Khosravi *et al.*
Egyptian Journal of Medical Human Genetics (2024) 25:8
https://doi.org/10.1186/s43042-024-00479-5

Egyptian Journal of Medical Human Genetics

RESEARCH

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In silico analysis of mutation spectrum of Ehlers–Danlos, osteogenesis imperfecta, and cutis laxa overlapping phenotypes in Iranian population

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Abstract

Background Ehlers–Danlos syndrome (EDS), osteogenesis imperfecta (OI), and cutis laxa (CL) are three rare and heterogeneous connective tissue disorders. Patients with these syndromes have similar manifestations and unpredictable prognosis, making a misdiagnosis highly probable. Some of their subtypes are inherited in autosomal recessive patterns, so they are expected to be prevalent in populations like Iran, where consanguineous marriages are common. In the current work, a cohort of Iranian patients with overlapping phenotypes of the EDS/OI/CL and their mutation spectrum was defined. Based on this, in silico analysis was conducted to anticipate further probable genetic variations. Pathogenicity of EDS, OI, and CL variants in Iranian patients was evaluated using Web servers. A protein interaction network was created by String database and visualized using a Python-based library. The Iranome database was used to predict other genetic mutations in all reported genes of EDS, OI, and CL syndromes.

Results In the EDS/OI/CL overlap phenotype, 32 variants in 18 genes have been involved. At least 59% of patients were from families with consanguineous marriages. Interaction analysis showed that *COL1A1*, *COL1A2*, *CRTAP*, *LEPRE1*, *PLOD1*, and *ADAMTS2* have the most significant impact within the protein network of EDS/OI/CL overlap phenotype. Analyzing the Iranome database revealed 46 variants of EDS, OI, and CL genes potentially disease causing.

Conclusion The overlapping phenotype of EDS, OI, and CL syndromes requires genetic testing (e.g., whole-exome sequencing) to reveal respective variants, which helps to diagnose more accurately and manage the disease more effectively. Particularly in populations with high rates of consanguineous marriages, such as Iran, genetic screening plays a crucial role in premarital and prenatal counseling to prevent the transmission of these rare connective tissue disorders.

Keywords Ehlers–Danlos syndrome, Osteogenesis imperfecta, Cutis laxa, Genetic database, Mutations, Genetic testing, Consanguineous marriages

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Background

Hereditary connective tissue disorders (HCTD) comprise a heterogeneous and pleiotropic group of genetic conditions with structural and functional disruptions in extracellular matrix (ECM) components. Dermal, ocular, and musculoskeletal manifestations, along with heart and lung defects, contribute to the burden of HCTDs [2]. From an epidemiological perspective, every HCTD is a rare disease, but combined, they are a notable part of human congenital disorders [9]. Studying these syndromes enhances our understanding of the nature of connective tissue (CT) and has the potential to lead to more effective treatments. Connective tissue is one of the mesodermal germ layer derivatives that exist in almost every part of the body. It connects biological structures and establishes the framework necessary for the normal functioning of organs. This tissue comprises three basic parts: soft CT, which surrounds internal organs; hard CT, including bone and cartilage; and liquid CT, which is blood. Extracellular matrix in CT consists of four components: collagens, elastic fibers, glycoproteins, and glycosaminoglycans [8, 30].

Collagens are fibrillary proteins that account for one-third of the human body's total protein. There are five types of classical fibrillary collagen: types I, II, III, V, and XI, which are different helical conformations of alpha-chain polypeptide strands coiling around each other. The alpha chain is made of an amino acid triplet repeat glycine-X–Y, where X and Y are commonly hydroxyproline and proline [12, 36]. While collagen fibrils are responsible for the strength of the structures, their resiliency is provided by elastic fibers. The process of elastin formation, also referred to as elastogenesis, is complex and not yet fully understood. Microfibers are the main building blocks of elastic fibers. They are a polymerized scaffold of fibrillins, a large protein with a molecular weight of 150 kDa [45]. Collagen, elastic fiber, and other ECM components like fibronectin and laminin interact to perform tissue morphogenesis, cell adhesion, migration, or differentiation.

Clinical management of HCTDs is faced with three challenges [35]: (1) Ambiguity: The ubiquitous presence of connective tissue throughout the human body contributes to the challenge of defining and observing the phenotypes of HCTDs in various organs. (2) Variability: patients with the same diagnosis of an HCTD can differ, even in intra-familial cases. (3) Unpredictability: phenotypes of an individual with an HCTD can change over the lifetime, and also they might have temporal manifestations. Therefore, a misdiagnosis at the early stages is highly probable.

Based on which component of ECM is dysregulated, HCTDs are categorized into two major classes: collagenopathies, including Ehlers–Danlos syndrome (EDS), osteogenesis imperfecta (OI), Alport syndrome, and chondrodysplasias. And elastinopathies, including cutis laxa (CL), Marfan syndrome, and pseudoxanthoma elasticum (PXE). These diseases are phenotypically varied and genetically heterogeneous. These diseases exhibit a wide range of phenotypic variations and genetic heterogeneity. A total of 20, 16, and 13 genes have been responsible for EDS, OI, and CL syndromes, up to now.

Ehlers–Danlos syndrome is a soft HTCD characterized by skin hyperextensibility, joint hypermobility, bone fragility and osteoporosis, atrophic scars, loose skin, and cardiovascular problems like mitral valve prolapse [28]. The prevalence of different subtypes is about 1 in 5000 to 1 in 20,000. Based on a 2017 international classification, classical EDS, arthrocalasis EDS, and cardiac valvular EDS are the three main subtypes of the syndrome [27].

Osteogenesis imperfecta has a prevalence of 1 in 20,000 live births. It mostly manifests with growth defects, bone fragility, osteopenia, dentinogenesis imperfecta, and blueish sclera. Up to 90 percent of IO cases are due to mutations in *COL1A1* and *COL1A2*. These two are also responsible for many EDS cases [24]. The initial step in diagnosing these two syndromes is identifying their similar clinical signs, which makes it challenging to provide follow-up care and genetic counseling. There is an extremely rare condition called EDS/OI overlap, which affects approximately 1 in every 1,000,000 individuals (based on Orphanet data). It was first described in 2013 when patients with combined symptoms were reported. Molecular analysis of this overlap revealed an association with N-terminal mutations in type 1 collagen [33].

An abnormal synthesis of elastic fibers can result in CL syndrome, characterized primarily by loose and redundant skin, developmental emphysema, cardiovascular issues like aortic aneurysm, hernia, delayed growth, and fragile bones. In some CL cases, patients mimic manifestations of EDS with similar skin hyper-elasticity, scarring, and joint laxity [13]. Furthermore, CL cases with mutations in RIN2 and ELN exhibit phenotypic similarities to EDS patients, including sparse hair and alopecia. [14, 48].

Genetic defects in CT components mostly manifest as phenotypic traits. In these three disorders, in addition to the CT nature, intermediate clinical phenotypes (e.g., blueish sclera in EDS and IO and bone fragility in all three) increase the probability of misdiagnosis. Consanguineous marriage (marriage between relatives) is commonly performed in Iran. The general rate of that is 38.6% throughout the country [44]. Thus, it has received

