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Elastin-Driven Genetic Diseases

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Abstract

Elastic fibers provide recoil to tissues that undergo repeated deformation, such as blood vessels, lungs and skin. Composed of elastin and its accessory proteins, the fibers are produced within a restricted developmental window and are stable for decades. Their eventual breakdown is associated with a loss of tissue resiliency and aging. Rare alteration of the elastin (ELN) gene produces disease by impacting protein dosage (supravalvar aortic stenosis, Williams Beuren syndrome and Williams Beuren region duplication syndrome) and protein function (autosomal dominant cutis laxa). This review highlights aspects of the elastin molecule and its assembly process that contribute to human disease and also discusses potential therapies aimed at treating diseases of elastin insufficiency.

Keywords

Elastin; supravalvar aortic stenosis; autosomal dominant cutis laxa; Williams Beuren syndrome; Williams Beuren duplication syndrome; emphysema

1. Introduction

Elastic fibers are essential elements of the extracellular matrix (ECM), providing recoil to a broad range of tissues. Elastin is the main component of elastic fibers. It is formed through multimerization and crosslinking of tropoelastin monomers in the presence of elastic fiber proteins including fibrillins, fibulins, and lysyl oxidases. Once deposited in the ECM, elastin is exceedingly stable, with a half-life of approximately 70 years [1]. Genetic alterations that impact either the quantity or quality of elastin deposited have the potential to impact the function of elastic tissues. This review aims to describe the medical conditions caused by rare variation in the *ELN* gene. To do so, it first introduces the elastin protein and highlights protein domains that contribute to the efficient assembly and appropriate function of the molecule. In the next section, the review provides a comprehensive report of the genetic variants that cause rare elastin mediated disease, while outlining the mechanism by which

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the various mutation types cause disease. Third, the review discusses the specific disease phenotypes produced by the different mutation mechanisms and finally it examines current and potential future treatment strategies for the care of affected individuals.

2. Elastin domain structure and expression

The human elastin gene contains 34 exons in its longest transcript (NM_0012789391.1, ENST00000358929.8). The canonical transcript, however, has 33 exons with exon 22 typically spliced out (NM_000501.3, ENST00000252034.11). The exons encode a series of repeated pairs of hydrophobic and crosslinking domains (Figure 1) [2, 3]. Crosslinking exons are important for binding tropoelastin monomers to one another through the formation of desmosine, isodesmosine, and lysinonorleucine crosslinks [4–6], while the hydrophobic regions are essential for generating the entropic forces that contribute to elastic recoil [7–11].

Small-angle X-ray and neutron scattering studies performed on un-crosslinked tropoelastin show that the monomer takes on boot-like configuration with an N-terminal asymmetric coil structure at the top and a protruding C-terminal foot domain at the end. In between are the spur regions and flexible bridge regions linking the two [12, 13]. Although the domain structure is repetitive, three regions deserve special discussion for their impact on elastic fiber assembly and disease.

First, exon 30, a hydrophobic exon, is believed to mediate the initial association of tropoelastin monomers with one another, in a process referred to as microassembly [14, 15]. Exon 30 of elastin contains a repeated (GGLG(V/A)) sequence that was shown by fourier transform infrared spectroscopy to form anti-parallel beta sheets [16]. With the addition of transmission electron microscopy and atomic force microscopy, Tamburro *et al* found that fibers were formed from self-association and alignment of primary cross beta structures formed by the exon 30 sequences. Glycine-rich hydrophobic sequences such as this are found in many molecules that form amyloid-like aggregates [16]. Although many regions in tropoelastin are hydrophobic in nature, exon 30 displays a relative paucity of proline residues. It is this lack of proline within the hydrophobic region that increases its ability to form amyloid structures [17]. In *in vitro* cells systems, when exon 30 was deleted from the elastin cDNA in a bovine assembly system, multimerization and assembly of elastin by cells was reduced [18]. More recent experiments using multiple tropoelastin constructs bearing genetic variations in exon 30 sequence upheld a role for exon 30 in guiding elastic fiber formation and also revealed the region's contribution to viscoelastic and tensile properties of the final crosslinked polymer[19]. Consequently, human mutations causing the loss of this region are expected to have decreased matrix accumulation of elastin. Miao *et al* also showed that single point variants within this exon have the potential to affect the mechanical properties of the resulting monomer, potentially affecting its ability to assemble [20].

The exon 16–17 region has also been implicated as a possible assembly domain. Like the exon 30 studies, cDNA constructs of human tropoelastin carrying an exon16–17 deletion failed to deposit elastic fibers when transfected into pigmented epithelial cells [21]. Mutant

protein was able to bind fibrillin-1 and fibulin-5 but showed an increased coacervation temperature suggesting decreased association between mutant tropoelastin monomers.

The final region of interest lies in the terminal exon of the human *ELN* gene. Although the 34th consecutive exon in the human gene, it is referred to as domain 36 in much of the literature. The numbering convention is used because the majority of species have two additional *ELN* exons, exons 34 and 35, that were evolutionarily lost in humans and higher primates [22]. This domain contains a group of basic amino acids (KxxxRKRK) that are important for cell surface heparin binding. Such interactions are thought to assist in growth/stabilization of the growing elastin multimer and potentially to aid in the release of tropoelastin from chaperones/binding proteins that assist in assembly [23–25]. When exon 36 was deleted in bovine cDNA constructs, the resulting elastin proteins were secreted and deposited in the extracellular space but showed reduced numbers of desmosine crosslinks [18, 26], potentially due to the absent interaction with other relevant matrix associated proteins. As such, mutations affecting the extreme C-terminus may affect the quality of the elastin produced.

