited from her mother (F7 II-1; Fig. 2 P,Q,R,S,T) who displayed severe varicose veins aged 53 years. Her cutaneous features were similar to the probands except from remarkable wrinkling of the skin at knees. She displayed generalised joint hypermobility (Beighton 4/9, age 53). Cardiovascular workup further revealed a superior mesenteric artery aneurysm (transverse diameter 14 mm; longitudinal diameter 36 mm). The presence of the variant was also confirmed in the maternal aunt of the proband (F7 II-2; Fig. 2 U-V) who also displayed a similar phenotype. She had no vascular abnormalities but was reported to suffer from chronic musculoskeletal pain.

Glutamic acid to lysine substitutions in the proα1(III)-collagen chain

Family 8

A woman from Turkish origin (F8 II-4, Fig. 4 A-C), followed at the Center for Medical Genetics in Ghent, received a clinical diagnosis of cEDS at young adulthood, but at the time, molecular analysis was limited to sequencing of *COL5A1* and *COL5A2* and revealed no pathogenic variants. Clinical re-assessment at 56 years revealed facial features most reminiscent of cEDS with a premature aged appearance, facial scars, palpebral skin redundancy, prominent eyes with greyish sclerae, and a fine nose and thin lips (Fig. 4 A). She presented generalised joint hypermobility (Beighton score 9/9), most pronounced at the distal joints and pedes plani. Her skin was remarkably doughy, soft and hyperextensible with a few dilated, atrophic scars on her arm and varicose veins at the lower limbs. At adult age, she suffered from chronic fatigue and widespread pain, and she reported bad dental quality. Medical history was negative for major vascular complications and echocardiographic investigations revealed no abnormalities. She had two full-term pregnancies with neither miscarriages nor any gestational complications. Both her children are healthy. Her parents are first-degree cousins and are reported to be healthy but two of her brothers are also reported to show features of connective tissue fragility, including skin fragility and easy bruising (not clinically or molecularly evaluated). An exome-based connective tissue gene panel revealed the presence of a *COL3A1* c.2110G>A, p.(Glu704Lys) substitution in the proband. This variant has an allele frequency in GnomAD of 3/152122 (0,00001972) (20). Additionally, a heterozygous variant of uncertain significance in *AEBP1* c.794G>A, p.(Arg265Lys) was identified. No second variant in *AEBP1* could be identified. Copy Number Variant (CNV) analysis, *COL5A1* null-allele testing, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of procollagens type I and III were all normal.

Family 9

The proband (F9 III-2) is a man of Belgian descent. He was born at term after an uneventful pregnancy and had a normal psychomotor development. His skin was hyperextensible and bruisable with increased thoracic vascular visibility. He once suffered from a muscle haematoma after minor trauma. Clinical evaluation at age 26 years revealed palpebral skin redundancy and a small chin. He presented generalised joint hypermobility (Beighton score unknown), most pronounced at the distal joints, without dislocations. There were several small atrophic scars at his knees and a haemosiderotic scar at his right lower leg. He also suffered from severe varicose veins at the lower limbs. He

