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Supplemental Data

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

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Supplemental Note

Case Reports

Subject A-II:1 is a 35-year-old white male of German and Panamanian ancestry who has been followed from a young age. He exhibited a complicated history of preterm birth and was noted to have several congenital abnormalities including cryptorchidism that was repaired at age 15, redundant skin, and joint laxity. He also reported delays in walking and difficulty with fine motor skills in his left hand. On recent examination, this individual was found to have normal motor function with no focal deficits. The subject exhibited increased wrinkles on his hands and feet, right shoulder and hip subluxations, hammertoes, chronic constipation, impaired temperature sensation, poor wound healing, and abnormal bleeding (Figure 1A-E). The subject was diagnosed with EDS at 15 years of age and previous genetic testing for variants in collagen genes was unrevealing.

A workup revealed no kyphoscoliosis or hernias, negative Gorlin sign, and normal colored sclera with possible keratoconus present. The subject's palms were hyperlinear and atrophic and the dorsal sides were atrophic, hyperlinear, and extensible (Figure 1). He has extremely extensible skin with retained elasticity at the elbows; his knees also exhibited extremely extensible skin with subtle laxity and redundancy noted when the knee was in the extended position (Figure 1). He has widened fishmouth-like atrophic scars on his left knee and back, pes planus, and piezogenic papules on his feet. The subject had a Beighton score of 8 out of 9.

The subject had a history of syncope in his youth and has intermittent palpitations particularly at night. He was found to have mitral valve prolapse on a previous evaluation. A vascular workup, which included magnetic resonance angiography (MRA) of the head, neck, and chest, revealed no evidence of stenosis, aneurysm, or dissection and normal vascular architecture with the exception of the left vertebral artery, which is diffusely small in caliber developmentally. There were no other similarly affected individuals in the subject's family (Figure 1L). Currently, he has no history of hypertension, hyperlipidemia, or diabetes.

X-ray examination of the spine showed straightening of the normal lumbar lordotic curve with lower lumbar facet arthrosis with minimal disc space narrowing and retrolisthesis L5-S1. There was also mild disc space narrowing L4-L5. The remaining disc spaces were preserved. There was proliferative degenerative wedging at the thoracolumbar junction. There was mild disc space narrowing and anterior subluxation at C5-C6 with similar changes at C6-C7. There was also lower cervical facet arthrosis with slight anterior subluxation and disc space narrowing at C7-T1. No fractures were observed.

Dual-energy X-ray absorptiometry (Lunar iDXA) revealed a hip bone mineral density that fell significantly below the expected range for the subject's age, consistent with osteoporosis (Figure S1). Total BMD for left hip is 0.781 g/cm^2,

with T-Score of -1.8 and Z-Score of -2.1; total BMD for right hip is 0.789 g/cm^2, with T-Score of -1.7 and Z-Score of -2.0; total BMD for left femoral neck is 0.816 g/cm2, with a T-Score -1.6 and Z-Score of -1.8; total BMD for right femoral neck is 0.884 g/cm^2, with a T-Score -1.1 and Z-Score of -1.3.

Whole exome sequencing was performed by GeneDx (Gaithersburg, MD) using the Agilent Clinical Research Exome kit to target the exonic regions and flanking splice junctions of the genome. These targeted regions were sequenced on an Illumina HiSeq 2000 sequencing system with 100 bp paired-end reads. Bi-directional sequence was assembled, aligned to reference gene sequences based on human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method was used to confirm all potentially pathogenic variants identified in this individual and their relatives. Sequence alterations were reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines. The exome was covered to a mean depth of 121x, with a quality threshold of 94.3%.