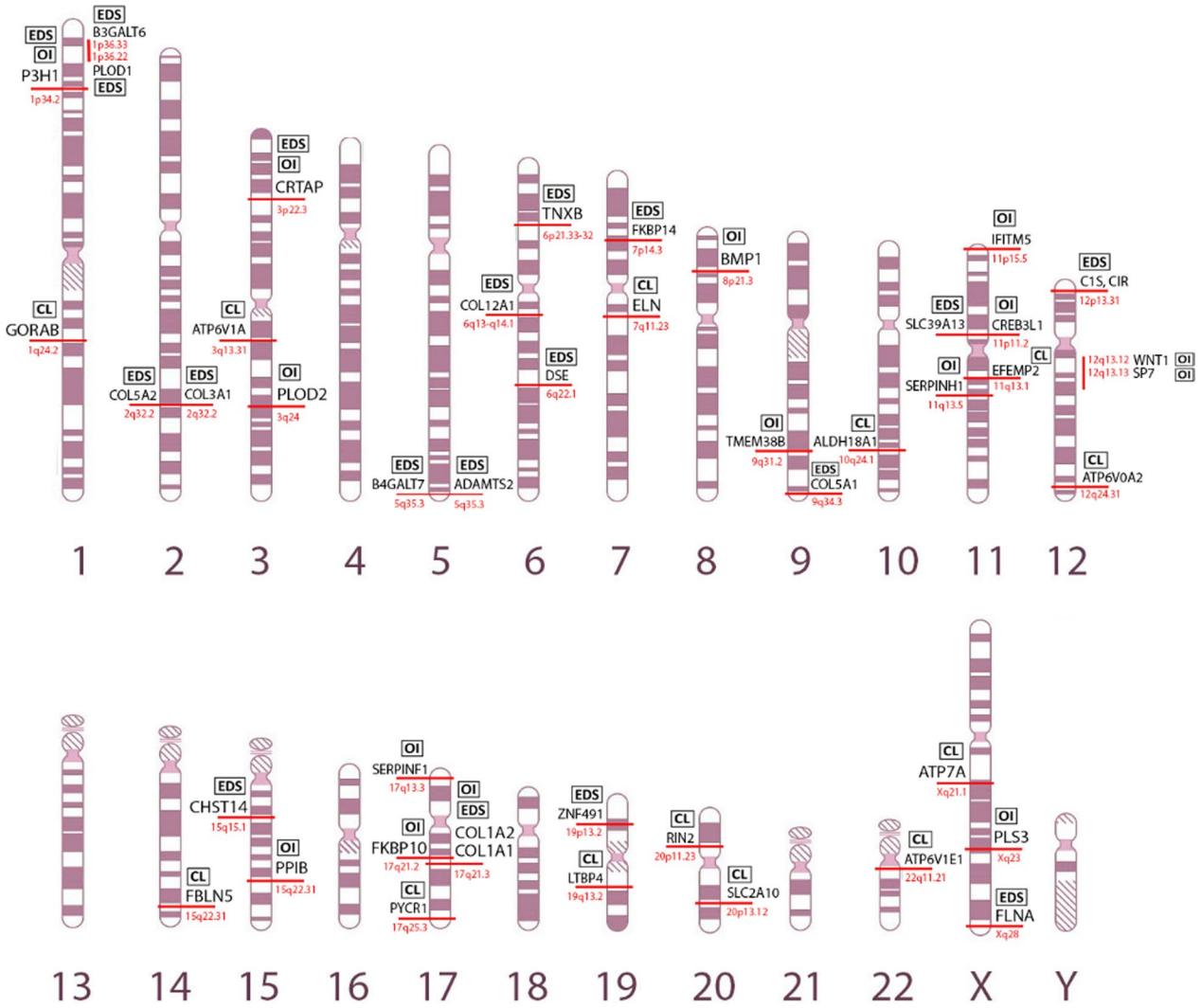

Fig. 1 Genes involved in overlap phenotype of EDS, OI, and CL demonstrated on their respective chromosomal region. None are located on chromosomes 4, 13, 16, 18, 21, and Y, while chromosomes 11 and 17 host five genes

great attention as a potential risk factor for many genetic-influenced health outcomes, especially autosomal recessive (AR) disorders.

According to NORD's database (https://rarediseases.org/), CL, ESD, and OI have various subtypes and inheritance patterns. For CL, subdivisions are as follows: acquired cutis laxa, ALDH18A1-related cutis laxa, ATP6V0A2-related cutis laxa, autosomal dominant cutis laxa (ADCL), autosomal recessive cutis laxa type 1A (ARCL1A), autosomal recessive cutis laxa type 1B (ARCL1B), autosomal recessive cutis laxa type 1C (ARCL1C), autosomal recessive cutis laxa type 2A (ARCL2A), autosomal recessive cutis laxa type 2B (ARCL2B), autosomal recessive cutis laxa type 3, Debre-type cutis laxa, EFEMP2-related cutis laxa, ELN-related cutis laxa, geroderma osteodyplasticum, LTBP4-related cutis laxa, MACS syndrome, PYCR1-related cutis laxa, RIN2-related cutis laxa, Urban–Rifkin–Davis syndrome, wrinkly skin syndrome. Most cases of autosomal dominant cutis laxa are caused by mutations in the elastin (ELN) gene and are also known as ELN-related cutis laxa or autosomal dominant cutis laxa type 1 (ADCL1). One case, classified as autosomal dominant cutis laxa type 2 (ADCL2), was caused by a mutation in the fibulin-5 (FBLN5) gene. Ehlers–Danlos syndrome subdivisions are as follows: classic EDS, classical-like EDS, cardiac valvular EDS, vascular EDS, hypermobile EDS, anthrochalasia EDS, dermatosparaxis EDS, kyphoscoliotic EDS, brittle

Table 1 Function, pathway, and ontology of the genes involved in overlap phenotype of EDS, OI, and CL. All OI-related genes have a role in collagen biosynthesis and function or bone development. Elastic fibers biosynthesis and function, different amino acid biosynthesis, and energy production are the main roles of CL-related genes. EDS-related genes have similar functions

	Gene	Full name	Function	Pathway	Ontology
OI	COL1A1	Collagen alpha-1(I) chain	Type 1 Collagen Structure	ECM–receptor interaction, PI3K-Akt signaling pathway, focal adhesion, platelet activation, relaxin signaling pathway, protein digestion and absorption	Identical protein binding and platelet-derived growth factor binding
	COL1A2	Collagen alpha-2(I) chain	Type 1 Collagen Structure	ECM–receptor interaction, PI3K-Akt signaling pathway, focal adhesion, platelet activation, relaxin signaling pathway, protein digestion and absorption	Identical protein binding and protein–macromolecule adaptor activity
	BMP1	Bone morphogenetic protein 1	ECM formation	Cytokine–cytokine receptor interaction, ovarian steroidogenesis	Calcium ion binding and growth factor activity
	CREB3L1	Cyclic AMP-responsive element-binding protein 3-like protein 1	COL1A1 and COL1A2 regulation in bone formation	Glucagon signaling pathway, cGMP-PKG signaling pathway, cAMP signaling pathway, PI3K-Akt signaling pathway, AMPK signaling pathway, TNF signaling pathway	DNA-binding transcription factor activity and chromatin binding
	CRTAP	Cartilage-associated protein	Collagen stabillizing	Collagen bisynthesis and modifying enzymes	Protein-containing complex binding
	FKBP10	FKBP prolyl isomerase 10	Collagen molecule cross-linking	PI3K signaling pathway	Calcium ion binding and FK506 binding
	SP7	Sp7 transcription factor	Embryonic bone development	RUNX2 regulates osteoblast differentiation	DEAD/H-box RNA helicase binding
	WNT1	Wnt family member 1	Osteoblast function, bone development, and bone homeostasis	Signaling pathways regulating pluripotency of stem cells, mTOR signaling pathway, Hippo signaling pathway	Signaling receptor binding and transcription cis-regulatory region binding
	PLOD2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2	Type 1 collagen synthesis	Lysine degradation, metabolic pathways	Oxidoreductase activity and oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen
	PLS3	Plastin 3	Bone development	PI3K/AKT signaling pathway, MAPK signalling pathway, TGF-β signaling pathway	Calcium ion binding and actin binding
	PPIB	Peptidylprolyl isomerase B	Type 1 collagen synthesis and terimerization	Collagen bisynthesis and modifying enzymes	RNA binding and unfolded protein binding
	IFITM5	Interferon-induced transmembrane protein 5	Type 1 collagen synthesis/ bone mineralization	Collagen synthesis pathway	NA
	P3H1	Prolyl 3-hydroxylase 1	Type 1 collagen synthesis and terimerization	Collagen synthesis pathway	Oxidoreductase activity and iron ion binding
	SERPINF1	Serpin family F member 1	Type 1 collagen synthesis	Wnt signaling pathway	Serine-type endopeptidase inhibitor activity
	SERPINH1	Serpin family H member 1	Type 1 collagen synthesis and terimerization	Collagen bisynthesis and modifying enzymes	RNA binding and serine-type endopeptidase inhibitor activity
	TMEM38B	Transmembrane protein 38B	Type 1 collagen synthesis/ intracellular calcium release	Collagen synthesis pathway	Potassium channel activity and cation channel activity

Table 1 (continued)