Elastin production is highly regulated in a temporal and tissue specific manner. The *ELN* promoter contains purported TGF-β, insulin and glucocorticoid responsive elements [27–30], but the regulation of elastin protein production is thought to occur mainly through the binding of microRNA (miR) regulatory elements both in the 3' UTR and in coding sequences of *ELN* [31, 32]. It is suggested that high expression of miRs-29 and -15 is responsible for posttranscriptional repression of elastin in the adult aorta [32]. Binding of the miRs causes the degradation of ELN RNA. Tissue specific alternative splicing resulting in different tropoelastin isoforms have been identified and may play a role in the diversity of disease manifestations [20, 33–38]. In addition, deposition of elastin into the ECM is known to be impacted by the relative quantity of other ECM glycoproteins (e.g. versican, fibrillins, fibulins, MFAPs, etc) [18, 39–46].

2.1 Rare variation in the ELN gene:

Most described mutations within the ELN gene cause one of two OMIM-designated diseases, supravalvar aortic stenosis (SVAS-MIM #185500) and autosomal dominant cutis laxa (ADCL-MIM#123700). More than 100 pathogenic or presumed pathogenic variants have been described in *ELN* to date in the literature, in the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar) and in the Human Gene Mutation Database (http://www.hgmd.cf.ac.uk).

Variants causing the SVAS phenotype are generally expected to cause a decrease in total elastin. Reported variants include missense mutations affecting the initiation methionine, non-sense changes leading to a premature stop codon prior to exon 30, and insertion/deletion changes that produce frameshift transcripts expected to be destroyed by non-sense mediated decay (Figure 2 and Supplemental Table 1).

In addition, splice site mutations are also reported to cause the SVAS phenotype (19 of the 104 reported variants are thought to impact splicing). Because all elastin exons are in the same frame, exon skipping has the potential to produce an in frame, although shorter, protein

product. Miao *et al* showed that changing the domain structure of tropoelastin by adding and subtracting exons from the molecule affected both its hydrodynamic radius and tensile mechanical properties, suggesting that exon skipping may impact the function of the protein product [20]. However, it is not clear whether in most cases, the mutant protein is made. For one variant, two groups evaluated the same gene change, c.800-3C>G, in skin fibroblasts from different patients. This variant causes a change in intron 15, affecting the splice site. Wachi *et al* found stable expression of a message missing exons 16 and 17 in the mutant fibroblasts [21], while Urban *et al* noted that the variant led to the generation of a cryptic splice that ultimately led to frameshift and reduced expression [47]. Unfortunately, because of the primers chosen by each team, the groups did not identify the transcript shown by the other laboratory. The group that found stable expression of the mutant Δ16–17 mRNA followed those studies with *in vitro* work using an expression system that showed exon 16–17 to be important for tropoelastin self-association. An additional study by Park *et al* showed that the loss of 11 in frame amino acids from exon 17 also led to the SVAS phenotype [48], potentially due to dysfunctional assembly. However, the authors were unable to find an aberrant protein by western.

As for the remaining splice variants, most of the references do not contain functional studies to determine the impact of the gene change. Given the number of such variants, it would seem more likely that the majority would either extend into an intron where a premature stop is encoded or serve to generate cryptic splices leading to aberrant message. In each case, the expectation is that the transcripts will largely be destroyed by nonsense mediated decay. It is possible, however, that like the Δ16–17 story described above, some of the abnormal splices generate shorter variants that delete additional assembly domains. More work is needed to determine this empirically.

In addition to these single base pair/short variations, a translocation [49], a deletion that removes the C-terminus [50], a deletion of exons 2–28 [51] or loss of the entire ELN gene [52] can cause SVAS. These findings suggest that loss of one functional copy of the elastin gene and decreased elastin protein production may contribute to the SVAS phenotype.

The most common genetic alterations affecting the elastin gene, however, are large deletions that remove one copy of *ELN* in addition to the neighboring 25–27 genes as part of the recurrent microdeletion disorder Williams-Beuren syndrome (WBS)[53–55]. Patients with WBS have vascular features similar to SVAS, but have additional phenotypes owing to the loss of other genes in the deletion [56–61]. Patients with WBS/SVAS mutations deposit less total elastin but the elastic fibers typically appear normal, if a bit less organized [62, 63]. These findings, together with phenotyping data from murine models outlined below suggest that the SVAS phenotype is caused by elastin haploinsufficiency.

A few of the reported SVAS mutations deviate from the above convention. These include missense mutations at amino acids p.Ala55Thr (exon 3), p.Pro220Leu (exon 13), p.Gly610Gln (exon 26), and p.Ala707Asp (exon 30) that cause an SVAS phenotype. The p.Ala55Thr variant occurs as the last basepair in exon 3 and likely contributes to aberrant splicing, as variation in the next two nucleotides in the intron also produce the SVAS phenotype. Both exon 13 and 26 are alternatively spliced and it is possible that increased

splicing occurs in the setting of these variants that prevents the accumulation of mutant protein. Dosage could be affected if tissue or timing specific requirements for particular isoforms were critical. The p.Ala707Asp variant is of particular interest due to its location in the putative exon 30 microassembly domain [18], a region also potentially involved in posttranscriptional regulation of message stability [31, 32]. The finding of missense variation at these locations suggests a change to the structure of the tropoelastin monomer that inhibits multimerization or stability of the tropoelastin transcript that leads to functional haploinsufficiency.