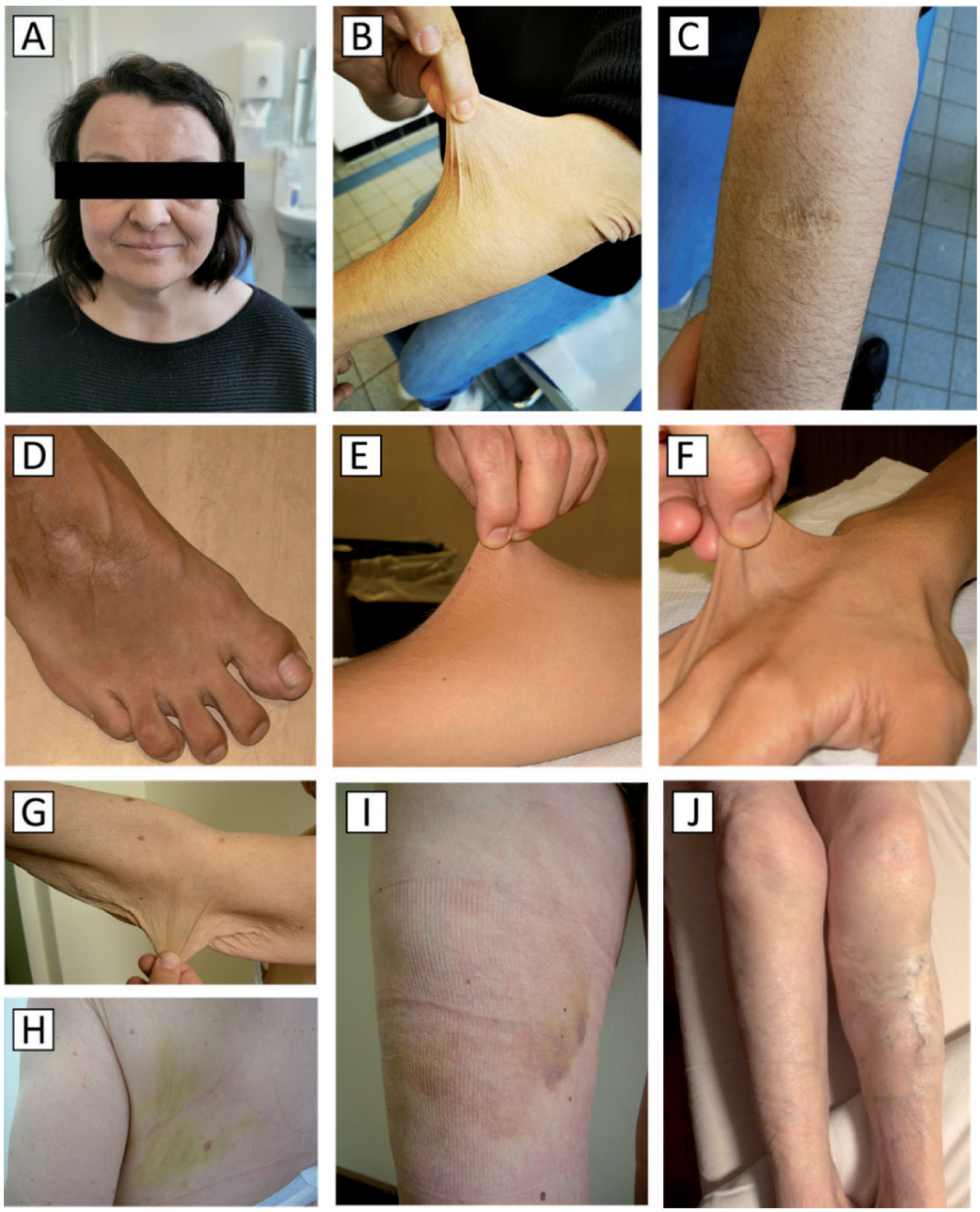

Fig. 4. Clinical pictures of individuals harbouring glutamic acid to lysine substitutions in the proα1(III)-collagen chain. F8 II-2 showing some facial scars and an aged appearance (A), skin hyperextensibility (B) and a dilated atrophic scar (C). F11 II-1 with an atrophic scar on the foot (D) and skin hyperextensibility (E, F). F12 III-6 with skin hyperextensibility (G), bruising (H, I) and varicose veins (J).

suffered from chronic pain and fatigue and reported poor dental quality. Based on the clinical presentation he was suspected to have cEDS. SDS-PAGE of procollagens I and III was within normal ranges and a *COL5A1* null-allele test excluded *COL5A1* haploinsufficiency. NGS sequencing analysis of the EDS gene panel revealed the presence of a c.2791G>A, p.(Glu931Lys) substitution in *COL3A1*. This variant was absent in GnomAD (20). Moreover, a variant of uncertain significance in *COL5A1* c.3370 G>A, p.(Glu1124Lys) was identified. Family history revealed that several relatives on his paternal side shared a similar phenotype. None of the (affected) relatives, however, were available for segregation analysis or clinical evaluation.