	Gene	Full name	Function	Pathway	Ontology
CL	FBLN4 (EFEMP2)	EGF-containing fibulin extracellular matrix protein 2	Elastic fiber formation in aorta	Extracellular matrix organization and integrin pathway	Calcium ion binding and extracellular matrix structural constituent
	FBLN5	Fibulin 5	Elastic fiber formation in skin, lung, and vasculature	Autophagy pathway and extracellular matrix organization	Calcium ion binding and transmembrane signaling receptor activity
	ATP6V0A2	ATPase H + transporting V0 subunit A2	a2 subunit of the V-type H + ATPase	Oxidative phosphorylation, metabolic pathways, synaptic vesicle cycle, epithelial cell signaling in helicobacter pylori infection	ATPase binding and proton-transporting ATPase activity, rotational mechanism
	ALDH18A1	Aldehyde dehydrogenase 18 family member A1	P5CS mitochondrial protein	Biosynthesis of amino acids, arginine and proline metabolism, metabolic pathways	RNA binding and NADP binding
	LTBP4	Latent transforming growth factor beta binding protein 4	Elastic fiber formation	Apoptotic pathways in synovial fibroblasts and extracellular matrix organization	Calcium ion binding and glycosaminoglycan binding
	ELN	Elastin	Extracellular matrix organization and Integrin Pathway	Protein digestion and absorption	Extracellular matrix constituent conferring elasticity
	GORAB	Golgin, RAB6 interacting	A protien of golgin family	P53 signaling pathway	NA
	PYCR1	Pyrroline-5-carboxylate reductase 1	Proline synthesis	Arginine and proline metabolism, metabolic pathways, biosynthesis of amino acids	Identical protein binding and oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor
	ATP7A	ATPase copper-transporting alpha	Cell homeostasis of copper	Platinum drug resistance, mineral absorption	Nucleotide binding and ATPase-coupled cation transmembrane transporter activity
	RIN2	Ras and Rab interactor 2	Regulates the adhesion of ECs to ECM proteins via its Rab5 and Ras binding domains	Vesicle-mediated transport and Rab regulation of trafficking	GTPase activator activity and GTPase regulator activity
	SLC2A10	Solute carrier family 2 member 10	Facilitative glucose transporter required for the development of the cardiovascular system	Nuclear receptors meta-pathway	Transmembrane transporter activity and glucose transmembrane transporter activity
	ATP6V1E1	ATPase H + transporting V1 subunit E1	Acidify intracellular compartments in eukaryotic cells	Oxidative phosphorylation, metabolic pathways, mTOR signaling pathway, epithelial cell signaling in helicobacter pylori infection	ATPase binding and P-type proton-exporting transporter activity
	ATP6V1A	ATPase H + transporting V1 subunit A	Hydrolyze ATP to provide energy for transporting H +	Oxidative phosphorylation, metabolic pathways, mTOR signaling pathway, epithelial cell signaling in helicobacter pylori infection	Acyltransferase activity and choline O-acetyltransferase activity
EDS	COL1A1	Collagen type I alpha 1 chain	Type 1 collagen structure	ECM–receptor interaction, PI3K-Akt signaling pathway, focal adhesion, platelet activation, relaxin signaling pathway, protein digestion and absorption	Identical protein binding and platelet-derived growth factor binding

Table 1 (continued)

Gene	Full name	Function	Pathway	Ontology
COL3A1	Collagen type III alpha 1 chain	Provides instructions for making type III collagen	PI3K-Akt signaling pathway	Integrin binding and SMAD binding
PLOD1	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	Encodes the 125-kDa catalytic subunit of DNA polymerase delta	Lysine degradation, metabolic pathways	Protein homodimerization activity and iron ion binding
TNXB	Tenascin XB	Provides instructions for making a protein called tenascin-X	PI3K-Akt signaling pathway and extracellular matrix organization	Heparin binding and collagen binding
COL5A2	Collagen type V alpha 2 chain	Provides instructions for making a component of type V collagen	PI3K-Akt signaling pathway and collagen chain trimerization	Extracellular matrix structural constituent and SMAD binding
COL5A1	Collagen type V alpha 1 chain	Provides instructions for making a component of type V collagen	PI3K-Akt signaling pathway and collagen chain trimerization	Heparin binding and extracellular matrix structural constituent
COL1A2	Collagen type I alpha 2 chain	Type 1 collagen structure	ECM–receptor interaction, PI3K-Akt signaling pathway, focal adhesion, platelet activation, relaxin signaling pathway, protein digestion and absorption	Identical protein binding and platelet-derived growth factor binding
ADAMTS2	ADAM metallopeptidase with thrombospondin type 1 motif 2	Cleaves the propeptides of type I and II collagen prior to fibril assembly	Collagen synthesis pathway, o-glycozilation of TSR domain-containing proteins	Peptidase activity and metallopeptidase activity
P3H1	Prolyl 3-hydroxylase 1	Type 1 collagen synthesis and terimerization	Collagen synthesis pathway	Oxidoreductase activity and iron ion binding
B4GALT7	Beta-1,4-galactosyltransferase 7	Required for the biosynthesis of the tetrasaccharide linkage region of proteoglycans, especially for small proteoglycans in skin fibroblasts	Glycosaminoglycan biosynthesis—chondroitin sulfate / dermatan sulfate, glycosaminoglycan biosynthesis—heparan sulfate / heparin, metabolic pathways	Glycosyltransferase activity and galactosyltransferase activity
B3GALT6	Beta-1,3-galactosyltransferase 6	Beta-1,3-galactosyltransferase that transfers galactose from UDP-galactose to substrates with a terminal beta-linked galactose residue	Glycosaminoglycan biosynthesis—chondroitin sulfate / dermatan sulfate, glycosaminoglycan biosynthesis—heparan sulfate / heparin, metabolic pathways	Galactosyltransferase activity and UDP-galactosyltransferase activity
CHST14	Carbohydrate sulfotransferase 14	Encodes a member of the HNK-1 family of sulfotransferases	Glycosaminoglycan biosynthesis—chondroitin sulfate / dermatan sulfate	Culfotransferase activity and N-acetylgalactosamine 4-O-sulfotransferase activity
DSE	Dermatan sulfate epimerase	Converts D-glucuronic acid to L-iduronic acid (IdoUA) residues	Retrograde endocannabinoid signaling, glycosaminoglycan biosynthesis—chondroitin sulfate / dermatan sulfate, metabolic pathways	Chondroitin-glucuronate 5-epimerase activity
FKBP14	FKBP prolyl isomerase 14	PPIase which accelerates the folding of proteins during protein synthesis	IL-6/STAT3 signaling pathway, notch pathway, Wnt/β-catenin signaling pathway	Calcium ion binding and FK506 binding
SLC39A13	Solute carrier family 39 member 13	Acts as a zinc influx transporter	Nuclear receptors meta-pathway and metal ion SLC transporters	Protein homodimerization activity and zinc ion transmembrane transporter activity
FLNA	Filamin A	Promotes orthogonal branching of actin filaments and links actin filaments to membrane glycoproteins	Cytoskeletal signaling, MAPK signaling pathway, focal adhesion	RNA binding and transcription factor binding

Table 1 (continued)

Gene	Full name	Function	Pathway	Ontology
C1S	Complement C1s	C1s B chain is a serine protease that combines with C1q and C1r to form C1, the first component of the classical pathway of the complement system	NA	Calcium ion binding and serine-type endopeptidase activity
C1R	Complement C1r	C1r B chain is a serine protease that combines with C1q and C1s to form C1, the first component of the classical pathway of the complement system	Initial triggering of complement and notch signaling	Calcium ion binding and serine-type peptidase activity
ZNF49	Zinc finger protein 546	May be involved in transcriptional regulation	NA	Nucleic acid binding and DNA-binding transcription factor activity
COL12A1	Collagen type XII alpha 1 chain	Encodes the alpha chain of type XII collagen	Protein digestion and absorption	Extracellular matrix structural constituent conferring tensile strength
CRTAP	Cartilage-associated protein	Necessary for efficient 3-hydroxylation of fibrillar collagen prolyl residues	Collagen synthesis pathway	Protein-containing complex binding

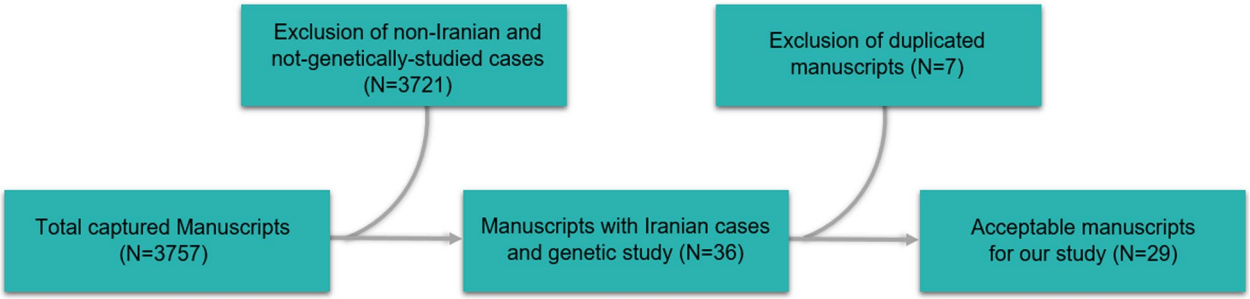

Fig. 2 Diagram of Data Collection. In most studies, lack of genetic evaluation was observed

cornea syndrome, spondylodysplastic EDS, musculocontractural EDS, myopathic EDS, periodontal EDS. Among these syndromes, classical-like, cardiac valvular, dermatosparaxis, and kyphoscoliotic types are inherited in an AR manner. Myopathic EDS has both AD and AR inheritance, while the remaining types are AD.