By contrast, ADCL is predominantly caused by frameshift mutations in exons 30–34 (Figure 2 and Supplemental Table 1). Elastic fibers deposited by these individuals are abnormal and display fiber fragmentation along with reduced deposition [64, 65]. In general, mutant mRNA in ADCL patients is stable and in some, protein containing the frameshifted alleles has been detected in the matrix using an antibody to the frame-shifted product [64], suggesting a dominant negative mechanism of disease. In addition, Callewaert *et al* suggested that ADCL variants generate tropoelastin monomers that are aberrantly folded and induce endoplasmic reticulum stress and apoptosis [64]. Of note, due to alternative splicing of exon 32, ADCL mutations in this domain may be phenotypically less severe [64]. Tissues where exon 32 is typically spliced out, for example, would be expected to show no phenotypic consequences of the genetic alteration.

Reported variants not fitting the above mutational prescription for ADCL include a frameshift mutation (c.914_930del17insGCT; p.Ala305Glyfs) that was reported in exon 17 by a clinical lab in ClinVar. The indication for the test was reported to be cutis laxa but no additional clinical details are available. Care should be taken in interpretation of this variant. One additional variant, c.1708C>T (c.1621C>T in [66] which uses NM_000501.3 rather than the full length transcript) has been reported to cause ADCL like features in one patient and SVAS-like features in others [66–68]. The variant changes in the last base pair of exon 25. In the ADCL-like proband and his apparently very mildly affected father, the variant induced in frame splicing out of exon 25 in a fraction of the skin derived RNA. While the proband had quantitatively normal amounts of ELN message (suggesting equal parts WT and splice form), the father had reduced message (suggesting haploinsufficiency). Interestingly, in the transcripts where splicing of exon 25 does not occur, a new stop codon is generated by the exon 25 mutation and the first two base pairs in exon 26, leading to p.Arg570Ter. The child's skin fibroblasts showed ADCL-like features of scant and small fragmented elastic fibers while the father's findings were subtler. Consequently, it appears that some with this genotype express a predominantly haploinsufficiency mediated SVAS phenotype (the father in this case (albeit with decreased penetrance) and SVAS cases from [68, 69]), while others (the proband in this case) have increased exon 25 splicing and the retention of aberrant protein leading to ADCL. Exon 25 has been implicated in interchain crosslinking [70]. Of note, this proband had additional features such as agenesis of the corpus collosum and seizures, features not typically seen with ADCL. Sequencing was only performed for *ELN*, *ARX*, and *FBLN5* and *FBLN4*. Other genetic changes could be responsible for the unique phenotypic presentation of this proband.

Of note, a homozygous missense mutation in exon 12, p.Pro211Ser, was reported to cause a mild form of autosomal recessive cutis laxa in two related consanguineous families [71]. The phenotype was more severe in a family member who carried biallelic *ELN* variants and an additional variant in the *FBLN5* gene. The carrier parents were clinically unaffected in all cases except one mother who possessed a single ELN p.Pro211Ser mutation and the *FBLN5* variant—she possessed mild features of cutis laxa. These findings may suggest that more minor missense changes in non-critical portions of the *ELN* gene may have limited phenotypic effects when present in isolation. However, when the burden of *ELN* or other cutis laxa gene variants increases, a clinical phenotype may be appreciated due to the cumulative effect of the total variant load. However, because this testing was done using a candidate gene approach (only *ELN* and *FBLN5* were sequenced), re-evaluation using current technologies (exome or whole genome sequencing) may provide an alternative explanation for the disease features in these patients.

In addition to the SVAS and ADCL phenotypes, the rare *ELN* point mutation c.2318G>A resulting in p.Gly773Asp (exon 34) was identified in a large pedigree with severe early onset chronic obstructive pulmonary disease (COPD) [72, 73]. This mutation results in a non-conservative amino acid change in a position that is conserved across species, and is predicted to be probably damaging by *in silico* testing. This variant was identified in one further proband following screening of ~1300 individuals genotyped through the Boston Early-Onset COPD Study and the National Emphysema Treatment Trial but other causative variants in *ELN* have not been identified in the numbers evaluated [72].

The conditions above discuss the impact of reduced or aberrant elastin, but a small number of individuals are known to possess increased elastin gene dosage due to a duplication event of the WBS region. Patients with this condition have three copies of the ELN gene and mild cardiovascular phenotypes including aortic dilation. Because the vascular phenotype of the WBS duplication patients appears inverse to the stenotic phenotype of the deletion patients, scientists have speculated that increased production of elastin from the 3rd allele leads to subtle changes in aortic mechanics that produce to dilation over time [74].

3. Disease manifestations

Individuals with genetic changes affecting the elastin gene have phenotypes in a range of elastic tissues, although features vary between genetic changes affecting gene dosage and those leading to dominant negative production of aberrant protein.

3.1 Elastin Insufficiency (WBS and SVAS)

3.1.1 Vessel disease- Arteriopathy—In the vasculature, elastic lamellae are organized circumferentially in the media and are layered between sheets of smooth muscle cells. Studies performed on arteries from elastin insufficient mice and humans show increased numbers of smooth muscle and elastic lamellae (Figure 3A and B); each layer, however, has decreased elastin content [63, 69]. These changes impact the biomechanical properties of the vessel wall, leading to a vessel with a smaller lumen, thicker wall and decreased compliance [75, 76]. *Eln*$^{-/-}$ mice show total disorganization of the smooth muscle layers and obliteration of the luminal space by cells, a process reported to be independent

of blood flow, hemodynamic stress, endothelial damage, thrombosis, inflammation, or fibrosis [77], suggesting that the presence of elastin in the extracellular matrix and cell proliferation/orientation are tightly linked [62, 78–80]. *Eln*$^{+/-}$ mice showed ~50% reduction in Eln mRNA and had ~25–35% more elastic lamellae and smooth muscle in their arteries. Adult hemizygous mice have higher systolic blood pressure, increased arterial stiffness and smaller caliber vessels, with longer segmental length than WT littermates, [69, 75, 76]. Tortuosity is noted in multiple tissue beds ([81] and Figure 3D and E). Gene dosage seems to be important as transgenic mice expressing human elastin from a bacterial artificial chromosome containing the human ELN gene on a *Eln* $^{-/-}$ null background (*hELN* BAC; *mEln*$^{-/-}$) deposit ~35% of normal elastin content and show higher blood pressure than Eln$^{+/-}$ mice and the ascending aorta is increasingly thickened with more poorly organized lamellae than *Eln*$^{+/-}$ mice (Figure 3 C). Vessel caliber is markedly reduced and some decrease in longevity was noted [82].