Subject B-II:1 is a 41-year-old white male of Italian ancestry who had been followed since birth for congenital hip dislocation, easy bruising, and repeat dislocation of his shoulders. He had surgical correction at 18 months for dislocated hip, which healed with a large scar (Figure S2). The subject's scars are unusual and appear widened, spread, hyperpigmented, and atrophic (Figure

1). He developed elbow bursitis at the age of 21, which required surgical evacuation. At 27 years of age he was hospitalized for ruptured bowel, which was thought to be due to diverticulosis. The surgeon noted abnormal tissue during the procedure and repeated attempts to re-anastomose the bowel failed and a colostomy was required (Figure 1G). A large ventral hernia developed at one of the surgical sites and severe scarring was apparent. The subject later had his colostomy reversed and ventral hernia closed. Additionally, the subject has micrognathia, downsloping shoulders, soft, hyperextensible skin, piezogenic papules on his feet, severe pes planus, sacral dimple, and hypertriglyceridemia (Table 1). When the subject presented to medical genetics, vascular-type EDS was considered based on the presence of hollow organ rupture, impaired wound healing, abnormal scaring, and joint dislocations. The subject had a Beighton score of 8 out of 9. Sequencing analysis and deletion/duplication studies of *COL3A1* revealed no variants in this gene. The subject's family does not have a history of connective tissue disease (Figure 1M).

When this individual was 32 years of age, he had an echocardiogram that showed a hyperdynamic left ventricle that was normal in size with an estimated ejection fraction of 65-70%. The right ventricle was normal. The mitral valve was normal in structure and function but had a slight focal prolapse posterior leaflet with mild mitral regurgitation. The tricuspid valve was normal with trace regurgitation. Measurements of the great vessels showed an aortic annulus

measuring 2.2 cm, the sinuses of Valsalva measuring 3.8 cm, the sino-tubular junction measuring 3.8 cm and the ascending aorta measuring 3.6 cm.

A repeat echo at 36 years of age showed aortic root dilation with other parameters largely unchanged from the previous exam. Measurements of the great vessels showed an aortic annulus measuring 2.3 cm, the sinuses of Valsalva measuring 4.0 cm, the sino-tubular junction measuring 2.6 cm and the ascending aorta measuring 3.1 cm. The aortic root diameter was calculated to be 4.0 cm.

Contrast MRA was also performed on this individual at this time and confirmed a dilated aortic root measuring 4.2 cm. The descending aorta was normal in size and the aortic artery was unremarkable as was the descending thoracic aorta. The abdominal aorta including the celiac artery, superior mesenteric artery, and a separate common hepatic artery between the celiac and superior mesenteric artery were normal with no evidence of aortic aneurysm. These measurements are indicative of aortic dilation. Correspondence with this individual after the NIH study was closed revealed that his aortic root had dilated further and surgery was required.

MRI of the brain and spine revealed an empty sella as well as upper thoracic scoliosis with degenerative disease and facet arthrosis of spine. Dual-energy X-ray absorptiometry at 36 years of age showed a hip BMD that fell below the

expected range for this individual's age, consistent with osteoporosis. His total BMD was 1.002 g/cm^2 with a T-Score -2.7 and Z-Score of -2.1. Total BMD for left hip was 0.747 g/cm^2, with T-Score of -2.5 and Z-Score of -2.0; total BMD for right hip was 1.059 g/cm^2, with T-Score of -0.3 and Z-Score of 0.2; total BMD for left femoral neck is 0.808 g/cm^2, with a T-Score -2.0 and Z-Score of -1.5; total BMD for right femoral neck is 1.135 g/cm^2, with a T-Score 0.5 and Z-Score of 1.0. He later developed severe degenerative disease of the hips requiring hip replacement.