Osteogenesis imperfecta types I to XXI are subtypes of OI. Types I to V exhibit AD inheritance, while the remaining types are inherited in an AR manner. These three rare HCTDs have multiple AR subtypes, which are another complexity next to their clinical overlap. As depicted in Fig. 1, a total of 45 genes have been identified globally to be associated with the overlapping phenotype of EDS, OI, and CL. About half are on chromosomes 1, 11, 12, and 17. Details like function, related pathway, and ontology of these genes are listed in Table 1. The aim of this study is to address the clinical and genetic complexities of the overlapping phenotypes of Ehlers–Danlos syndrome (EDS), osteogenesis imperfecta (OI), and cutis laxa (CL). The research question seeks to determine the extent to which specific genetic variants contribute to the clinical features of these disorders within the Iranian population, which is characterized by a high consanguineous marriage rate [16, 49]. It is hypothesized that a clear genetic basis of these diseases can aid in the development of more precise diagnostic and therapeutic strategies like whole-exome sequencing or RNA therapeutics [5, 22]. The genetic diversity of the Iranian population is leveraged in this study to fill a critical knowledge gap in understanding the pathogenesis of these syndromes and to propose potential targets for intervention in populations with similar genetic backgrounds.

Methods

Data collection

Investigation of EDS, IO, and CL patients in Iranian patients to draw a spectrum of their mutation was performed in a systematic search. For that purpose, the keywords 'osteogenesis imperfecta,' 'ehlers danlos,' and 'cutis laxa' along with 'Iran' (or Iranian) in both English and Farsi were used. PUBMED, Web of Science, Scopus, Cochrane Library databases, Google Scholar, and Scientific Information Database (SID, an Iranian medical database) were used as search engines. The search for data was up to March 2023, and there was no more restriction. Afterward, the manuscripts were filtered to reach the ones in which genetic tests reported the variants. Also, duplicated ones were removed. Moreover, the HGMD Professional 2021.4 database was utilized for each gene to evaluate the number and types of mutations.

In silico prediction of pathogenicity and stability of single nucleotide variants

The obtained spectrum of gene mutations of Iranian patients with EDS, CL, and OI was analyzed by in silico tools to predict their pathogenicity and protein stability. For that purpose, ACMG (https://franklin.genoox.com/clinical-db), CADD (https://cadd.gs.washington.edu/), SIFT (https://sift.bii.a-star.edu.sg/), polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), and Fathmm (http://fathmm.biocompute.org.uk/) were used. Also, the effect of missense variants on protein stability was evaluated using I-Mutant 2.0 (https://folding.biofold.org/i-mutant/i-mutant2.0.html) and MUpro (https://mupro.proteomics.ics.uci.edu/) Web servers. Moreover, the manuscripts were mined and the reported effects of splice site, deletion, and duplication variants.

Protein interaction analysis using NetworkX Python package

The NetworkX package (https://github.com/networkx/networkx), a Python language-based library, was employed to explore and visualize complex networks [59]. The protein interaction data set was obtained from the STRING database, according to the latest NGS panel for each syndrome. Subsequently, a list of 50 proteins was created. Our model of pairwise relations between proteins of this package was based on the graph theory perspective of NetworkX.

Table 2 Review of Revealed Genes and Variants in Iranian Patients with EDS, OI, or CL Syndromes, up to November 2022

Syndrome	Gene	Mutation (N = novel)	Protein	Reported disorder	Number of patients	Type of marriage	Genetic test	Study
CL	FBLN5	c.679 T > C	p.Ser227Pro	Autosomal recessive cutis laxa	1	Consanguineous	PCR/ direct sequencing	Elahi et al. [11]
	PYCR1	c.345delC	p.Arg116fs	Autosomal recessive cutis laxa type 2B	1	Consanguineous	PCR/ direct sequencing	Nouri et al. [46]
	FBLN5	c.544G > C (N)	p.Ala182Pro	Autosomal recessive cutis laxa	2	Consanguineous	WES	Gharesouran et al. [15]
	GGCX	c.373 + 3G > T (N)	p.Phe73_Gly125del	Cutis laxa	5	Undeclared	WES	Kariminejad et al. [28]
	LTBP4	c.533-1G > A	NA	Autosomal recessive cutis laxa type 1C	1	Undeclared	WES	Mazaheri et al. [36]
	FBLN5	c.907C > T (N)	p. Gln303X	Autosomal recessive cutis laxa type A1	1	Consanguineous	WES	Malakan Rad et al. [48]
	PYCR1	C.797G > A	p.Arg266Gln	Autosomal recessive cutis laxa type 2	1	Consanguineous	PCR/ direct sequencing	Rahmati et al. [49]
	PYCR1	c.722C > A	p.Ala241Asp	Autosomal recessive cutis laxa type 2B	1	Consanguineous	WES	Nikfar et al. [45]
	PYCR1	c.566C > T	p.Ala189Val	Autosomal recessive cutis laxa type 2B	5	Consanguineous	Whole-genome sequencing	Vahidnezhad et al. [61]
	RIN2	c.2251dup (N)	Leu751Profs*9	RIN2 syndrome	1	Consanguineous	WES	Kameli et al. [26]
	ATP6V0A2	c.1936_2055del	p.E646_685del	Autosomal recessive cutis laxa type 2	1	Undeclared	PCR/ direct sequencing	Hucthagowder et al. [24]
	GSN	c.654G > A	p.Asp187Asn	Gelsolin amyloidosis	1	Undeclared	Undeclared	Shokouhi et al. [3]
	ATP6V1E1	c.383 T > C	p.Leu128Pro	Cutis laxa	2	Undeclared	WES	Van Damme et al. [8]
EDS	PLOD1	c.1302 C > G (N)	p.Thr434X	Ehlers–Danlos syndrome type VI	1	Consanguineous	WES	Kariminejad et al. [27]
	B3GALT6	c.619G > C	p.Asp207His	Recessive Ehlers–Danlos	1	Consanguineous	PCR/ direct sequencing	Malfait et al. [35]
	B3GALT6	c.619G > C	p.Asp207His	Recessive Ehlers–Danlos	1	Non-consanguineous		
	B3GALT6	c.649G > A	p.Gly217Ser	Recessive Ehlers–Danlos	1	Non-consanguineous		
	B3GALT6	c.323_344del // c.619G > C	p. Ala108Glyfs ∗ 163 // p.Asp207His	Recessive Ehlers–Danlos	2	Non-consanguineous		
	ADAMTS2	c.669_670dupG (N)	p. (Pro224Argfs*24)	Ehlers–Danlos syndrome	1	Consanguineous	PCR/ direct sequencing	Van Damme et al. [7]
	FKBP14	c.143 T > A (N)	p.(Met48Lys)	Kyphoscoliotic Ehlers–Danlos syndrome	1	Consanguineous	PCR/ direct sequencing	Giunta et al. [16]

Table 2 (continued)

