Discovery of *AEBP1*-related Ehlers-Danlos Syndrome

The Ehlers Danlos Society

Expansion of understanding of EDS & Related Disorders

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SCIENTIFIC MEETING - RARER TYPES
TOKYO 2019

REPORT

Biallelic Alterations in *AEBP1* Lead to Defective Collagen Assembly and Connective Tissue Structure Resulting in a Variant of Ehlers-Danlos Syndrome

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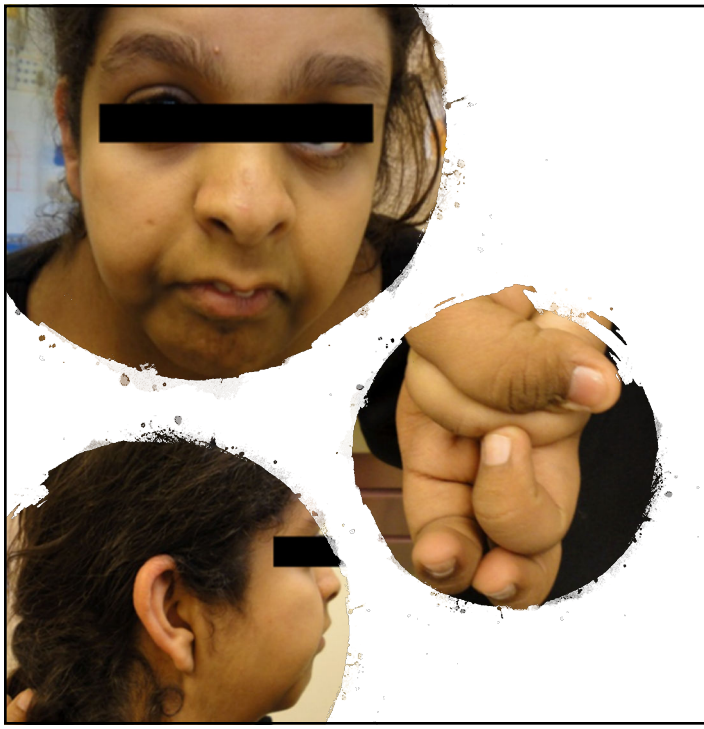
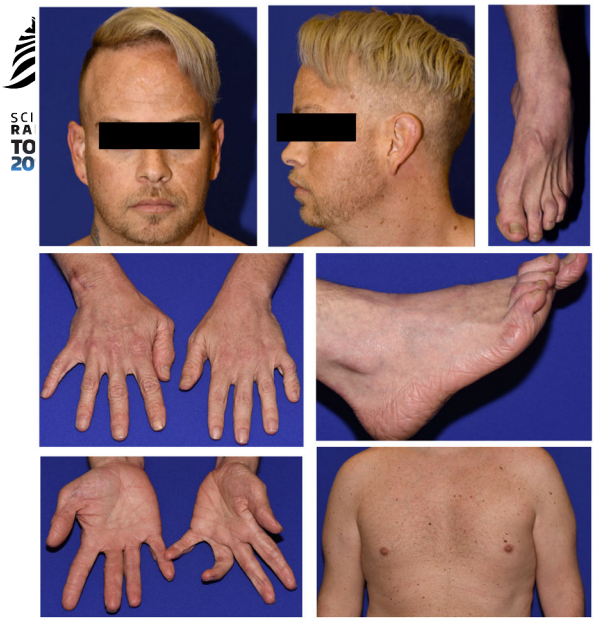
Am J Hum Genet. 2018;102(4):696–705. doi:10.1016/j.ajhg.2018.02.018

Outline

- Presentation of collaborative story & clinical cases
- Background on *AEBP1*, AEBP1/ACLP
- Future Directions



- Alazami et al. 2016 reported on two siblings with a homozygous c.1630+1G>A splice site pathogenic variant in *AEBP1*
- The siblings were the product of consanguineous union born to first cousin parents
- Presented with severe joint and skin laxity, dysmorphic facial features, severe osteopenia, soft and redundant skin, and dislocated hips, knees and ankles
- This was the first report of an *AEBP1*-related disorder causing a human phenotype