Genomic DNA was extracted from whole blood collected from subject B-II:1 and both parents and was fragmented and subjected to whole-exome capture with the SureSelect Human All Exon 50Mb kit (Agilent Technologies) following the manufacturer's protocols. Exome capture libraries were then sequenced on the Illumina HiSeq 2000 platform according to the manufacturer's instructions, and 100-bp paired-end reads were generated. Sequencing and subsequent analysis including alignment and variant calling were performed at the Beijing Genomics Institute (BGI Inc., Shenzhen, China). Potentially pathogenic variants were confirmed by Sanger sequencing on the ABI Genetic Analyzer 3130xl (Applied Biosystems, Foster City, CA). Absence of variants in *COL3A1*, *COL5A1* and *PLOD1* was confirmed by Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA) using probes set P155 (EDS probemix, MRC-Holland, Amsterdam, Netherlands).

Subject C-IV:6 initially described in Alazami et al. (Family 1 ID: 14DG1601) was born post-term by C-section at 42 weeks gestation with a birth weight of 4.3 kg. She was admitted to intensive care because of hypotonia and poor feeding and was noted to have severe joint and skin laxity and talipes deformity. She had delayed motor milestones that improved with age. She walked at 20 months but attained speech at an appropriate age. There is family consanguinity with parents being double 1st cousins with 7 siblings; one brother (IV:4) manifested with similar features which were diagnosed as myopathy of unknown etiology. Genetic testing showed that he was also homozygous for the c.1630+1G>A splice site variant in *AEBP1*.

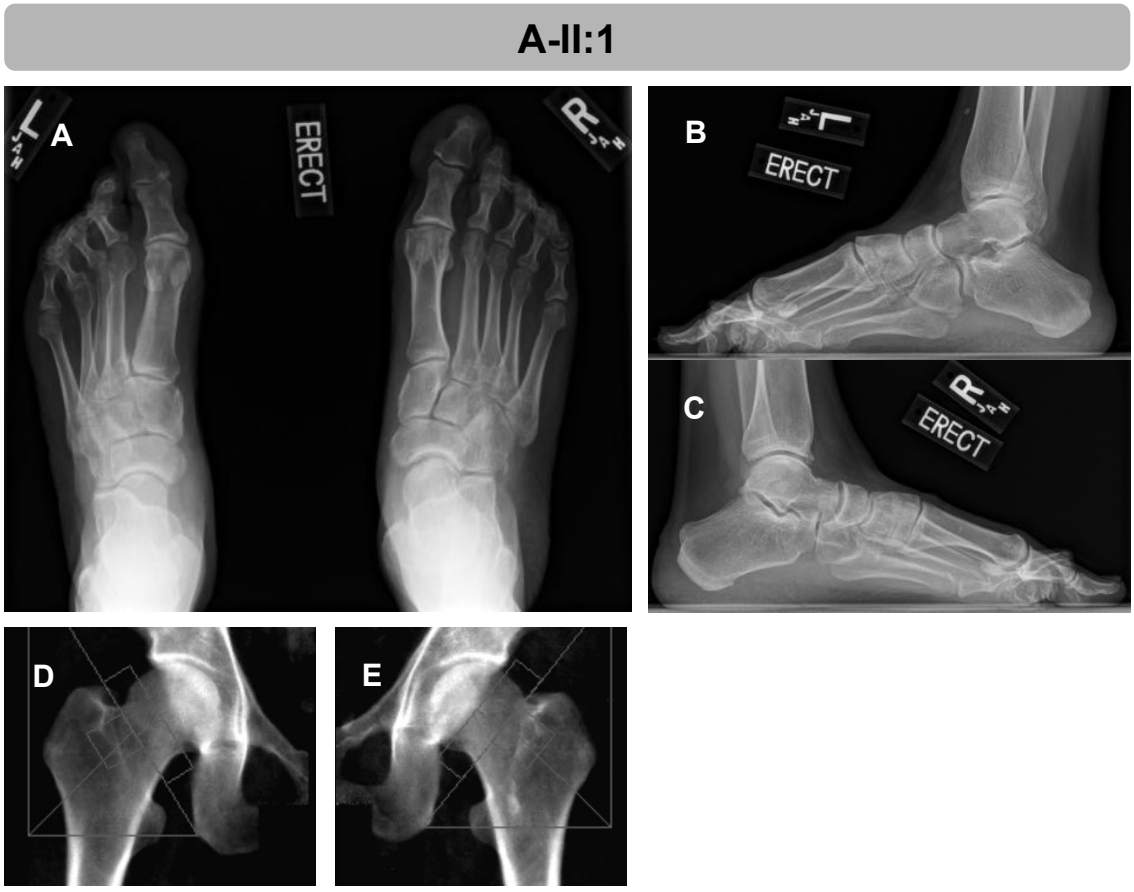
At 10 years of age the subject had a weight of 62 kg (>95th percentile), height of 142.5 cm (75th percentile), and OFC of 50 cm (~25th percentile). She had coarse facial features as well as severe skin and joint laxity. There was a generalized skin redundancy with sagging cheeks, micrognathia with high arched palate and gross dental misalignments, bilateral ptosis with thinning of the eyebrows. The neck was short and webbed with low hairline and large ears. The phenotype of the small and large joints included dislocations involving the hips, knees, ankles, and interphalangeal (IP) joints. She also had multiple variable-sized keloids.