A Belgian woman (F10 II-2) presented at age 49 years with recurrent joint dislocations and chronic widespread pain. Her skin was fragile, hyperextensible and had a soft and doughy aspect with remarkable translucency. There were no atrophic scars. She reported severe bruising tendency, and varicose veins at a young age. She presented generalised joint hypermobility (of both distal and proximal joints) with a Beighton score of 7/9. Although she had prominent eyes, she did not show the typical facial features of vEDS, or acrogeria. She had no children but reported to have had four first-trimester miscarriages. There was no history of major vascular complications. Based on her clinical presentation, she was initially suspected to have hypermobile EDS. SDS-PAGE of procollagens I and III was normal and molecular analysis of *COL5A1*, *COL5A2* and *COL3A1* revealed the c.1387G>A, p.(Glu463Lys) variant in *COL3A1* which was absent in GnomAD (20). MRA head-to-pelvis showed no aneurysms nor arterial tortuosity. Segregation analysis also showed

Table IV. Summary of the clinical features of the 35 reported individuals harbouring *COL1A1* c.934C>T, p.(Arg312Cys) and the 27 individuals harbouring glutamic acid to lysine substitutions in the proα1(III)-collagen chain, including the individuals from this report.

	COL1A1 c.934C>T, p.(Arg312Cys)	Glu-to-Lys substitutions in the proα1(III)-collagen chain
Families	12	14
Individuals	35	25
Gender	12M, 23F	14M, 11F
Mean age (years)	35.8	38.9
Skin		
Soft, doughy texture	25/35 (7 U)	17/25 (8 U)
Skin hyperextensibility	30/35 (2 U)	25/25
Skin fragility	25/35 (6 U)	9/25 (14 U)
Atrophic scarring	32/35 (2 U)	17/25 (5 U)
Translucent skin	8/35 (12 U)	7/25 (15 U)
Musculoskeletal		
Generalised joint hypermobility	27/35 (2 historical, 0 U)	20/25 (1 historical) (3 U)
Joint luxations / subluxations	15/35 (4 U)	11/25 (11 U)
Scoliosis	6/35 (13 U)	1/25 (18 U)
Pectus deformity	4/35 (13 U)	3/25 (excavatum) (14 U)
Hand/foot deformities	20/35 pes planus, 7/35 hallux valgus (6 U)	10/25 pes valgus, 1/25 hallux valgus (12 U)
Recurrent fractures	2/35 (10 U)	1/25 (18 U)
Cardioascular		
Vascular complications/abnormalities	10/35 (4 artery dissection, 3 aneurysms (1 requiring embolisation), 2 AR dilation, 1 AA dilation, 3 arterial tortuosity)	4/25 (2 artery dissections/ruptures, 3 aneurysms (1 requiring surgery), 1 AR dilatation, 1 hepatic artery dilation, 1 subclavian vein dilation)
Easy bruising/haematoma	32/35 (1 U)	25/25
Varicose veins	12/35 (6 U)	6/25 (18 U)
Cardiac/valvular abnormalities	11/35 (8 MV and 2 AV regurgitation, 4 MV prolapse, 1 LV wall thickening) (4 U)	3/25 (2 MV prolapse, 1 LV dilatation)
Miscellaneous		
Pneumothorax	1/35	2/25
Gastro-intestinal ruptures/perforations	0/35	6/25 (1 esophageal, 2 colon, 1 rectal, 2 small bowel)
Abdominal wall herniations	7/35 (6 inguinal, 1 umbilical) (1 U)	5/25 (6 inguinal, 1 incisional) (14 U)
Females: pregnancy-related complications	5/15 perineal tearing (3 individuals), 0/15 severe pregnancy-related complications	1 multiple miscarriages, 1 grade 2 perineal tear, 0/11 severe pregnancy-related complications

M: male; F: female; U: not reported/unknown; LV: left ventricle; AR: aortic root; AA: ascending aorta; MV: mitral valve; AV: aortic valve.

the presence of the variant in her mother (F10 I-1), who was not clinically evaluated but who had a history of premature varicose veins, a cerebrovascular accident at 72 years and orthopaedic problems with bilateral hip prothesis.