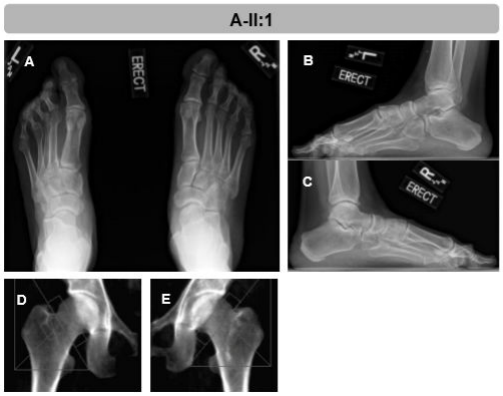
P1 Proband Phenotype:

- 34-year-old male
- Joint hypermobility and joint pain.
- Shoulder and hip subluxations
- Born with contractures involving left hand and cannot straighten all of his fingers on either hand
- High arches in feet and hammertoes affecting both feet
- Excess wrinkled skin on his hands and feet.
- Mitral valve prolapse, syncope, cryptorchidism, chronic constipation, impaired temperature sensation, poor wound healing, abnormal (prolonged) bleeding, and chronic dry eyes

- Exome: Compound heterozygous truncating variants in *AEBP1* in trans
- c.1470delC, p.Asn490LysfsX6
- c.1743C>A, p.Cys581Ter

P1 Bone Findings

Supplemental Figure 1. Bone densitometry and partial skeletal survey of subject A-II:1



(A-C) Standing X-rays of left and right foot. This individual was noted to have bilateral lesser hammertoe and hallux valgus deformities. Moderate bilateral great toe metatarsophalangeal joint osteoarthritis, right greater than left was also noted with diffuse mild osteoarthritis throughout both mid feet and forefeet. An osteochondral lesion of the right talar head can be seen on the dorsal plantar radiograph. (D,E) Dual-energy X-ray absorptiometry (Lunar iDXA) revealed a hip bone mineral density (BMD) that fell below the expected range for this individual's age, consistent with osteoporosis.