In humans, the prototypical defect associated with elastin insufficiency is focal stenosis of the large elastic arteries (Figure 3F and G). This narrowing can occur in any elastic artery, but is most frequently described in supravalvar aorta and branch pulmonary arteries [83, 84]. Vascular disease varies from person to person with ~30% of individuals requiring surgical intervention for their stenosis and ~20% having no appreciable stenosis. In addition to the aortic and pulmonary disease, narrowing and anatomical abnormalities of more distal vasculature have been described. Coronary artery abnormalities are also reported in SVAS and WBS and may contribute to sudden death in this population [83, 85–88]. Strokes have been reported in individuals with WBS [89–91], and with isolated SVAS [92], both in the presence and absence of cerebral artery stenosis or aneurysms. A small study performing magnetic resonance angiography in patients with WBS *vs*. controls found no noticeable stenosis in cerebral arteries. However, they did note that the posterior communicating segment of the anterior cerebral artery was longer in patients with WBS [93]. In addition to focal stenosis, patients with elastin insufficiency have globally narrow vasculature, even in locations without focal stenosis. Recent studies suggest that deficient circumferential growth during development may play a role in this generalized arteriopathy [94]. The vessels show increased stiffness [95, 96]. This stiffness may increase risk of negative cardiovascular outcomes [97–99].

It is important to note that none of the mouse models develop the pathognomonic hourglass appearing SVAS seen in many human patients. Instead, the mouse models show more homogeneous long segment wall thickening and narrowing, with the *mEln*$^{-/-}$*; hBAC ELN* mouse revealing the most profound arch narrowing. Consequently, although endothelial damage and hemodynamic stress may not be necessary for the increased smooth muscle layers seen in both humans and mice, the focal aortic stenosis that typically worsens postnatally in humans may depend on additional mechanical, genetic or environmental factors that are currently incompletely understood.

Hypertension is an important health problem in individuals with elastin insufficiency. Renal artery stenosis may contribute to hypertension in this population [83, 100–103]. However, hypertension often occurs independently of renal artery stenosis in WBS/SVAS. Studies in humans and mice have shown that hypertension is less common in WBS patients with

larger deletions that include the *NCF1* gene [96, 104–106]. *NCF1* encodes p47phox and is a component of the NADPH oxidase complex. The finding that patients with reduced capacity to generate oxidative stress are protected from hypertension suggests that increased reactive oxygen species, generated in response to altered mechanical stresses may be important in the pathology of hypertension in elastin insufficiency.

3.1.2 Lungs—In the lungs, elastin is located within the arteries and elastic cartilages but is also present at the tips of alveolar septa. During expiration, the diaphragm relaxes, allowing the elastic recoil of the lungs to move air out of the air spaces. Lungs of $Eln^{+/-}$ pups displayed a 50% reduction in tropoelastin, significantly fewer microvessels, including lung capillaries, and two-fold increase in collagen-1 and lysyl oxidase [107]. No difference in alveolar size and number has been found [107, 108]. The *hELN* BAC+ m$Eln^{-/-}$ mice, however, have ~65% decrease in elastin level, and present with congenital emphysema characterized by enlarged thoracic cavities, large distended lungs and massively dilated airspaces on microscopy [82]. The lungs in $Eln^{-/-}$ mice demonstrated arrest of development at the level of the distal airways. These mice had dilated distal air sacs and attenuated septae demonstrated even before alveologenesis [109].

Respiratory symptoms in human patients with ELN insufficiency are similarly mild and may include cough, dyspnea and wheezing. Spirometry in 16 young adults with WBS was largely normal [110]. Although uncommon, early onset emphysema has been described in several WBS patients [110–112]. It is unclear whether the severe disease presented in these studies is as a result of their elastin insufficiency alone or if they, in fact, have variations in additional modifiers genes that contribute to the severe phenotype. Additional studies in older individuals with elastin insufficiency are needed to more completely understand the impact on lung physiology. Studies in $Eln^{+/-}$ mice exposed to cigarette smoke suggest that environmental toxins such as smoke are particularly damaging in the face of elastin insufficiency and should be avoided in affected individuals [108]. The differences in phenotypes found in mice and humans may represent the species specific variations in elastin content in tissues [113].

3.1.3 Skin & Integument—Several skin and integument findings have been described in the WBS patient population including, soft skin, dry skin, wrinkles, and loose periorbital connective tissue [114–117]. Elastin insufficiency results in decreased diameter of oxytalan fibers and mature elastic fibers in the skin [118]. Likewise, Urban and colleagues found that individuals with WBS had reduced deposition of amorphous elastin when viewed under electron micrograph despite having a similar distribution of the elastic network when compared to controls [119]. Biomechanical skin properties studied in WBS individuals revealed diminished skin viscoelasticity relative to controls [116].