Family 11

The proband of family 11 (F11 II-1; Fig. 4 D-F) is a man of native American descent who was clinically diagnosed with EDS at the age of 18 years. Clinical evaluation at 31 years revealed a soft, doughy and hyperextensible skin and several small atrophic scars at his forehead, left arm and right leg. There was pronounced easy bruising of the skin, but there were no major vascular complications and echocardiographic investigations were normal. He presented generalised joint hypermobility (Beighton 5/9) with distal hypermobility and pedes plani. His family history was negative for connective tissue disorders. The *de novo COL3A1* c.2791G>A, p.(Glu931Lys) variant was identified with a multigene panel including the EDS-associated genes.

Family 12

The proband (F12 III-6) is an Italian woman with a clinical diagnosis of EDS in infancy (with initial suspicion of cEDS, but also vEDS because of family history of sudden death). A molecular diagnosis, however, was made at age 75 years. At clinical evaluation, historical joint hypermobility was noted with remaining distal hypermobility and recurrent dislocations of small joints and shoulders. She also presented moderate scoliosis, halluces valgi, pedes cavi and piezogenic papulae. Her skin was soft, doughy, and hyperextensible (almost cutis laxa-like) with increased fragility and multiple atrophic scars. She showed no increased skin translucency, but there was easy bruising with a history of haematomas. Besides varicose veins and a carotid stenosis at age 62 years, there were no other vascular complications and echocardiography and echo-doppler of the supra-aortic vessels showed no abnormalities besides a mild mitral valve prolapse. Her facial features included mild hypertelorism, light blue sclerae and a premature aged appearance. She did not have any miscarriages and had two full-term uneventful pregnancies. Furthermore,

she reported chronic fatigue, generalised pain, early osteoarthritis, osteopenia, and recurrent gingivitis.

The c.1342G>A, p.(Glu448Lys) variant in *COL3A1* was identified with a multigene panel including the EDS-associated genes. This variant was absent in GnomAD (20). SDS-PAGE of procollagens I and III was within normal ranges, multiplex ligation-dependent probe amplification of *COL5A1* and CNV analysis were also normal. One of her daughters, her mother, her two brothers and her maternal grandfather were reported to have the same phenotypic features. No relatives were available for clinical evaluation or segregation analysis.

Family 13

A French boy (F13 III-2) presented at the age of 16 years with a soft, doughy and hyperextensible skin and multiple atrophic scars. He reported easy bruising with spontaneous haematomas, but no major vascular complications. Echocardiographic investigations revealed no abnormalities. His brother (F13 III-3), aged 19 at clinical evaluation, presented with a similar phenotype including a soft, doughy and hyperextensible skin. He also experienced delayed wound healing with atrophic scarring. He did not report easy bruising and no increased skin translucency was noted. He did show generalised joint hypermobility (Beighton score 7/9) with pronounced distal joint hypermobility and temporomandibular subluxations. Furthermore, clinical evaluation revealed mild scoliosis, pectus deformity, and pedes plani. Echocardiographic investigations revealed a mitral valve prolapse. The father of the two brothers (F13 II-2) also had the same phenotypic features. At age 47 years, he reported no severe vascular complication and echocardiography was normal. His skin was hyperextensible and had a soft doughy texture. There was no apparent skin fragility, but he reported problematic wound healing and multiple atrophic scars were noted. No increased skin translucency was noted, but he had a lower-limb haematoma after a trauma requiring surgery for evacuation. His medical history further included diverticulosis requiring surgery and bilateral inguinal hernia. Initially, cEDS was clinically diagnosed but genetic investigations including all EDS-associated genes revealed the presence of the *COL3A1*: c.2791G>A, p.(Glu931Lys) variant in the proband. Genetic confirmation in the father (F13 III-3) and brother (F13 II-2) is still ongoing.