Skeletal survey showed severe osteopenia, both fore feet were abducted, and hind feet were in valgus position. There was lateral subluxation of middle

interphalangeal joint of the 2nd toe. There is bilateral hallux valgus and distal narrowing of the interpedicular distance. The iliac bone also had a squared appearance. Abdominal US showed hepatomegaly with severely contracted gallbladder.

Supplemental Figures

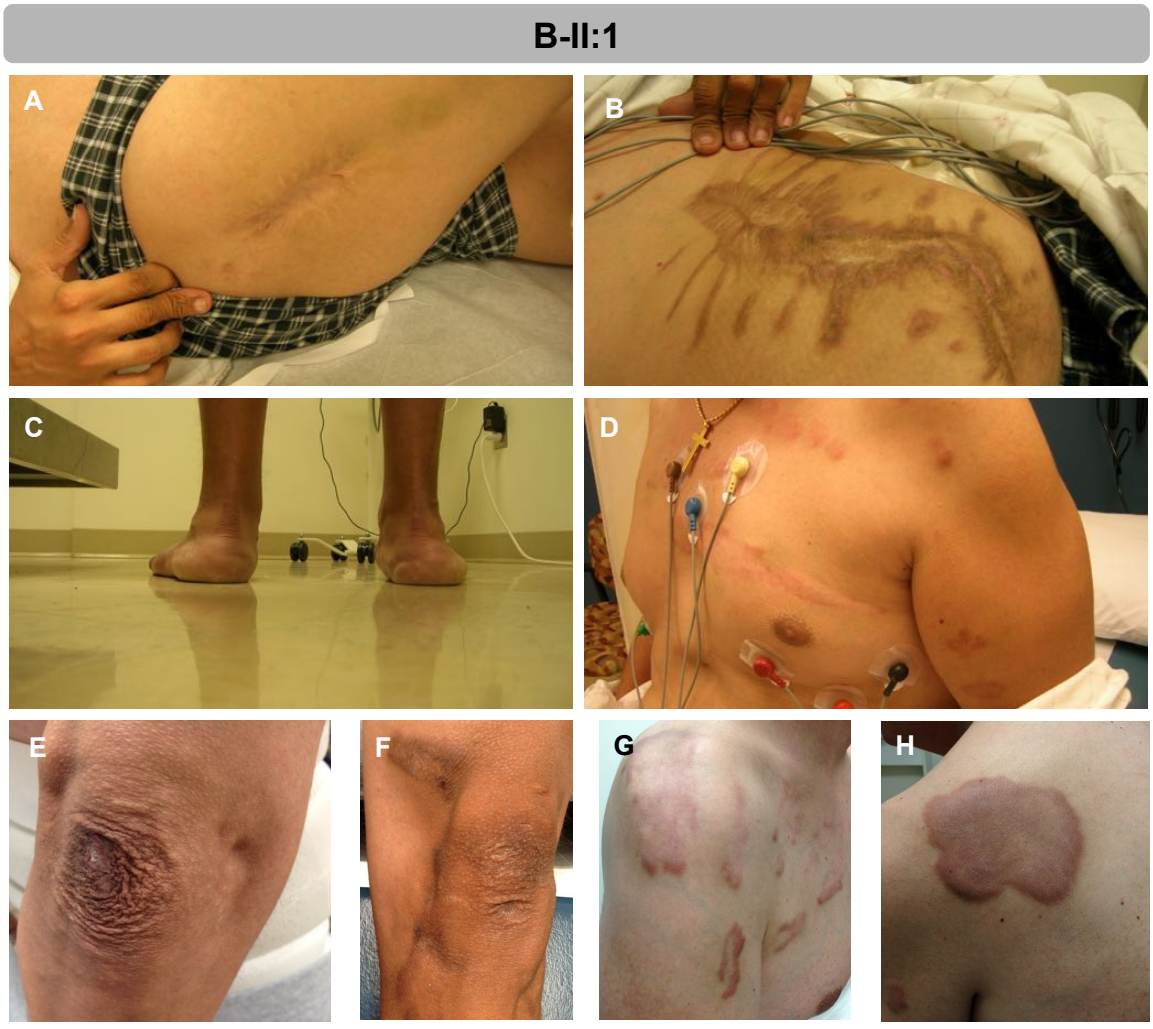
Supplemental Figure 1. Bone densitometry and partial skeletal survey of subject A-II:1