3.1.4 Gastrointestinal—Feeding difficulties and gastrointestinal complaints have been frequently described in individuals with WBS. The most common findings include chronic abdominal pain, gastroesophageal reflux, vomiting, chronic constipation, and rectal prolapse. It is unclear to what extent pain and motility issues can be attributed to vascular and connective tissue complications of the disease versus changes in innervation owing to the deletion of genes other than *ELN* in the WBS critical region. Diverticulosis and

diverticulitis are common in adults with WBS [114, 120–122]. Although there are data to suggest increased elastin in the longitudinal smooth muscle of colonic samples with diverticular disease, there is little information about the effect of elastin haploinsufficiency in the intestinal wall [123].

3.1.5 Genitourinary—Like the other organs discussed, the bladder also undergoes repeated cycles of stretch and recoil. The smooth muscle there deposits relatively high amounts of elastin and *Eln*$^{+/-}$ mice revealed decreased bladder compliance and capacity, and increase in contractility [124].

In patients with elastin insufficiency, lower urinary tract symptoms such as urgency, frequency, incontinence and enuresis are common [125–127]. Bladder diverticula, undescended testis, retractile testis, and inguinal hernias are also seen [125, 126, 128–130]. Some of these symptoms may be attributed to neurologic and developmental factors in WBS, but the role of elastin insufficiency should be considered given its relatively high expression in the bladder wall.

3.1.6 Voice—Elastin is found in human vocal cord lamina propia (VCLP) as oxytalan and elaunin in the superficial layer, and as mature elastic fibers in the deeper layers [131, 132], making up 9% of total protein present in the VCLP [132]. Elastin plays an important role in vocal cord vibration, and biomechanics of voice quality. Hoarse voice is a common finding in individuals with WBS [121, 133–135]. The voice quality of individuals with SVAS/WBS has been found to be rough, hoarse, and to have a lower pitch when compared to normal controls [136]. Histological evaluation of vocal cord on autopsy specimen of a WBS patient revealed decreased elastin when compared to an age-matched control [135]. Mice heterozygous for deletions in the *Eln* gene showed decreased elastin within the vocal folds when compared to *Eln* WT mice [137].

3.1.7 Hearing—Audiological findings in individuals with WBS include sensorineural hearing loss (SNHL), conductive hearing loss and hyperacusis [114, 138, 139]. Increased sensitivity to sound has been reported in up to 95% of individuals with WBS [138] and is associated with high-frequency hearing loss, similar to noise-induced hearing loss [140, 141]. 60–70% of school-aged-children with WBS display mild to moderate high frequency hearing loss or mixed hearing loss [139, 141]. The pattern of hearing loss is progressive with up to 92% of adults with WBS having some hearing loss [114, 140]. Hearing loss phenotype and hyperacusis were reported in two related individuals with non-syndromic SVAS, suggesting that elastin haploinsufficiency may contribute to the pathogenesis of SNHL in WBS [139]. Conductive hearing loss is related to excessive cerumen buildup [114, 139], and middle ear pathology [140]. Distortion product otoacustic emissions findings were suggestive of subclinical cochlear pathology even in children with WBS with normal behavioral hearing threshold [140].

Different mechanisms for hearing loss related to elastin insufficiency have been proposed including poor cochlear perfusion secondary to vascular stenosis, increased rigidity of the basilar membrane, dysregulation of cochlear cell proliferation and disruption of middle ear mechanics [139, 140, 142]. Hyperacusis may be caused by deficiency of acoustic reflex

from auditory nerve dysfunction [141]. More recently, LIMK1 haploinsufficiency has been proposed to be responsible for the auditory phenotype in WBS [143]. Further research into the role of elastin in warranted.

3.2 Dominant negative mediated elastic fiber disease (autosomal dominant cutis laxa)

3.2.1 Skin—Skin findings are the major clinical feature of ADCL, with loose, redundant, inelastic skin and appearance of premature aging being present in 100% of affected individuals. Electron micrographs of skin from affected individuals show disorganized elastin with abnormal branching and fragmentation of elastic fibers [64, 65].

Individuals with ADCL have normal wound healing despite abnormal and decreased dermal elastin. Transgenic mice expressing human tropoelastin with a single nucleotide deletion in exon 30 (c.2012delG) previously described in patients with ADCL, hBAC^{CL}, in addition to *mELN*$^{+/+}$ showed accumulation of the mutant protein within the skin and skin laxity, requiring half as much force to be displaced when compared with WT and hemizygous mice [36]. Biomechanical testing in skin of individuals with various types of cutis laxa, including ADCL, confirmed that reduced force was necessary to displace patient skin, and showed abnormal and prolonged recoil [144].

3.2.2 Vessel Disease—ADCL was initially thought to be a mild disease with findings limited to the skin [145]. However, multiple individuals have been described with aortopathy, valvulopathies and significant pulmonary disease. Aortic root dilation has been described in 30–50% of patients with ADCL [64, 146]. Aortic dilation can range from mild to severe aneurysms or aortic rupture [147]. Cardiac valve abnormalities, namely bicuspid aortic valve, aortic regurgitation, mitral regurgitation and mitral valve prolapse are common in affected individuals [64, 146, 148]. Studies with hBAC^{CL}; *mEln*$^{+/+}$ mice showed limited incorporation of the mutant protein into the aorta and no effect on vessel compliance. Combined with the skin findings presented above, these data suggest that tissue specific alternative splicing may occur that modifies the phenotypic presentation in different organs [36].