Syndrome	Gene	Mutation (N = novel)	Protein	Reported disorder	Number of patients	Type of marriage	Genetic test	Study
	FKBP14	c.2 T > G	NA	Kyphoscoliotic Ehlers–Danlos syndrome	1	Undeclared	WES	Colman et al. [6]
	PLOD1	c.1471–1 G > A	NA	Kyphoscoliotic Ehlers–Danlos syndrome	1	Consanguineous	PCR/ direct sequencing	Rohrbach et al. [52]
	COL3A1	c.2194G > A	p.Gly732Arg	Vascular Ehlers–Danlos syndrome	1	Consanguineous	WES	Colman et al. [5]
	B3GALT6	c.545A > G	p.Tyr182Cys	Spondylodysplastic Ehlers–Danlos	1	Consanguineous	WES	Van Damme et al. [9]
OI	FKBP10	c.976delA (N)	p. Met326Trpfs * 39	Osteogenesis imperfecta type XI (Bruck syndrome)	1	Consanguineous	WES	Seyedhassani et al. [55]
	COL1A1	c.2298 T > C (N)	p.Thr766Thr	Osteogenesis imperfecta	1	Consanguineous	WES	Talebi et al. [59]
	COL1A1	c.3313delA	(p.Arg-1105GlufsX3	Osteogenesis imperfecta	1	Non-consanguineous	Conformation-sensitive gel electrophoresis (CSGE)	Nwosu et al. [47]
	GLUT2	C.685_701del (N)	p.A229QfsX19	Osteogenesis imperfecta/ Fancoli–Bickel syndrome	1	Undeclared	WES	Shafaghati et al. [56]
	FKBP10	c.1257-2A > G // IVS7-2A > G (N)	p.H420PfsX12	Osteogenesis imperfecta type XI (Bruck syndrome)	1	Consanguineous	WES	Maghami et al. [32]
	COL1A2	c.14929-14930TG > GT	NA	Osteogenesis imperfecta	1	Undeclared	PCR	Moshref et al. [41]
	FKBP10	c.204delCinsAAA (N)	p.His68Glnfs*92	Osteogenesis imperfecta type XI (Bruck syndrome)	1	Consanguineous	WES	Moravej et al. [39]
	MESD	c.676C > T	p.Arg226 *	Osteogenesis imperfecta type XX	1	Undeclared	WES	Tran et al. [60]

Prediction of probable damaging variants using Iranome database

The reported genes of CL, EDS, and OI syndromes were evaluated using the Iranome Genomic Database (http://www.iranome.ir/) to predict probable damaging variants. This database was established by whole-exome sequencing (WES) data of 800 healthy Iranian individuals from eight major Iranian populations, including Iranian Arabs, Azeris, Persians, Lurs, Baluchs, Persian Gulf Islanders, Kurds, and Turkmen. Iranome discovered more than 1,500,000 variants, more than 300,000 of which were novel [7, 31]. The pathogenicity of these variants was investigated using six tools, including SIFT, Polyphen2, MutationTaster, MutationAssessor, FATHMM, and FATHMM MKL, as listed on the Iranome Website. Here, the missense heterozygous alleles of all reported EDS, OI, and CL genes in the database were obtained; then, inclusion and exclusion criteria were established to predict which variant has the most probability of causing one of the three syndromes. The criteria are as follows: A variant with A) three or more times predicted as damaging via the six mentioned Web servers. B) More than 10 number of heterozygotes. C) CADD score of 20 or more.

Table 3 Types of Mutations in Genes Involved in Overlap Phenotype of EDS/OI/CL in Iran, According to the HGMD database

Gene	Missense/ nonsense	Splicing	Regulatory	Small deletions	Small insertions	Small indels	Gross deletions	Gross insertions	Complex	Repeat	Total
Mutations											
ADAMTS6	6	1	–	–	–	–	–	–	–	–	7
ATP6V0A2	20	10	–	18	7	–	3	1	–	–	59
ATP6V1E1	2	–	–	–	–	–	–	–	–	–	2
B3GALT6	32	–	–	7	1	1	3	2	–	–	46
COL1A1	617	274	4	275	83	19	30	4	2	–	1308
COL1A2	523	69	–	28	19	6	19	2	1	2	669
FBLN5	26	–	–	1	–	–	–	1	–	–	28
FKBP14	3	–	–	5	2	–	–	–	–	–	10
GGCX	52	10	–	5	1	1	–	–	–	1	70
SLC2A2	44	21	7	18	5	4	–	1	1	–	101
GSN	25	1	–	2	3	1	1	–	–	–	33
LTBP4	17	4	–	7	6	1	–	1	–	–	36
MESD	1	–	–	3	1	–	–	–	1	–	6
PLOD1	30	8	–	9	5	–	5	2	1	–	60
PYCR1	33	7	–	5	1	–	2	–	–	–	48
RIN2	2	–	–	3	2	–	–	–	–	–	7

Results

Data collection

Based on initial keyword research in the six search engines, 416, 1593, and 1748 manuscripts were found for CL, EDS, and IO, respectively. Further in-detailed mining of papers and removing duplicated studies revealed that in 13, 8, and 8 manuscripts, homozygous variants of cases with CL, EDS, and IO were reported (Fig. 2). Statistically, 32 variants were found in 18 genes as a result of genetic tests in 43 patients. The novelty status of variants, reported disorders, applied genetic test, and type of marriage are summarized in Table 2. HGMD Professional 2021.4 database also revealed that most of the mutations in these genes are missense/nonsense, splicing, and small deletion (Table 3).

Pathogenicity and stability of EDS/OI/CL overlap phenotype variants

The results of both pathogenicity and stability analysis by Web servers are listed in Table 4, divided into two sections—one for missense variants and another for splice site, deletion, and duplication variants. From eight OI variants, only one is missense (*COL1A1*: c.2298T > C). Three CL and one EDS variants were inconsistent with the reported phenotype. FBLN5: c.544G > C was found in two patients and, according to I-Mutant 2.0, has a positive effect on protein stability. The Web server also reported the same effect for PYCR1: c.722C > A. One patient with CL type 2 had PYCR1: c.797G > A, which was identified as benign by Polyphen2. It also reported *FKBP14*: c.143T > A as a benign variant in an EDS patient.

Protein interaction analysis using NetworkX Python package

A list of 50 proteins involved in EDS/OI/CL overlap phenotype was created using NetworkX. Figure 3 shows an undirected weighted graph in which the nodes and edges represent proteins and their interactions, respectively, so that each edge's length shows the interaction score. The degree of the graph (defined as the average number of edges connected to each node) equals 4.47. NetworkX package applied the concept of 'betweenness centrality' to the graph. It is a measure in graph theory to demonstrate which nodes are more important (or their absence causes more disruption in the network) based on the shortest paths. In this graph, the size of the nodes indicates the betweenness centrality. Also, a color range from dark green to white indicates the degree; greener nodes have more degrees (more connected edges) and bigger nodes have more impact on the network. Moreover, an edge with more width and greener color shows more interaction scores between a pair of proteins. The graph shows that COL1A1, COLA1A2, CRTAP, LEPRE1, PLOD1, and ADAMTS2 have the biggest impact on the protein network of the overlapping phenotype.

Table 4 Evaluation of Pathogenicity and Effect on Protein Stability in Variants of Overlap Phenotype of EDS/OI/CL in Cohort of the Study

	Gene	Variant	Protein	ACMG	Fathmm	CADD	SIFT	POLY PHEN 2	MUpro	I-Mutant 2.0
Missense variants										
Cutis laxa	FBLN5	c.679 T > C	p.Ser227Pro	Likely pathogenic	Damaging (-3.01)	25.6	Damaging	Probably damaging	Decrease (DDG: -1.37)	Decrease (DDG: -1.08)
	FBLN5	c.544G > C	p.A182P	VUS	Damaging (-2.08)	27.9	Damaging	Probably damaging	Decrease (DDG: -1.31)	Increase (DDG: 0.68)
	FBLN5	c.907C > T	p. Gin303*	Likely pathogenic	N/A	26.7	Damaging	Probably damaging (0.993)	N/A	N/A
	PYCR1	c.797G > A	p.Arg266Gln	Likely pathogenic	Damaging (-1.89)	33	Damaging (0.04)	Benign (0.350)	Decrease (DDG: -0.57)	Decrease (DDG: -0.70)
	PYCR1	c.722C > A	p.Ala241Asp	Likely pathogenic	Damaging (-2.42)	26.3	Damaging (N/A)	Probably damaging (1.000)	Decrease (DDG: -1.07)	Increase (DDG: 0.33)
	PYCR1	c.566C > T	p.Ala189Val	VUS	Damaging (-1.95)	27	Damaging (N/A)	Probably damaging (0.781)	Decrease (DDG: -0.75)	Decrease (DDG: -0.14)
	ATP6V1E1	c.383 T > C	p.Leu128Pro	VUS	N/A	27.1	Damaging (0.03)	Probably damaging (1.000)	Decrease (DDG: -1.25)	Decrease (DDG: -0.23)
Ehlers–Danlos	B3GALT6	c.619G > C	p.Asp207His	VUS	Damaging (-3.11)	29.4	Damaging (NA) 0	Probably damaging (1.000)	Decrease (DDG: -1.18)	Decrease (DDG: -0.37)
	B3GALT6	c.619G > C	p.Asp207His	VUS	Damaging (-3.11)	29.4	Damaging (NA) 0	Probably damaging (1.000)	Decrease (DDG: -1.18)	Decrease (DDG: -0.37)
	B3GALT6	c.649G > A	p.Gly217Ser	VUS	Damaging (-4.25)	29.5	Damaging (NA) 0	Probably damaging (1.000)	Decrease (DDG: -1.03)	Decrease (DDG: -1.62)
	B3GALT6	c.545A > G	p.Tyr182Cys	VUS	Damaging (-5.48)	29.5	Damaging (NA) 0	Probably damaging (1.000)	Decrease (DDG: -1.17)	Decrease (DDG: -0.21)
	FKBP14	c.143 T > A	p.(Met48Lys)	VUS	Damaging (-3.01)	26.1	Damaging (NA) 0	Benign (0.000)	Decrease (DDG: -1.75)	Decrease (DDG: -0.75)
	COL3A1	c.2194G > A	p.Gly732Arg	Pathogenic	Damaging (-5.49)	31	Damaging (NA) 0	Probably damaging (1.000)	Decrease (DDG: -0.61)	Increase (DDG: 0.40)
Osteogenesis	COL1A1	c.2298 T > C	p.Thr766Thr	Benign	Tolerated	10.96	Tolerated (1.00)	NA	N/A	N/A