Genetic Analysis P1 – Exome Report

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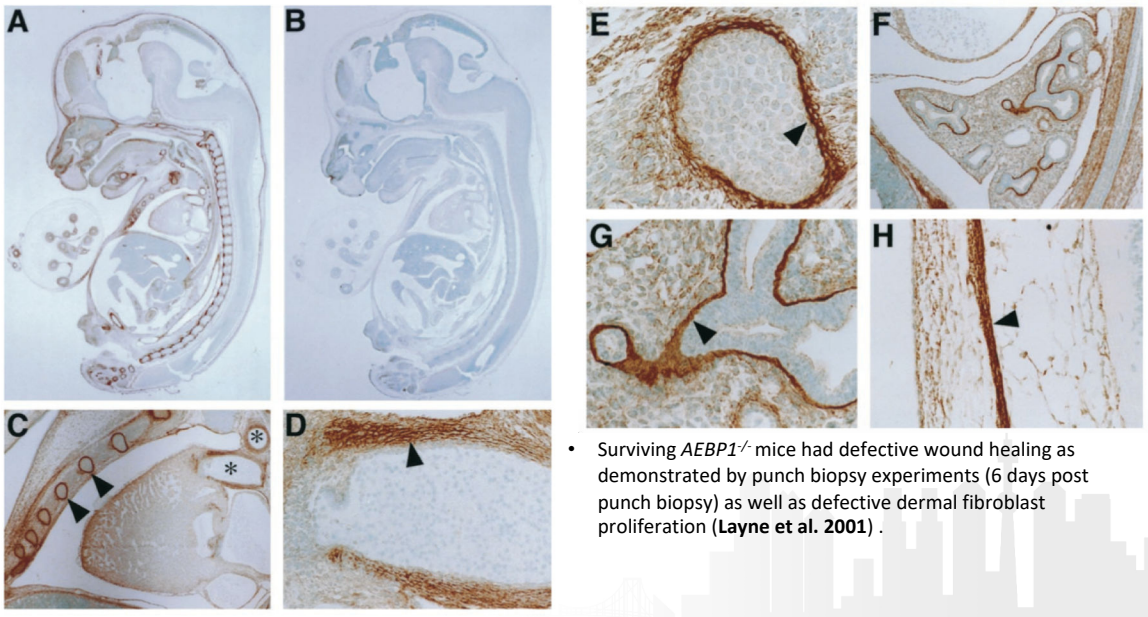
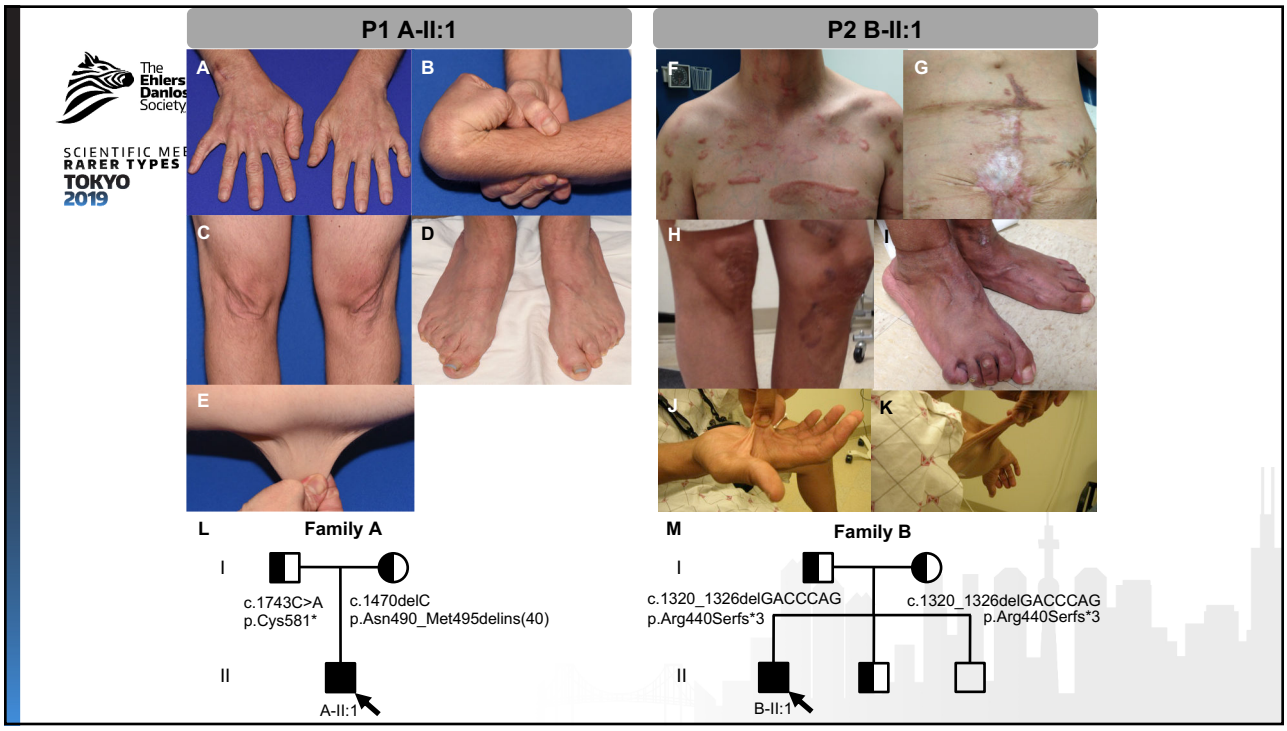
AEBP1 **COMPOUND HET** **p.N490KfsX6 (Mother), p.C581X (Father)**
Disease: None currently described
ESP & ExAC: ESP AA: N/R; ESP EA: N/R; ExAC: N/A
ESP AA: N/R; ESP EA: N/R; ExAC: 1/113984
***In silico* (SIFT/Polyphen/Mutation Taster):** N/A
Location: Exon 12 of 21
Exon 15 of 21