Family 14

This family harbouring the *COL3A1* c.1351 G>A, p.(Glu451Lys) variant has been previously described by Ghali *et al*. 2019. The *COL3A1* c.1351 G>A, p.(Glu451Lys) variant is absent in GnomAD (20) and was classified as variant of unclear significance as no other individuals with this specific variant have been identified at that time. The proband (F14 III-5) presented with cutaneous features reminiscent of cEDS including a soft, doughy, hyperextensible skin with presence of atrophic scars (without haemosiderin deposits). Aged 48 years, a Beighton score of 4/9 was noted with distal joint hypermobility. His facial features were evocative of both cEDS and vEDS. He also had a history of an inguinal hernia and incisional hernia, both requiring repair and he suffered from a spontaneous perforation of a sigmoid colon diverticulum at age 41. Vascular complications include a right renal artery dissection and an internal iliac aneurysm (16mm). He further reported easy bruising with frequent haematomas requiring drainage twice, and increased gum bleeding. He inherited the variant from his father who had a similar phenotype and had a colonic perforation during a polypectomy at the age of 48.

Table V. Reported arginine to cysteine substitutions in proα1(I)-collagen chain with the associated phenotypes.

COL1A1 variant			
Phenotype	References	c-notation	p-notation
c.934C>T	p.(Arg312Cys)	Classical-like EDS	(12-18) + this report
c.1720C>T	p.(Arg574Cys)	Osteopenia with vascular rupture	(16)
c.2752C>T	p.(Arg918Cys)	Caffey disease	(35)
c.2872G>T	p.(Arg958Cys)	Mild osteogenesis imperfecta type I	(36)
c.3040C>T	p.(Arg1014Cys)	Caffey disease	(37)
c.3106C>T	p.(Arg1036Cys)	Osteogenesis imperfecta/EDS overlap	(26)
c.3196C>T	p.(Arg1066Cys)	Osteogenesis imperfecta/EDS overlap	(25)
c.3277C>T	p.(Arg1093Cys)	Osteopenia with vascular rupture	(16)

Discussion

In fibrillar collagens, reported substitutions of non-glycine residues with documented or suspected pathogenicity often involve Arg-to-Cys substitutions in the Xaa- or the Yaa-position of the triple helical domain (21-23). One of the first non-glycine substitutions in the proα1-chain of type I procollagen to be clearly linked to a connective tissue disorder was p.(Arg312Cys). It was first reported in two children with typical skin and joint features of cEDS (F1 II-1 and F2 II-4 in this report) (17), but later also in an adult woman who was initially suspected to have vEDS, in view of a rupture of medium-sized arteries, and more vEDS-like skin features (16). Since then, this variant has been found in several individuals (12-15, 18) and it remains unclear whether to classify the phenotype as cEDS, vEDS or as a distinct EDS type. We add 22 novel individuals from seven families to this series, bringing the total reported individuals with this variant to 35 affected individuals from 12 distinct families (the phenotypic features are summarised in Table IV).

Most fulfil the major clinical criteria for cEDS and indeed are clinically indistinguishable from individuals with pathogenic type V collagen defects. The risk for severe vascular complications however appears slightly higher than for 'true' (type V collagen-associated) cEDS, as four individuals (11.4%) (3F/1M) suffered from arterial dissection (including the iliac arteries in three individuals and the vertebral artery in one), all between the age of 37 and 44 years. There were also three individuals with aneurysms including a young girl in whom a superior mes-

entery artery aneurysm was embolised at the age of twelve years. In addition, three individuals showed arterial tortuosity (vertebral or retinal arteries), two had aortic root dilatation, and ten had mitral/aortic valve regurgitation/prolapse (albeit mild). Out of 15 reported pregnancies, five were complicated by perineal tearing, and one by a dissection of the right iliac artery following Caesarean section. Other features evoking a suspicion of vEDS included premature varicose veins and a translucent skin, but no gastro-intestinal ruptures were reported.