	Gene	Variant	ACMG	Reported protein effect	MUpro	I-Mutant 2.0
Splice site, deletion, and duplication variants						
Cutis laxa	GSN	c.654G > A	NA	Conformational change in gelsolin protein resulted in aggregation as amyloid plaques (p.Asp187Asn)	Decrease (DDG: -0.79)	Decrease (DDG: -1.57)
	GGCX	c.373 + 3G > T	Likely pathogenic	A deletion of 53 amino acids (p.Phe73_Gly125del)	N/A	N/A
	LTBP4	c.533-1G > A	NA	-	N/A	N/A
	RIN2	c.2251dup	Likely pathogenic	p.Leu751Profs*9	N/A	N/A
	ATP6V0A2	c.1936_2055del	Uncertain	p.E646_685del	N/A	N/A
	PYCR1	c.345delC	Pathogenic	Frame shift and premature termination of translation	N/A	N/A
Ehlers–Danlos	B3GALT6	c.323_344del // c.619G > C	Pathogenic // VUS	(p.Ala108Glyfs ∗ 163) // (p.Asp207His)	Decrease (DDG: -1.87)// decrease (DDG: -1.18)	Decrease (DDG: -1.27) // decrease (DDG: -0.37)
	ADAMTS2	c.669_670dupG	Likely pathogenic	p.Pro224Argfs*24	Decrease (DDG: -0.28)	Decrease (DDG: -1.03)
	PLOD1	c.1471-1G > A	Likely pathogenic	Activation of a cryptic splice site within exon 14, causing an out of frame deletion of the first 55 bp of exon 14 leading to a premature stop in exon 17	N/A	N/A
	FKBP14	c.2 T > G	VUS	-	N/A	N/A
	PLOD1	c.1302C > G	Likely pathogenic	p.Thr434X	N/A	N/A

Table 4 (continued)

	Gene	Variant	ACMG	Reported protein effect	MUpro	I-Mutant 2.0
Osteogenesis	COL1A1	c.3313delA	Likely pathogenic	p.Arg1105GlufsX3	N/A	N/A
	SCL2A2 (GLUT2)	C.685_701del	Likely pathogenic	p.A229QfsX19	N/A	N/A
	FKBP10	c.1257-2A > G // IVS7-2A > G	NA	p.H420PfsX12	N/A	N/A
	COL1A2	c.14929-14930TG > GT	NA	–	N/A	N/A
	FKBP10	c.204delCinsAAA	Likely pathogenic	p.His68GInfs*92	N/A	N/A
	FKBP10	c.976delA	Pathogenic	p.Met326Trpfs∗39	N/A	N/A
	MESD	c.676C > T	VUS	p.Arg226∗	N/A	N/A

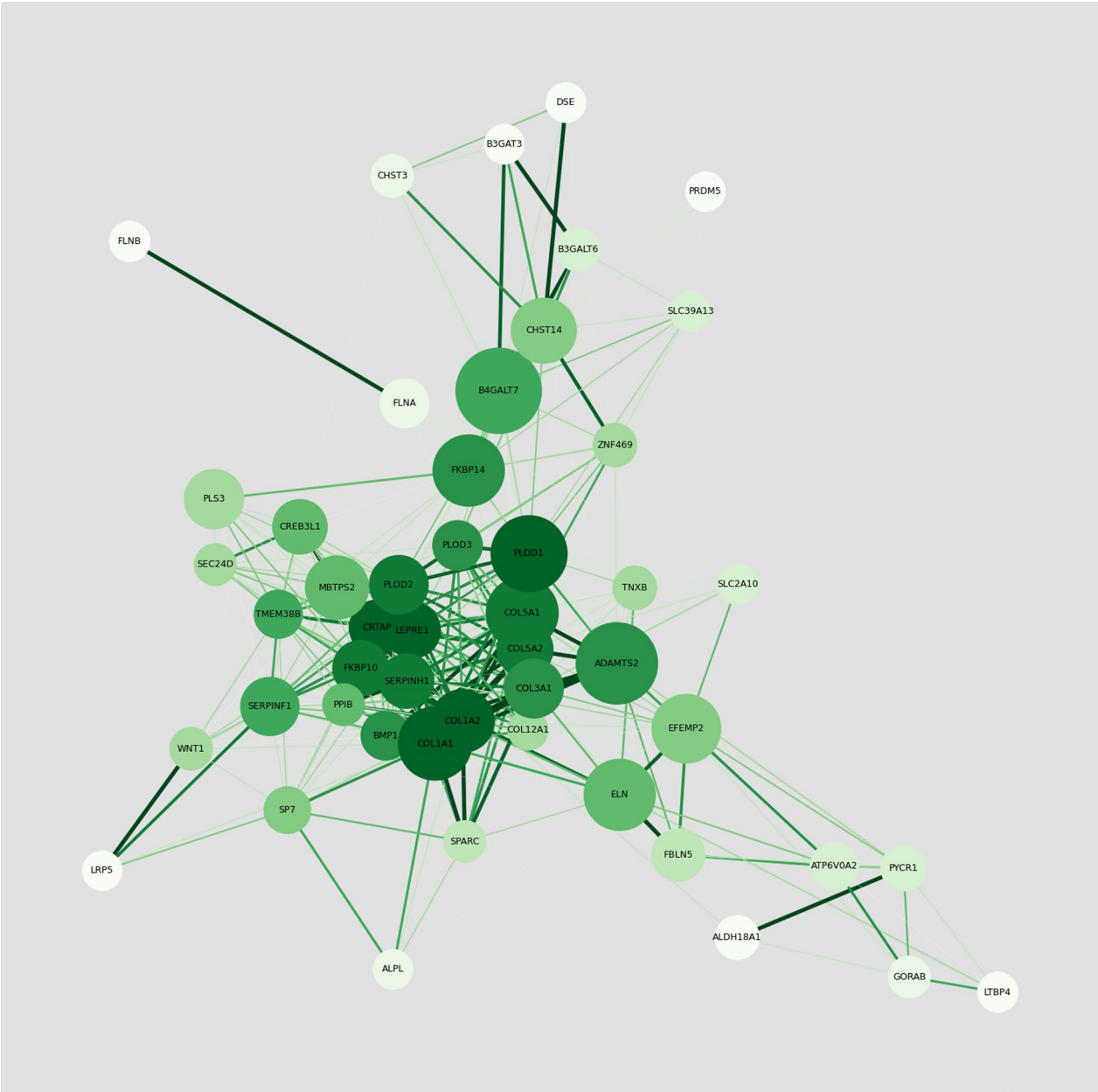

Fig. 3 Protein Interaction Network in EDS/OI/CL Overlap Phenotype. Using NetworkX Python Library, an Undirected Unweighted Graph is Visualized Based on the Betweenness Centrality

Iranome database reveals 46 probable disease-causing variants for EDS, OI and CL

A total number of 46 genes were investigated using the Iranome Database. They were previously reported to be related to CL, EDS, and OI syndromes. Searching a gene in Iranome provides all discovered variants—deletion, duplication, splice region, intronic, and single-nucleotide variants. Later on, missense variants of each gene were selected for further evaluation. Missense variants with more than 10 heterozygotes, a CADD score of at least 20 (which indicates the variant is one of the 1% most deleterious variants in the genome) [41], and at least three times reported as damaging in pathogenicity Web servers are considered as probable damaging variants. From all evaluated genes, 46 variants in 18 genes were found to have a probable damaging effect. They are listed in Table 5, along with populations with the most and the least frequency of the alleles in Iran.