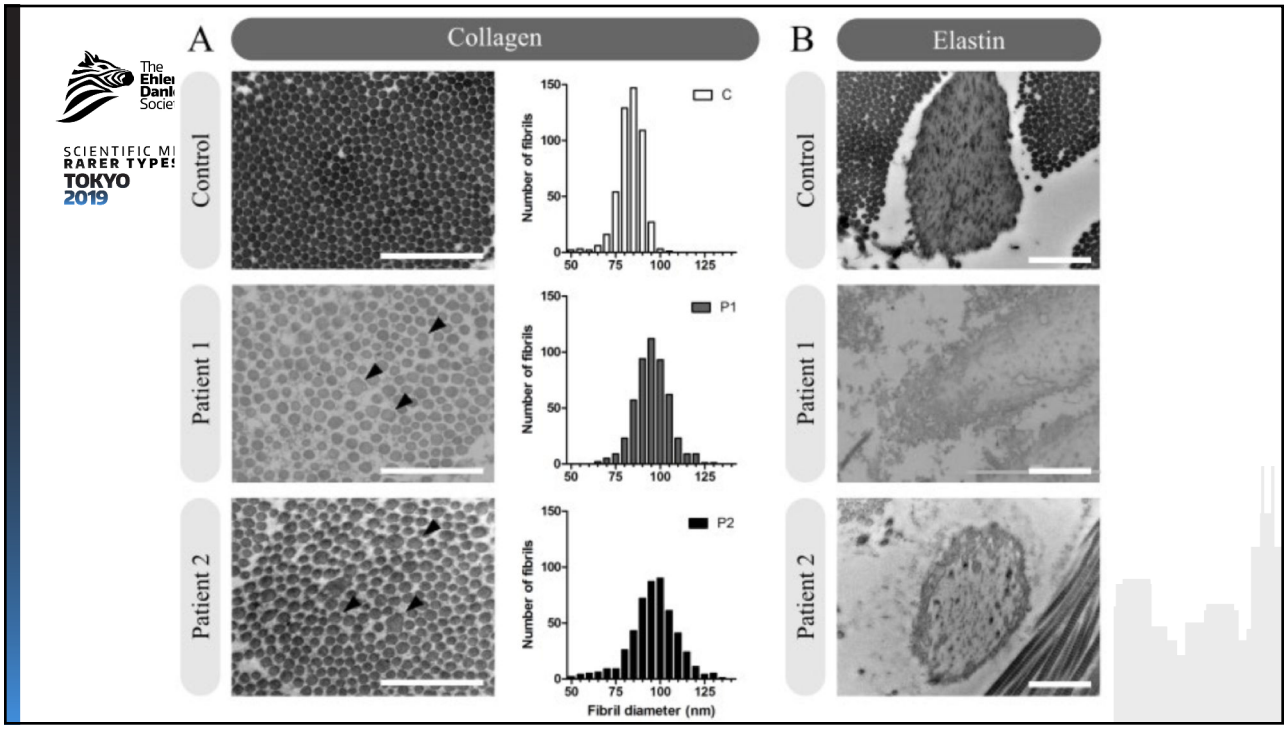
Comments: *AEBP1* encodes adipocyte enhancer binding protein (ABEP1). ***AEBP1* is alternatively spliced** and has **two isoforms** that display differential expression patterns (Feldkamp et al., 2012). The shorter of the two isoforms, (**AEBP1**) is an **intracellular protein involved in proinflammatory function** and is thought to play a key role in **apoptosis and cell survival** (Feldkamp et al., 2012).
The larger isoform, **aortic carboxypeptidase like protein (ACLP) associates with the extracellular matrix** and appears to play an important role in development and **adipogenesis through expression in vascular smooth muscle**, **the skeleton, lungs, and dermal layer of the skin** (Feldkamp et al., 2012; Ro et al., 2007). ***Aclp*$^{-/-}$ animal models develop gastroschisis, deficient wound repair, and poor fibroblast proliferation** (MIM# 602981).

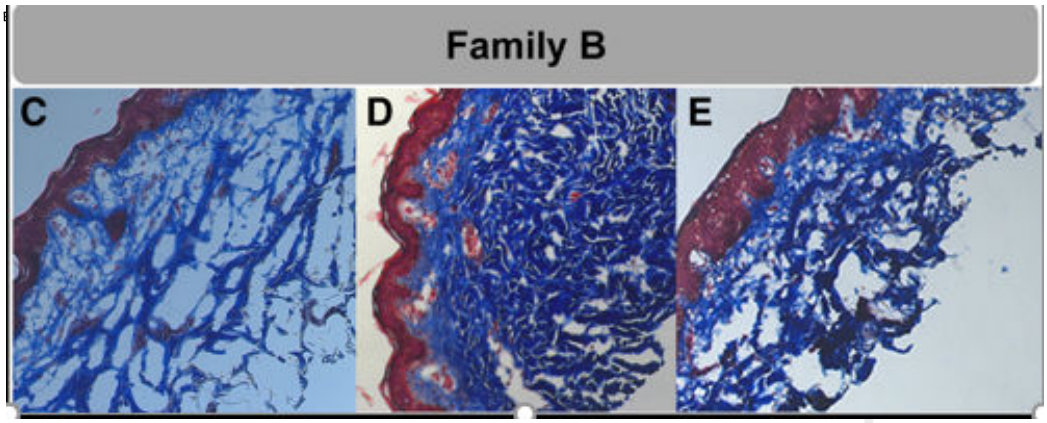
AEBP1$^{-/-}$ Mouse Model



- Surviving *AEBP1*$^{-/-}$ mice had defective wound healing as demonstrated by punch biopsy experiments (6 days post punch biopsy) as well as defective dermal fibroblast proliferation (**Layne et al. 2001**) .