3.2.3 Lung—A recent literature review states 28% of individuals with *ELN* variants resulting in ADCL have pulmonary findings [148]. Prior reports state that approximately 35% of patients with ADCL have severe emphysema [64, 146, 149]. Transgenic mice expressing a 25-nucleotide deletion in exon 30 (c.2114_2138del25) as described in a family with ADCL, in the presence of *mEln*$^{+/+}$ developed severe emphysema and had increased mortality [150]. Both this and the Sugitani *et al* study which followed [36] showed the co-polymerization of mutant with WT elastin as well as the numerous imaging studies that show abnormal elastic fibers[64, 65], underscore the claim that ADCL is caused by a dominant negative mechanism.

3.2.4 Genitourinary—Genital prolapse has been described in at least 2 individuals with ADCL caused by *ELN* variants [146, 149]. Biopsy from cardinal and uterosacral ligaments in a patient with cutis laxa and genital prolapse revealed decreased elastin and increased

collagen type VI, suggesting a role for extracellular matrix components in the stability of the pelvic floor [151].

3.3 Increased ELN gene dosage

In contrast to individuals with elastin haploinsufficiency, individuals with three copies of the *ELN* gene as a result of the Williams Beuren duplication syndrome have an increased rate of aortic dilation, ranging from mild to moderate, and mostly affecting the ascending aorta [74, 152–154]. Two individuals, a mother-son kinship harboring a 2.53Mb duplication that included the WBS region in its entirety, along with 18 additional genes, have required surgical intervention for aortic dilation/aneurysm [154]. In another cohort, possessing a triplication of just the ELN and neighboring LIMK1 gene, 10/11 affected family members exhibited ascending aortic aneurysm [155]. Patent ductus arteriosus and atrial septal defects have also been described in individuals with 7q11.23 microduplication [74, 153, 156]. The ductus arteriosus is a vessel that connects the aorta and pulmonary artery in fetal life. This vessel should normally close after birth to accommodate postnatal circulation [157]. The anatomical closure of the ductus arteriosus depends on vascular smooth muscle cell proliferation, migration and patterning [157–159]. Because elastin haploinsufficiency has been associated with increased smooth muscle cell proliferation [62, 69, 76], one could speculate that elastin overexpression could decrease smooth muscle cell proliferation or alter smooth muscle contractile phenotype, hindering the physiologic closure of the ductus arteriosus. Human studies have not presented expression or tissue based data to confirm or refute this hypothesis. However, work in animal models highlights the importance of elastin/smooth muscle cell interaction to maintain the ductus arteriosis [160–162]. It is possible that overexpression of other genes in the WBS region influence ductus closure such as Baz1b, a gene implicated in neural crest cell function[163].

A single patient with WBS duplication has been reported to have supravalvular aortic stenosis with post-stenotic dilation on echocardiogram, along with several other congenital defects not usually described as part of this syndrome. This child was the product of consanguineous parents which raises concern for additional genetic disorder [164]. Cutis marmorata, a condition with increased vascular predominance on the skin's surface, has been described in the WBS duplication patient population, but there has been no description of biomechanical properties of the skin in individuals with WBS duplication [153, 165, 166]. No lung findings have been reported to date.

As in the haploinsufficiency model, overexpression of elastin produces a less severe phenotype in mice. In a transgenic mouse expressing human elastin in addition to normal mouse elastin (h*ELN*$^{+}$ BAC, *mEln*$^{+/+}$), overexpression of WT human elastin resulted in no detectable lung disease [150]. Vascular changes were minimal [150] and there was no change in physiological parameters, blood pressure, vessel wall remodeling or compliance [82]. These differences could represent relative differences in gene expression, location, or timing between humans and mice. Alternatively, it could suggest that the cardiovascular phenotypes in the WBS duplication are arising from a different gene. However, data from the *ELN-LIMK1* triplication family presented above would seem to limit the gene dosage effects to *ELN* or *LIMK1*.

4. Treatment

Genetic changes in elastin cause multi-organ dysfunction and intervention is warranted to improve outcomes. Current treatment for individuals with elastin defects is largely symptomatic. Individuals with elastin insufficiency are monitored regularly for evidence of arterial stenosis and hypertension, as well as the other health concerns described above [84, 167, 168]. If identified hemodynamically significant, stenosis is treated with catheter based dilation or surgical repair. Hypertension is managed with anti-hypertension medications, however limited information is available regarding the best choice of antihypertensive for this population [169, 170]. Research studies are only beginning to assess the role for arterial stiffness and aberrant flow generated by narrow caliber and tortuous vessels over the course of a lifetime [96, 171].

Similarly, patients with ADCL are monitored for cardiac, lung, and urinary symptoms [147, 172]. Emphysema and aortic dilation are managed with the same techniques used for non-cutis laxa induced forms of these conditions. Some individuals with ADCL may choose to undergo plastic surgery to correct the appearance of loose skin [173, 174]. However, results may not be permanent. In both SVAS and cutis laxa, avoidance of environmental toxins such as smoking is recommended [110, 175]. Cutis laxa patients are advised to avoid sun bathing, which can damage the skin.

Currently, there are no FDA approved treatments aimed at the molecular cause of these conditions. Investigational treatments in elastin insufficiency have focused on two targets: increasing elastin production and decreasing smooth muscle proliferation.

4.1 Increased elastin production

The ability to increase elastin deposition is particularly intriguing. Due to its long half-life, an effective treatment may only need to be administered over a short therapeutic window to produce life-long beneficial effects. To improve elastin production, two potential therapies have been tested in mice or tissues: 1) inhibition of microRNA (miR) 29 and 2) K_{ATP} channel openers.