Table 5 Probable Disease-Causing Variants of EDS/OI/CL Overlap Phenotype based on Iranome Database

Syndrome	Gene	Variant	Number of heterozygotes	CADD score	N of 6 damaging prediction	Population with the least frequency of allele	Population with the least frequency of allele
OI	P3H1 (LEPRE1)	c.1045G > A	77	31	6	Persian (0.025)	Persian (0.025)
	SERPINF1	c.395C > G	52	22	3	Baloch (0.005)	Lur (0.055)
CL	GSN	c.385G > A	47	34	5	N/A	Turkmen (0.075)
	GSN	c.1688C > G	67	21.8	4	N/A	Azeri (0.07)
	ATP6V0A2	c.440C > T	10	23.3	3	N/A	Persian Gulf Islander (0.045)
	ATP6V0A2	c.2438C > T	55	35	6	Lur, Persian, Turkmen (0.015)	Arab, Azeri, Persian Gulf Islander (0.07)
	ALD18A1	c.1115C > A	68	24.8	4	Baloch (0.005)	Arab, Kurd (0.065)
	ALD18A1	c.896C > T	177	25.4	3	Baloch (0.06)	Kurd (0.18)
	LTBP4	c.1903C > G	10	29.6	4	N/A	Turkmen (0.03)
	LTBP4	c.3419C > T	388	20.7	-	Kurd (0.36)	Baloch (0.525)
	LTBP4	c.4496A > T	23	23.4	-	Turkmen (0)	Arab (0.04)
	GORAB	c.958G > A	400	25.9	-	Persian Gulf Islander (0.36)	Turkmen (0.575)
	PYCR1	c.685C > T	127	28.2	5	Persian Gulf Islander (0.45(	Persian (0.135)
	ATP7A	c.2299G > C	185	26.7	5	Arab (0.285)	Persian Gulf Islander (0.45(
	RIN2	c.232G > A	13	28.4	5	Baloch (0)	Lur (0.02)
	RIN2	c.1789G > A	14	28..4	5	Baloch (0)	Lur (0.02)
EDS	COL3A1	c.1804C > A	23	23.2	4	Lur (0)	Arab, Azeri, Persian Gulf Islander, Turkmen (0.02)
	COL3A1	c.2002C > A	10	23.5	6	Persian, Persian gulf Islander (0)	Azeri, Kurd, Lur, Turkmen (0.01)
	PLOD1	c.391G > A	31	23.1	5	Turkmen (0.005)	Persian Gulf Islander (0.04)
	PLOD1	c.1675C > T	10	24.2	4	Lur, Turkmen (0)	Azeri (0.015)
	TNXB	c.12547G > A	76	33	5	Turkmen (0.01515)	Persian Gulf Islander (0.1222)
	TNXB	c.12520G > A	89	31	6	Persian (0.0641)	Tukrmen (0.25)
	TNXB	c.12224G > A	14	34	6	Baloch, Turkmen (0)	Lur (0.02)
	TNXB	c.12170A > T	335	22.4	4	Baloch (0.18)	Azeri (0.43)
	TNXB	c.11962C > A	32	23.5	4	Persian, Turkmen (0)	Persian Gulf Islander (0.1316)
	TNXB	c.11539G > A	29	24.9	3	Turkmen (0)	Azeri (0.05618)
	TNXB	c.10723 T > C	261	23.7	4	Turkmen (0.135)	Persian Gulf Islander (0.22)
	TNXB	c.8740G > A	11	33	5	Arab, Lur, Azeri, Persian, Persian Gulf Islander (0)	Baloch (0.045)
	TNXB	c.8542G > A	16	28.7	5	Persian Gulf Islander, Turkmen (0)	Baloch (0.05)
	TNXB	c.8111G > A	80	26.7	4	Arab (0.025)	Baloch (0.115)
	TNXB	c.7235C > T	73	29.5	5	Arab (0.025)	Baloch (0.115)
	TNXB	c.6379G > A	298	25.3	3	Turkmen (0.1758)	Baloch (0.385)
	TNXB	c.2485G > A	18	25.4	3	Persian Gulf Islander (0)	Arab, Lur, Persian (0.2)
	TNXB	c.607G > A	140	24	4	Persian Gulf Islander (0.08)	Arab (0.135)
	COL5A2	c.1081A > C	41	23.7	3	Kurd, Lur (0.015)	Arab (0.05)
	COL5A2	c.1378C > T	22	24.6	3	Azeri (0)	Arab, Persian, Persian Gulf Islander (0.2)

Table 5 (continued)

Syndrome	Gene	Variant	Number of heterozygotes	CADD score	N of 6 damaging prediction	Population with the least frequency of allele	Population with the least frequency of allele
	COL5A2	c.1535 T > C	21	22.8	3	Persian Gulf Islander (0)	Kurd (0.035)
	COL5A2	c.2498C > T	22	25.9	6	Persian Gulf Islander (0)	Kurd (0.04)
	COL5A1	c.4135C > T	14	25.8	5	Baloch, Lur (0)	Kurd (0.2)
	COL5A1	c.1588G > A	61	24.9	4	Persian Gulf Islander (0.2)	Persian (0.07)
	DSE	c.158C > T	68	28.5	3	Baloch (0.02)	Turkmen (0.07)
	DSE	c.266G > A	12	19.57	4	Persian, Kurd (0)	Arab, Baloch (0.015)
	DSE	c.901A > G	41	23.6	3	Turkmen (0.01)	Arab, Baloch (0.05)
	C1S	c.356G > A	138	25.1	5	Baloch (0.035)	Kurd (0.12)
	COL12A1	c.9172G > A	345	27.8	6	Baloch (0.57)	Turkmen (0.69)
	COL12A1	c.6479A > T	35	26.8	5	Baloch (0)	Kurd (0.045)

Discussion

Due to the clinical overlap between CL, EDS, and OI, it is difficult to provide proper follow-up care and genetic counseling. Their similar phenotypes increase the likelihood of misdiagnosis. The phenotypic overlap is likely due to the functional roles and interactions of genes associated with these syndromes. Consequently, traditional clinical guidelines and methods are no longer sufficient to differentiate between them. Whole-exome sequencing (WES) has the potential to enhance diagnostic capabilities significantly. This widely used next-generation sequencing (NGS) method is cost-effective, requires fewer sequencing reagents, and enables faster bioinformatic analysis compared to whole genome sequencing. Data collection revealed that genetic tests such as WES are rarely conducted in case studies, despite their potential to facilitate more precise diagnosis and more effective patient management. This study included 43 patients exhibiting the overlap phenotype of EDS/OI/CL, with a total of 32 genetic variants. Among these unrelated families, the rate of consanguineous marriage (CMR) was approximately 59%, with 12.5% reporting non-consanguineous marriages and 28.5% not disclosing their marital status. Out of the 32 variants identified, 12 were previously unreported and considered novel. In approximately 94% of cases, a sequencing method (direct, whole exome, or whole genome) was employed, successfully identifying the genetic variant. Figure 4 provides a graphical representation of all the reported variants in this cohort. Variants of *PYCR1* (a protein that helps mitochondrial proper functioning and synthesis of proline), *B3GALT6* (an enzyme essential for the manufacturing of ECM components), *FKBPs* (a family of chaperons that perform folding on proline-containing proteins), *FBLN5* (which has a variety of roles in ECM and also play a role in arteries development), and collagen genes were identified in more patients than others. There were 8, 6, 5, and 4 patients with mutations in *PYCR1*, *B3GALT6*, *FKBPs*, and collagen genes, respectively. The result of NetworkX interaction analysis also showed that these genes, along with *ADAMTS2*, *COL1A1*, *COLA1A2*, *CRTAP*, *LEPRE1*, *FBLN5*, *ATP6V0A2*, and *PLOD1*, have the most impact on their protein network. In addition to the direct roles these genes play in the production and structure of connective tissues, they also exert regulatory influence over each other's expression and function. This intricate network of regulatory interactions highlights the complexity of connective tissue homeostasis and the challenges in pinpointing the specific genetic defect responsible for each case. *PYCR1*, a transcription factor, orchestrates the expression of genes involved in collagen synthesis and remodeling. Mutations in *PYCR1* are linked to hypermobile Ehlers–Danlos syndrome (hEDS), characterized by loose joints and hyperextensibility, as well as Cutis Laxa type 2B [42]. *B3GALT6*, encoding beta-galactoside 3-O-acetyltransferase, contributes to the synthesis of glycosaminoglycans (GAGs), essential components of the extracellular matrix. Deficiencies in *B3GALT6* lead to brittle bone disease with severe skin, joint, and eye involvement (BBSJI), demonstrating the intricate relationship between GAGs and connective tissue health [37]. FKBPs, a family of heat shock protein (HSP) binders, safeguard cells from stress-induced damage. Mutations in *FKBP* genes are associated with Ehlers–Danlos