A few other Arg-to-Cys substitutions in the proα1(I)-collagen chain have sporadically been associated with different phenotypes (Table V). p.(Arg574Cys) and a p.(Arg1093Cys) were each found in one individual with isolated vascular ruptures (16), p.(Arg958Cys) was found in a patient with mild OI (24), p.(Arg1036Cys) and p.(Arg1066Cys) were identified in families associated with an EDS/OI overlap phenotype (25, 26) and, intriguingly, p.(Arg1014Cys) and p.(Arg918Cys) were found in a series of individuals with Caffey disease or infantile cortical hyperostosis without any overt signs of OI or EDS (27, 28). The exact pathogenic link between these Arg-to-Cys substitutions and the different phenotypes remains yet to be elucidated. Cysteine residues are normally not present in the triple helical domain of type I collagen, but in presence of an Arg-to-Cys, half of the procollagen molecules are expected to have one aberrant chain with the cysteine in the triple helical domain and one quarter are expected to have two aberrant chains. These mutant chains might form inter-chain disulfide bonds (since the amino acids in the Xaa- and Yaa-position have their side chains pointing outwards) during trafficking through the Golgi apparatus when the molecules align in register into prefibrils (29). This could disrupt the staggered alignment in mature fibrils and affect their integrity, causing ultrastructural collagen fibril abnormalities, as has been observed in skin biopsies of individuals with these defects (17). In addition to the structural abnormalities of the connective tissue, the different phenotypes however are presumably also caused by alterations in signaling pathways due to the (spatiotemporal) disruption of specific interactions of other intra-or extracellular ligands with type I (pro)collagen molecules containing one or two aberrant proα1(I)-chains. p.(Arg312Cys) is located in a major hot spot for interaction with interleukin-2, α1β1 and α2β1 integrins (30) and its substitution by cysteine could disrupt interactions with these ligands and disturb downstream signaling pathways in the ECM. Intracellular interactions could lead to the accumulation of procollagen aggregates, which could interfere with normal cell homeostasis, eliciting for instance an unfolded protein response. Further studies are however needed to dissect the exact pathogenic consequences of the specific Arg-to-Cys substitutions.

The pathogenic mechanisms underlying the Glu-to-Lys substitutions in the proα1(III)-collagen chains are even less clear. In 2019 Ghali *et al*. (19) reported 18 individuals from seven independent families, all harbouring Glu-to-Lys substitutions (either at positions 241 (2 probands), 682 (3 probands) or 1171 (2 probands). The probands were initially suspected to have cEDS but no pathogenic defects in *COL5A1*, *COL5A2* or *COL1A1* were found. The Glu-to-Lys variants, which segregated with the phenotype in these families, were initially reported as VUS, but it was noticed that several affected family members showed vEDS-like features, including arterial aneurysm, dissection or rupture, gastro-intestinal rupture, pneumothorax, muscle haematoma and/or premature varicose veins. We describe the phenotype of seven individuals from seven families, with confirmed Glu-to-Lys substitutions at positions 448, 451, 463, 704 (each one proband), and 931 (three probands) (Fig. 5). 6/7 probands were clinically diagnosed with cEDS but molecular analysis of an EDS gene panel excluded (likely)pathogenic variants in *COL5A1*, *COL5A2* and *COL1A1*. In one female proband (F10 II-2), the skin features were more subtle, and she was diagnosed as hypermobile EDS. In