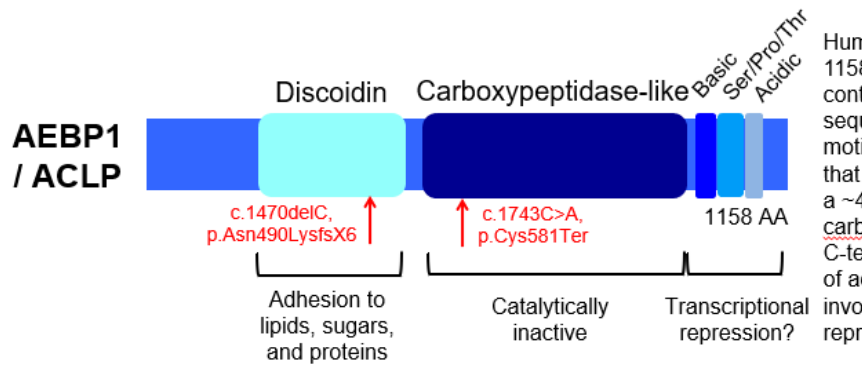
(C–E) Masson's trichrome staining of skin biopsies from family B show decreased dermal collagen in the proband. (C) B-II:1, the affected proband; (D) carrier brother; (E) carrier mother. Blue, dermis containing collagen bundles; red, epidermis. Original magnification, 100×.

Electron Microscopy

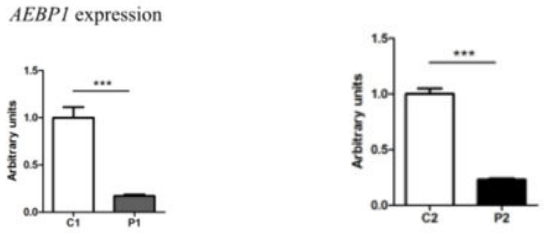
Collagen fibrils show moderate variation in size and scattered collagen flowers (composite collagen fibrils)

Overall decreased dermal collagen

Have a frayed/ragged moth-eaten appearance

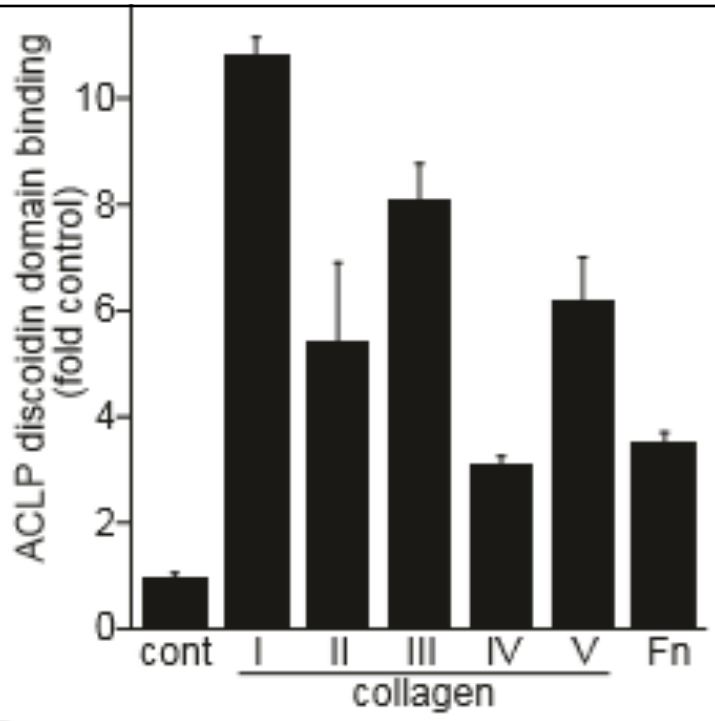


Human AEBP1 is made up of 1158 amino acids (AA), and contains a 25 AA signal sequence, an 11 AA repeating motif, a discoidin-like domain that is ~156 AA in length, and a ~422 AA inactive carboxypeptidase domain. The C-terminal domain is made up of acidic residues and is likely involved in transcriptional repression.