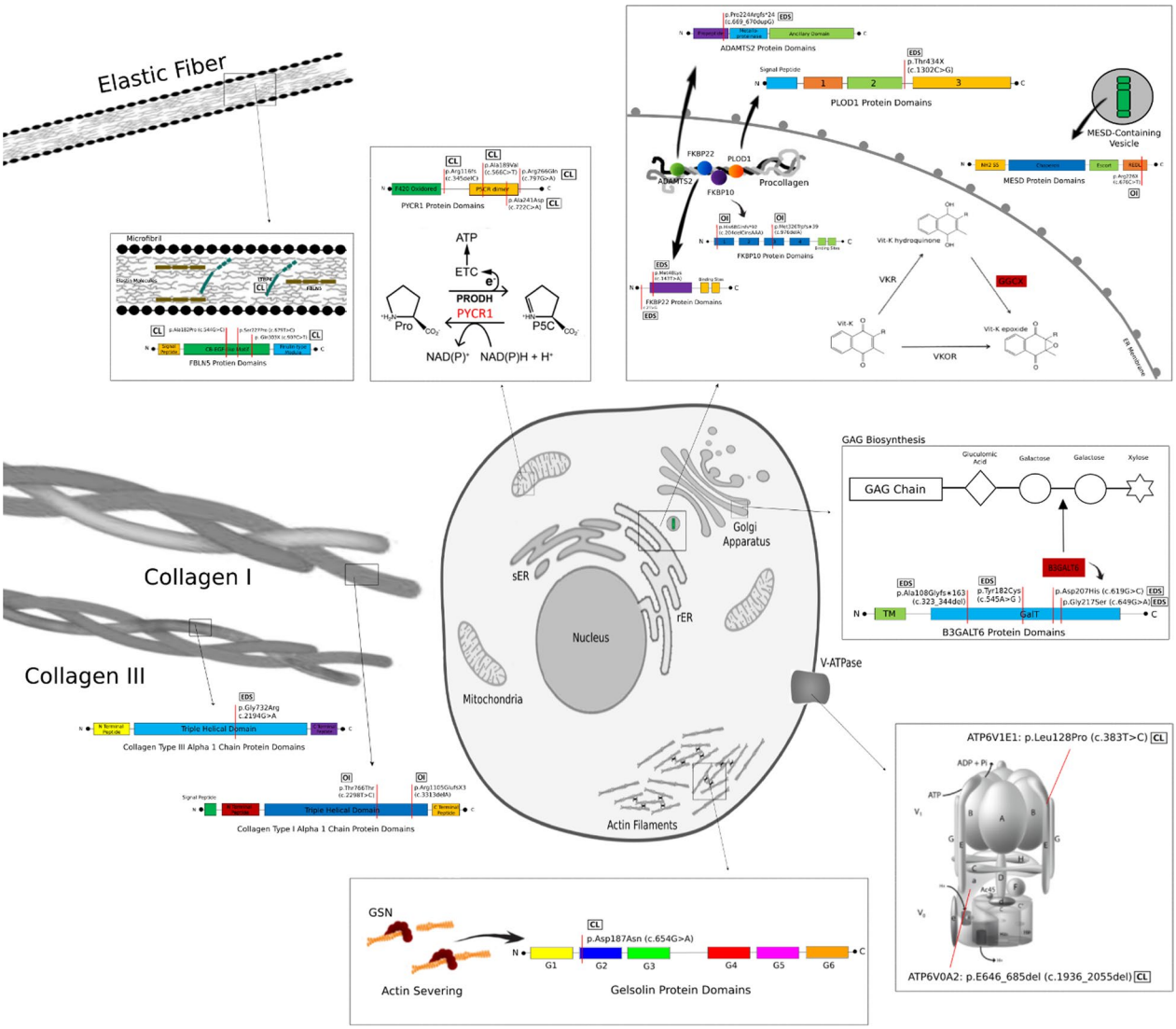

Fig. 4 Schematic Illustration of Genes and Variants Involved in EDS/OI/CL Overlapping Phenotype. (Red lines show the position of variants at the protein domains)

syndrome type VII, highlighting the importance of HSPs in connective tissue homeostasis [58]. *ADAMTS2*, encoding a collagen-cleaving enzyme, regulates collagen fiber degradation, influencing tissue flexibility and strength. Mutations in *ADAMTS2* are linked to classical Ehlers–Danlos syndrome (cEDS), characterized by hyperextensibility, easy bruising, and fragile skin [3]. *COL1A1* and *COLA1A2*, encoding the alpha 1 and alpha 2 chains of type I collagen, the most abundant type of collagen in the body, are essential for connective tissue integrity. Mutations in these genes are associated with various EDS subtypes, including cEDS, dermatosparaxis, and osteogenesis imperfecta type VI, emphasizing the critical role of type I collagen in connective tissue function [18]. Moreover, our investigation of all reported EDS, OI, and CL genes using the Iranome database reveals 46 variants that are dormant in heterozygous carriers with different frequencies in each ethnic group. Considering the high CMR in the country, it is probable for these heterozygous variants to rise in the next generation as a homozygous form, especially for populations with a high frequency of disease-causing alleles [1, 15]. For example, Baloch, Iranian Arab, and Kurd populations have the highest allele frequency for more than 6 variants of EDS. In this regard, carrier screening could be an effective strategy to prevent the birth of affected offspring [23]. Also, less than 1 percent of reported patients have undergone genetic study. This fact, which limited our cohort number, necessitates

performing genetic tests on more patients in future studies.

The literature data on EDS, OI, and CL overlap phenotypes are limited and may have some biases, as studies may have been conducted in specific populations or may have focused on particular clinical presentations. Future studies should aim to recruit more diverse patient cohorts and utilize standardized clinical diagnostic criteria to enhance the generalizability of findings.

While in silico tools and databases can serve as valuable resources for identifying potential disease-causing variants, it is crucial to acknowledge their limitations. These tools are still under development and may not always accurately predict pathogenicity, particularly for rare or novel variants. The reliability of these predictions can be enhanced by validation through experimental data, such as functional studies or animal models. It is important to note that the present study primarily encompasses the Iranian population. Therefore, the findings may not be entirely representative of other ethnic or geographical groups. Future research endeavors should aim to investigate these phenotypes in a broader range of populations to enhance the universality and applicability of the results. This will contribute to a more comprehensive understanding of EDS, OI, and CL overlap phenotypes across diverse populations.

Acknowledgements
Thanks to Human Genetics department of Golestan University of Medical Sciences for their support.

Author contributions
TK involved in methodology, investigation, formal analysis, software, visualization, writing of original draft. KN took part in investigation, software, formal analysis. FV involved in methodology, investigation, writing of original draft. AMF took part in methodology. MO involved in conceptualization, supervision, writing (review & editing), validation, data curation.

Funding
This study was financially supported by Golestan University of Medical Sciences (Grant Number: 111672).

Availability of data and materials
The datasets generated during and/or analyzed during the current study are available upon request.

Declarations

Ethics approval and consent to participate
The present study is approved by Ethics Committee of Golestan University of Medical Sciences (Code: IR.GOUMS.REC.1399.382).

Consent for publication
The consent for publication is not applicable for this study.

Competing interests
Authors of the study declared no conflict of interest.

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Received: 6 May 2023 Accepted: 16 January 2024
Published online: 23 January 2024

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