As described previously, elastin protein production is controlled largely by the rate and degree of binding of several miRs to the elastin transcript. The miR 29 family, in particular, has been a particularly interesting candidate as it has fourteen binding sites within the *ELN* exons and 3’ untranslated region [32]. Binding of miRs to a mRNA causes translational repression and mRNA degradation. Consequently, the loss of a miR might be expected to increase its usual target’s mRNA translation and protein production. Given this finding, Zhang *et al* chose to treat cells and engineered vessels from patients with elastin insufficiency with miR 29 inhibitors [176]. They showed that when cells were treated with miR 29 mimics, ELN transcript levels decreased, while treatment with miR inhibitors increased ELN expression levels above the untreated control levels and resulted in increased elastin in the ECM [176]. Challenges for the clinical use of these reagents include delivery and specificity. The miR 29 family, like most miR’s binds multiple mRNAs [177]. In addition to elastin, miR 29 family members have been shown to regulate fibronectin, laminin, integrin-B1, multiple collagens and matrix metalloproteinase-2 and have been

implicated heavily in fibrosis [177–179]. Consequently, use of anti-miRs as therapeutics requires the ability to tune the miR to mRNAs of interest and the ability to deliver the medication to a specific location in the body so as to avoid inappropriate production of ECM in tissues where it is not needed.

Minoxidil, a K_{ATP} channel opener and known vasodilator has been shown to increase elastin deposition [180–182]. Postnatal treatment of rats with lower levels of endogenous elastin and *Eln*$^{+/-}$ mice led to increased accumulation of elastin in the vasculature of those animals [171, 180]. Collagen content, as measured by hydroxyproline, however, was unchanged [171]. The medication also decreased blood pressure, increased lumen diameter, normalized pulse wave velocity and improved blood flow to end organs including the brain [171]. Cell work with this drug decreased proliferation of currently dividing cells [183], but evaluation of treated animals showed generally thicker vessel walls owing to increased matrix and relaxation of smooth muscle cells [171]. Treatment with other K_{ATP} openers (diazoxide and pinacidil) had similar effects [180] and subsequent work evaluating mechanisms for how K_{ATP} activators lead to increased elastin deposition points to increased calcium influx and Erk1/2 phosphorylation [184, 185]. More recent RNAseq experiments combined with distensibility data suggest that rather than minoxidil playing a specific role in inducing elastin production, it may instead cause outward remodeling and production of an overall larger vessel[171]. Elastin is deposited, but at a rate only modestly higher than other proteins in the vessel wall. However, because the lumen size does increase, this allows for improvement in blood flow to end organs, a potentially useful by-product. Minoxidil too, has side effects due to K_{ATP} channel expression in non-vascular tissues. Side effects in non-WBS/SVAS patients include hirsutism, cardiomegaly and edema. However, such changes were limited in the *Eln*$^{+/-}$ mouse treatment studies and changes in vascular diameter induced by minoxidil in treated Eln$^{+/-}$ vessels persisted at some level for at least 1 month after treatment was discontinued suggesting that the drug's role is not limited to simple vasodilation but that it has the capacity to "clamp the vessel in the relaxed condition" as previously suggested [186] and that chronic/continuous treatment may not be necessary. Like the miRs, use of K_{ATP} channel openers would be optimized by the identification of similar molecules with activity only in smooth muscle cells to limit side effects. Neither drug has been tested for its effect on existing focal stenosis.

4.2 Inhibition of smooth muscle proliferation

Because stenosis is the most obvious and life-limiting pathology associated with elastin insufficiency, the other major approach to treatment is through inhibition of smooth muscle proliferation. Work by multiple investigators has outlined the dramatic, primarily postnatal, increase in vascular smooth muscle numbers that occurs in elastin insufficient arteries [76, 79, 187, 188]. Evidence of smooth muscle cell proliferation was present as early as E15.5 in *Eln*$^{-/-}$ mice but happened closer to E18.0 in *Eln*$^{+/-}$. To inhibit hyperproliferation, investigators have administered mammalian target of rapamycin (mTOR) inhibitors and integrin β3 blockers to mice.

In the mTOR experiments [189], investigators noted increased mTOR signaling in arterial smooth muscle cells of elastin deficient (*Eln*$^{-/-}$) mice. They then administered rapamycin,

an mTOR inhibitor, to pregnant dams starting at E16.5. Pups showed decreased smooth muscle cell proliferation. *Eln*$^{-/-}$ revealed reduced obstruction but did not live longer. *Eln*$^{+/-}$ pups had reduced lamellar number relative to untreated mice and preserved vascular growth. However, in both *Eln*$^{+/-}$ and *Eln*$^{-/-}$ somatic growth was reduced. Subsequent work showed reduced collagen accumulation and stiffness in rapalog treated *Eln*$^{-/-}$*; hBAC ELN* mice [78]. In these experiments, treatment did not improve arterial narrowing per se but did alter the mechanosensor response to elastin insufficiency, highlighting the role of integrin and cell matrix interactions in the pathology of elastin insufficiency.

In the integrin study, Misra *et al* showed increased β3 integrin expression in *Eln*$^{+/-}$ aortas and in tissue taken from patients with WBS. To test whether inhibition of β3 integrin might serve as a useful therapeutic in elastin insufficiency, *Eln* mutants were raised in a β3 null genetic background. *Eln*$^{+/-}$*; Itgb3*$^{-/-}$ or *Itgb3*$^{+/-}$ mice showed decreased smooth muscle proliferation, improvement in smooth muscle cell alignment and retention of lumen size [79]. *Eln*$^{-/-}$*; Itgb3*$^{-/-}$ mice lived longer (from approximately p2 in the *Itgb3*$^{+/+}$ mice to p4 in the *Itgb3*$^{-/-}$ or *Itgb3*$^{+/-}$). Similarly, prenatal administration of the β3 blocking drug, cilengitide, led to less muscular arteries and reduced stenosis.