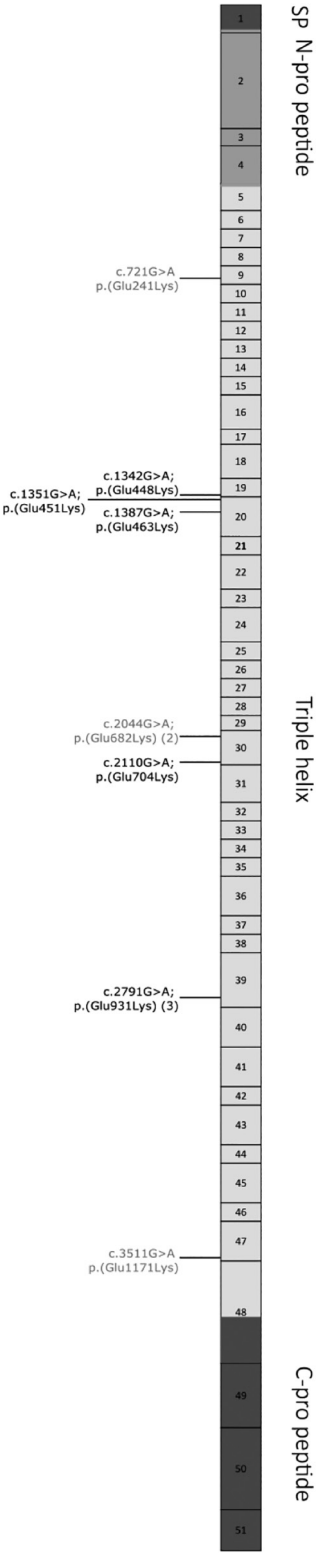

Fig. 5. Schematic representation of the hitherto reported glutamic acid to lysine substitutions in the proα1(III)-collagen chain on a schematic representation of the cDNA sequence of *COL3A1*. The variants from this report are depicted in black, the previously reported variants (19) are depicted in grey. When a variant has been identified in more than one family, this is depicted in brackets. SP: signal peptide.

view of premature varicose veins, remarkable bruising and skin translucency, an EDS gene panel was sequenced, and the proα1(III) p.(Glu463Lys) was classified as a VUS.

When looking at all reported individuals with confirmed proα1(III) Glu-to-Lys substitutions (n=25, 14 families, Table IV), most fulfil the major criteria for cEDS. Like for pro-α1(I) p.(Arg312Cys), skin translucency and/or premature varicose veins (features more evocative of vEDS) are also frequently present. In terms of severe complications of tissue fragility, one had an arterial rupture of the brachial artery and a dilatation of the hepatic artery at age 29 years, and one individual had a right renal artery dissection and a left internal iliac artery aneurysm. Furthermore, one proband had a mild aortic root dilatation and a celiac artery aneurysm, and one had a dilatation of the subclavian and the jugular veins. Mitral valve prolapse/floppy mitral valve was noted in two individuals. In contrast to proα1(I) p.(Arg312Cys), six individuals however had gastro-intestinal rupture/perforation (including oesophagus, small bowel, sigmoid and rectal), and two had a pneumothorax. In addition, several individuals were reported to have severe haematoma formation, including muscle haematoma's requiring drainage or hospitalisation. Out of 13 reported pregnancies, two were complicated with perineal tearing, but no other severe complications were reported. The pathomechanisms underlying these Glu-to-Lys substitutions have not yet been studied and for some of these variants, the true pathogenicity remains unclear due to the limited identified individuals with these variants.

To our knowledge, (likely) pathogenic Glu-to-Lys substitutions have not been reported for other fibrillar collagens. The previously reported substitutions all occur in the fairly common Gly-Glu-Arg sequence, while this is the case for only two of the five variants from this report (c.1342G>A, p.(Glu448Lys)). The other appear in Gly-Glu-Lys sequence (c.2791G>A, p.(Glu931Lys)), a Gly-Glu-Asp sequence (c.1387G>A, p.(Glu463Lys)), a Gly-Pro-Glu sequence (c.2110G>A p.(Glu704Lys)) and a Gly-Glu-Ala sequence (c.1351 G>A; p.(Glu451Lys)). When mapping the variant sites against the reported type III collagen interactome (31), none of the four new variants seemed to be part of integrin binding sites or any other known binding site.

Table VI. Surveillance and management recommendations for individuals harbouring the *COL1A1* c.934C>T, p.(Arg312Cys) variant and Glu-to-Lys substitutions in the proα1(III)-collagen chain.

	COL1A1 c.934C>T, p.(Arg312Cys)	*COL3A1*
Cardiovascular	baseline echocardiography + head-to-pelvis MRA repeated with a 3–5-year interval, or more frequently according to the findings	
	- role of cardioselective beta-blocker celiprolol unclear	- consider cardioselective beta-blocker celiprolol
Gastro-intestinal	- colonoscopy or gastroscopy do not seem to be contra-indicated	- immediate surgical intervention of bowel rupture - increased risk for colonoscopy-associated bowel perforation
Elective surgery	- surgical procedures are not contra-indicated, with awareness of the risk of vascular complications	- surgical procedures are to be avoided in favour of more conservative management strategies
Cutaneous	- avoidance of trauma and expertly closure of wounds	
Musculoskeletal	- physiotherapy and rehabilitation with awareness of the risk of vascular compli cations	
Pregnancies	- Increased risk of perineal tearing - managed as high-risk pregnancies?	