Loss of AEBP1 expression in P1 / P2

- The discoidin domain of ACLP binds to fibrillar collagen and enhances polymerization of collagen *in vitro*
- Fn = fibronectin



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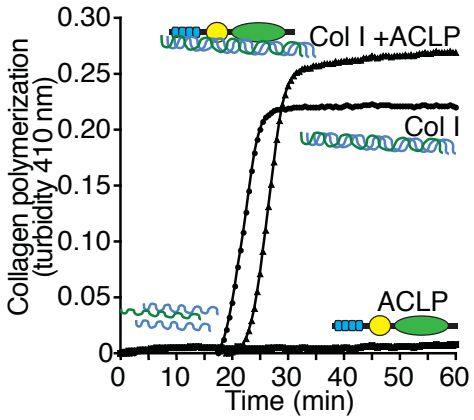

ACLP enhances in vitro collagen polymerization:

Denatured type I collagen was allowed to polymerize in PBS with or without rACLP for 1 h at 37°C. Collagen polymerization (turbidity at 410 nm) was measured at 30 s intervals. Note: increased collagen polymerization in the presence of ACLP.

Matthew Layne, BUSM

PBS = Phophate-buffered saline

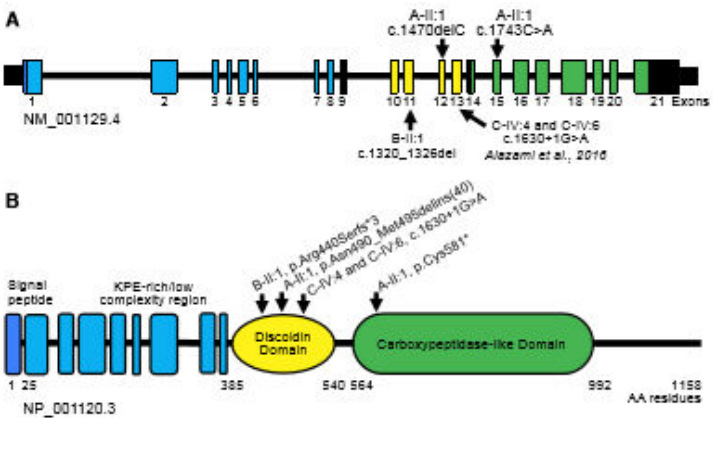

Figure 2. Schematic Representation of *AEBP1*, ACLP Protein Structure, and Summary of Known Mutations

(A) Exome sequencing revealed compound heterozygous (A-II:1) and homozygous (B-II:1) nonsense and small deletion frameshift variants in *AEBP1*. The homozygous splice site variant described in two siblings (C-IV:4 and C-IV:6) with similar clinical features by Alazami et al.[9] is also shown. *AEBP1* encodes the collagen binding protein.

(B) Aortic carboxypeptidase-like protein (ACLP) has several distinct domains that are thought to mediate different protein-protein interactions including a central discoidin domain that helps mediate binding and specificity for fibrillar collagens. ACLP also contains a catalytically inactive metallocarboxypeptidase-like domain. Mutations identified fell within the discoidin domain or immediately downstream and included nonsense, frameshift, and canonical splice site variants that were predicted to result in loss of function.