(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.

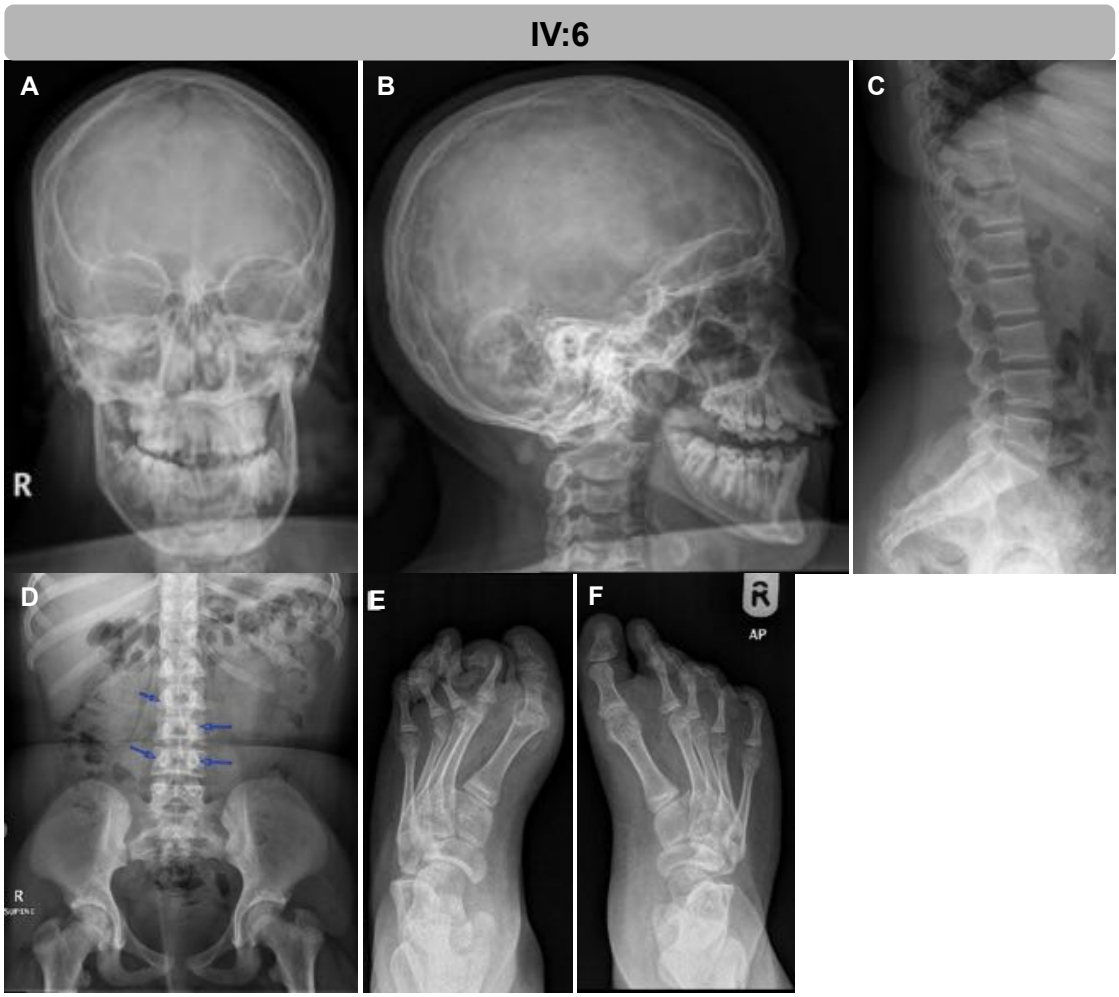
Figure S2. Additional photographs of subject B-II:1