MRA: magnetic resonance angiography.

Implications for diagnostic testing, classification, surveillance and management

The vast majority of reported individuals harbouring either the pro-α1(I) p.(Arg312Cys), or a proα1(III) Glu-to-Lys substitution exhibit skin and joint features (soft, doughy, hyperextensible skin with atrophic scarring, generalised joint hypermobility and dislocations) indistinguishable from those seen in type V collagen-associated cEDS. Several of them present in addition vEDS-like features such as skin translucency and premature varicose veins, and most importantly major complications such as arterial rupture or aneurysm (mainly associated with proα1(I) p.(Arg312Cys)) or gastro-intestinal rupture, pneumothorax and/or severe haematoma formation (mainly associated with proα1(III) Glu-to-Lys substitutions). This clinical overlap between the EDS types highlights the importance of gene panel testing and thorough evaluation of the identified (including non-traditional) variants. Identifying the molecular defect underlying the phenotype in an affected individual/family is of crucial importance for the development of a patient/family-tailored surveillance and management strategy, genetic counselling, predictive testing for at-risk family members and it opens the way towards preimplantation genetic diagnosis. With regards to classification, we propose to classify the proα1(I) p.(Arg312Cys) as a separate entity among the EDS types, since the risk for severe vascular complications appears to be higher than for type V collagen-associated cEDS (8). The number of reported individuals with pro-α1(I) p.(Arg312Cys) remains however small and there might be a diagnostic bias as individuals with vascular complications might undergo more thorough molecular workup. The risk for arterial complications however appears to be lower than for type III collagen-associated vEDS, and no other major complications (gastro-intestinal, uterine, spleen or liver rupture, muscle rupture) have been reported. Moreover, the pathogen-

ic mechanisms of this defect might differ from those resulting from type V or type III collagen defects, which eventually might be important for the development of therapeutic intervention. As such, the proα1(I) p.(Arg312Cys) is a separate entity that overlaps with, but is distinct from cEDS and vEDS. For individuals with the proα1(III) Glu-to-Lys substitutions we agree with Ghali *et al*. (19) that they should currently remain classified as vEDS. Affected individuals seem to have a substantial risk for rupture of the gastro-intestinal tract, pneumothorax and severe muscle haematoma, but the risk for arterial rupture currently appears smaller that for those harbouring proα1(III) glycine substitutions. The number of reported individuals is however still too small to draw firm conclusions.

For both groups we recommend a baseline ultrasound of the heart and head-to-pelvis MRA at time of diagnosis. These should be repeated with a 3–5-year interval, or more frequently according to the findings. Based on a previously published clinical trial demonstrating a beneficial effect of the cardioselective beta-blocker celiprolol in vEDS (32), this drug can be offered to individuals with a proα1(III) Glu-to-Lys substitution, but it remains unclear whether it is of benefit to individuals with proα1(I) p.(Arg312Cys). Surveillance and preventive or treatment measures for other major complications (such as gastro-intestinal rupture, elective surgery, muscle haematoma) should be done as per the vEDS guidelines (33)(for individuals harbouring a proα1(III) Glu-to-Lys substitution, but can probably be more relaxed for individuals harbouring proα1(I) p.(Arg312Cys). Colonoscopy or gastroscopy, for example, do not seem to be contra-indicated in the latter group when the right precautious measures are taken. For the management of the skin, the same measures as for cEDS can be advised with avoidance of trauma and expertly closure of wounds (34). As for treatment of musculoskeletal alterations, physiotherapy and rehabilitation are indispensable as for the other types of EDS, but with awareness of the risk of vascular complications. As such collision sports (such as boxing, ice hockey) and isometric training (such as weightlifting) should be avoided. The number of reported pregnancies is still small for both groups. There appears to be an increased risk for perineal tearing, and especially for proα1(I) p.(Arg312Cys), obstetricians and anaesthesiologists should be aware of an increased risk for arterial rupture. Table VI summarises the surveillance and management recommendations.

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