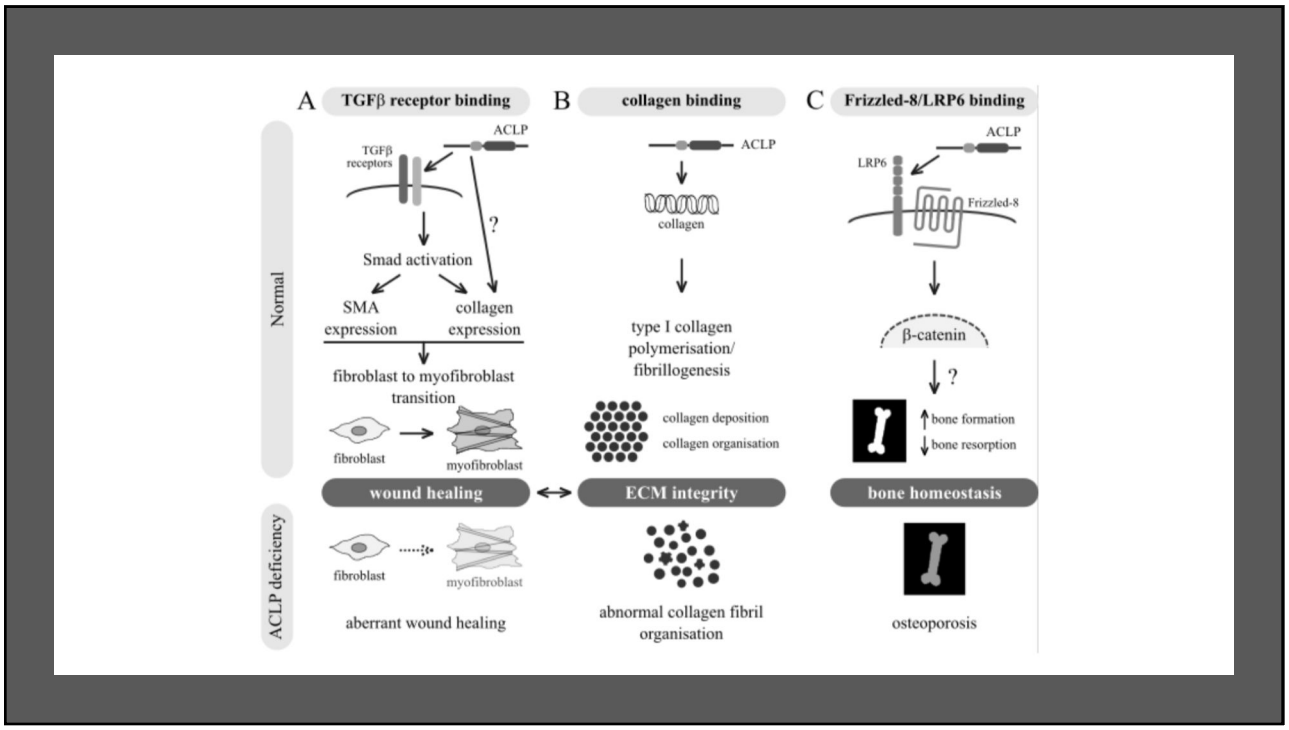

Table 1. Summary of Clinical Characteristics of Individuals with Autosomal-Recessive Mutations in *AEBP1*

	A-II:1	B-II:1	C-IV:6	C-IV:4
Citation	this report	this report	Alazami et al.[9]	Alazami et al.[9]
Sex	male	male	female	male
Ethnicity	white	white	Middle Eastern	Middle Eastern
Age at last evaluation	35 y	33 y	12 y	24 y
AEBP1 variant(s) (NM_001129.4)	c.1470delC; c.1743C>A; compound het	c.1320_1326del; homozygous	c.1630+1G>A; homozygous	c.1630+1G>A; homozygous
Protein change (NP_001120.3)	p.Asn490_Met495delins(40); p.Cys581*	p.Arg440Serfs*3	p.?	p.?
Beighton score	8 out of 9	8 out of 9	8 out of 9	unknown
Generalized joint hypermobility	+	+	+	+
Hip dislocation	hip subluxations reported	congenital hip dislocation, surgically corrected at 18 mo	dislocations reported	unknown
Shoulder dislocation	not reported	+	+	unknown
Foot deformities	pes planus, hallux valgus, hammer toes	pes planus, hallux valgus, hammer toes	pes planus, hallux valgus, hammer toes	pes planus, hallux valgus, toe deformities
Skin hyperextensibility	+	+	+	+
Excess skin/skin folding	increased wrinkles on hands and feet (acrogeria-like)	+	+	+
Delayed wound healing	+	+	+	+
Abnormal scarring	atrophic, widened scars	atrophic, widened scars with hyperpigmentation	hyperpigmented atrophic scars, multiple keloids	unknown
Easy bruising	+	+	+	unknown
Hernia	not reported	large ventral hernia developed at surgical sites secondary to ruptured bowel	umbilical, ventral, and inguinal	unknown
Genitourinary abnormalities	cryptorchidism, surgically corrected at 15 years of age	not reported	not reported	not reported
Gastrointestinal abnormalities	motility issues	bowel rupture	not reported	not reported

Table 1. *Continued*

	A-II:1	B-II:1	C-IV:6	C-IV:4
Skeletal anomalies	severe osteopenia involving the hips	hip replacement for severe osteopenia, upper thoracic scoliosis with degenerative disease and facet arthrosis of spine	skull shows 'copper beaten' appearance, severe osteopenia, narrowing of the interpedicular distance of the lumbar spines distally, iliac bones are short and squared, long bones of the lower extremities are remodeled	severe osteopenia
MRI findings	mild disc bulging at the C4-5 and C7-T1 levels	empty sella	not done	not done
Other	delays in walking and acquisition of fine motor skills, impaired temperature sensation, keratoconjunctivitis sicca, piezogenic papules on feet, right distal radioulnar joint dislocation, surgically repaired	elbow bursitis, piezogenic papules on feet, sacral dimple, and hypertriglyceridemia	uncontrolled diabetes mellitus, recurrent cellulitis	none

+ indicates clinical feature is present.