The subject had poor wound healing with atrophic scars that were both widened and hyperpigmented. (A) Widened atropic scar from hip surgery at 18 months for

dislocated hip. (B) Abnormal surgical scar from repair of a ruptured bowel at 27 years of age. Repeated attempts to re-anastomose the bowel failed and a colostomy was required. A large ventral hernia later developed and was repaired and the colostomy reversed. (C) Piezogenic papules were present bilaterally. (D) Abnormal keloid scars on the chest with bruises apparent. (E,F) Papyraceous scars at both the elbows and knees. (G,H) The skin on the chest and abdomen was translucent with veins visible beneath the skin. Multiple abnormal scars were also present, some with a keloid appearance.

Figure S3. Skeletal survey of 12-year-old proband C-IV:6



(A,B) Posteroanterior and lateral views of the skull showing 'copper beaten' appearance of the cranium and abnormal dental alignment. (C,D) Lumbar spine lateral and anteroposterior projection. The alignment of the spine is unremarkable, however narrowing of the interpedicular distance of the lumbar spines distally was observed. The iliac bones were also shortened and squared. There is no acetabular dysplasia or femoral head dislocation. (E,F) Standing X-rays of left and right foot showed bilateral lesser hammertoe and hallux valgus deformities.

Supplemental Tables

Table S1. Summary of *AEBP1* variant findings in each individual.

Subject	gDNA (GRCh37/hg19)	Exon	Transcript cDNA NM_001129.4	Coding Effect	Protein Change	Zygosity	gnomAD Allele Frequency	dbSNP	Reference
A-II:1	Chr7:44150393del	12	c.1470delC	Frameshift	p.Asn490_Met495 delins(40)	Het	N/R	N/R	This report
A-II:1	Chr7:44151132C>A	15	c.1743C>A	Nonsense SNV	p.Cys581*	Het	1/264694	rs777647845	This report
B-II:1	Chr7:44149865_44 149871del	11	c.1320_1326del	Frameshift	p.Arg440Serfs*3	Hom	N/R	N/R	This report
C-IV:4 and C-IV:6	Chr7:44150657G>A	13	c.1630+1G>A	Abolishes splice donor	p.?	Hom	9/271654	rs369016031	Alazami et al., 2016

Het, heterozygous; hom, homozygous; N/R, not reported.

Table S2. Comparison of *AEBP1*-related clinical phenotypes with EDS subtypes in the updated 2017 International Classification Guidelines.