Genes (Basel). 2019 Feb 12;10(2). pii: E135. doi: 10.3390/genes10020135.

Expanding the Clinical and Mutational Spectrum of Recessive *AEBP1*-Related Classical-Like Ehlers-Danlos Syndrome.

Ritelli M[1], Cinquina V[2], Venturini M[3], Pezzaioli L[4,5], Formenti AM[6], Chiarelli N[7], Colombi M[8].

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Abstract

Ehlers-Danlos syndrome (EDS) comprises clinically heterogeneous connective tissue disorders with diverse molecular etiologies. The 2017 International Classification for EDS recognized 13 distinct subtypes caused by pathogenic variants in 19 genes mainly encoding fibrillar collagens and collagen-modifying or processing proteins. Recently, a new EDS subtype, i.e., classical-like EDS type 2, was defined after the identification, in six patients with clinical findings reminiscent of EDS, of recessive alterations in *AEBP1*, which encodes the aortic carboxypeptidase-like protein associating with collagens in the extracellular matrix. Herein, we report on a 53-year-old patient, born from healthy second-cousins, who fitted the diagnostic criteria for classical EDS (cEDS) for the presence of hyperextensible skin with multiple atrophic scars, generalized joint hypermobility, and other minor criteria. Molecular analyses of cEDS genes did not identify any causal variant. Therefore, *AEBP1* sequencing was performed that revealed homozygosity for the rare c.1925T>C p.(Leu642Pro) variant classified as likely pathogenetic (class 4) according to the American College of Medical Genetics and Genomics (ACMG) guidelines. The comparison of the patient's features with those of the other patients reported up to now and the identification of the first missense variant likely associated with the condition offer future perspectives for EDS nosology and research in this field.

Hum Mol Genet. 2019 Jun 1;28(11):1853-1864. doi: 10.1093/hmg/ddz024.

Bi-allelic AEBP1 mutations in two patients with Ehlers-Danlos syndrome.

Syx D[1], De Wandele I[1], Symoens S[1], De Rycke R[2,3], Hougrand O[4], Voermans N[5], De Paepe A[1], Malfait F[1].

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Abstract

The Ehlers-Danlos syndromes (EDSs) are a clinically and molecularly diverse group of heritable connective tissue disorders caused by defects in a wide range of genes. Recently, bi-allelic loss-of-function mutations in the adipocyte enhancer-binding protein 1 (AEBP1) gene were reported in three families with an autosomal recessive EDS-like condition characterized by thin and hyperextensible skin, poor wound healing with prominent atrophic scarring, joint hypermobility and osteoporosis. Using whole exome sequencing, we identified novel bi-allelic AEBP1 variants in two unrelated adult patients, previously diagnosed with an undefined EDS type, which shows important clinical resemblance to several other EDS subtypes. Our patients present with similar cutaneous and musculoskeletal features as the previously reported patients. They also show unreported clinical features, including pectus deformity, premature aged appearance, sparse and frizzled hair, fatigue and pain. AEBP1 is ubiquitously expressed and encodes the secreted aortic carboxypeptidase-like protein (ACLP) that can bind fibrillar collagens and assist in collagen polymerization. Transmission electron microscopy studies on the patients' skin biopsies show ultrastructural alterations in collagen fibril diameter and appearance, underscoring an important role for ACLP in collagen fibril organization. This report further expands the clinical, molecular and ultrastructural spectrum associated with AEBP1 defects and highlights the complex and variable phenotype associated with this new EDS variant.

Summary

Described 4 affected individuals in 3 families with AEBP1 pathogenic variants

Additional 5 identified since publication (siblings in Germany) and 2 abstracts in prior slide

Present with recognizable pattern of connective tissue disorder that falls in EDS spectrum:

Joint hypermobility	Skin Laxity	Delayed wound healing	Abnormal Scarring	Hernias	Osteoporosis / reduction in bone mineral density

ACLP protein is expressed in skin, vasculature, other connective tissue and plays critical role in extracellular matrix maintenance and tissue repair

Contributors & Collaborators

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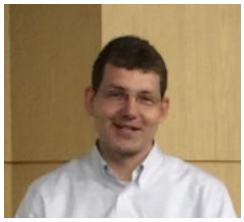

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Questions