For both the mTor and the cilengitide experiments, the drugs were given early in development and led to the prevention of smooth muscle proliferation and therefore would be predicted to prevent stenosis. The side effect profiles for these drugs are significant though and did not preserve life more than two days. Additional studies are needed to determine whether these drugs would be of benefit after the appearance of stenosis and whether chronic management with the medications would be needed to avoid the development of stenosis or if treatment during a sensitive period would be sufficient to prevent long term disease.

Conclusion:

Genetic alterations in the elastin gene cause disease in a variety of tissues with consequences ranging from mild to life threatening. Additional studies are needed to understand the mechanism by which both gene dosage and dominant negative effects of the protein cause disease and how to treat them. While this review focused on genetic forms of elastin mediated disease, the findings in these rare conditions are surely to impact the management of more common diseases such as vascular stiffness, skin changes and emphysema related to chronic damage to elastic fibers. Consequently, the treatments outlined here, once optimized, may be important for the treatment of a variety of age related illnesses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

ADCL	autosomal dominant cutis laxa
BAC	bacterial artificial chromosome
COPD	chronic obstructive pulmonary disease
ER	endoplasmic reticulum
ERK	extracellular signal–regulated kinases
hELN	human elastin gene
HGMD	Human Gene Mutation Database
Itgb3	Integrin beta-3
mEln	mouse elastin gene
miR	microRNA
mTOR	mechanistic target of rapamycin
SVAS	supravalvar aortic stenosis
TGFβ	transforming growth factor β
UTR	untranslated region
VCLP	vocal cord lamina propria
WBS	Williams Beuren Syndrome

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Highlights

- Rare ELN gene mutations affect vascular, lung, skin and genitourinary tissues.
- Elastin quantity (SVAS, WBS duplication) and quality (ADCL) changes cause disease.
- ELN variants predict important elastin assembly domains.
- Potential therapies may target elastin production and inhibition of proliferation.

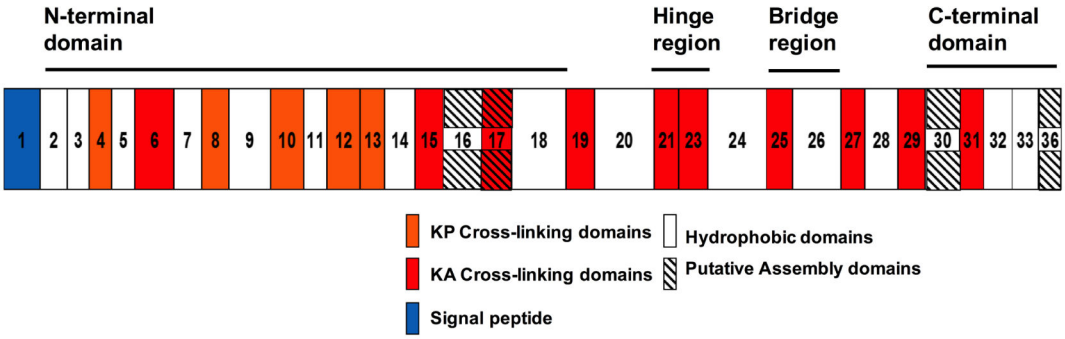

Figure 1. Elastin domain structure.

Pictured is the domain structure of the canonical form of human tropoelastin (NM_000501.3). Exon 22 is not included as it is commonly spliced out and is not part of this transcript. Domains 34 and 35 are also absent as they are not present in the human gene (but are present in other species). Domain 36 is encoded by human exon 34. Domains are scaled to the size of the exon and are color coded as noted in the key to depict domain type.

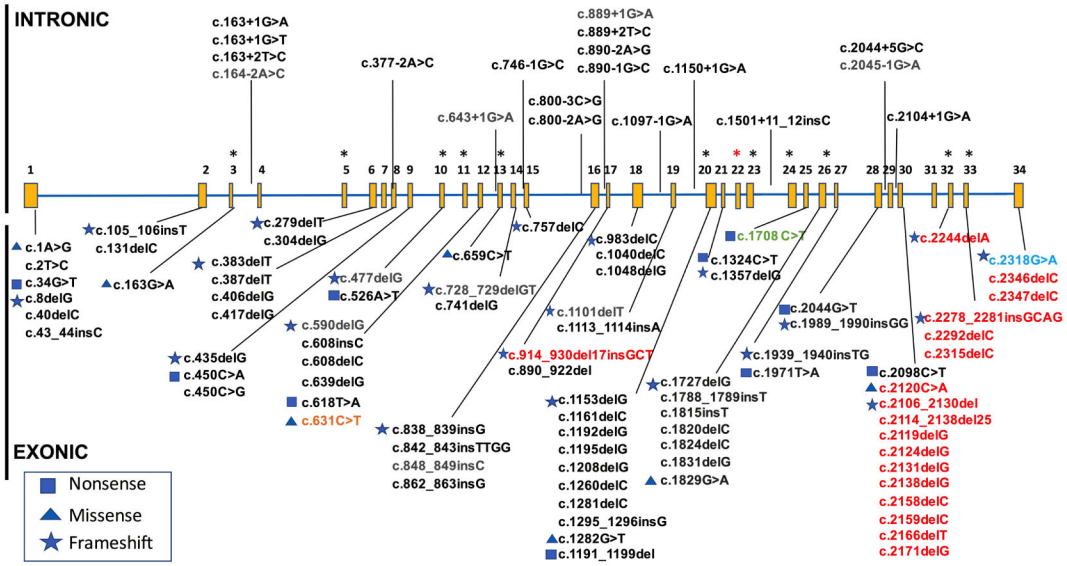

Figure 2. Elastin gene structure and pathogenic variants to date.

Known exonic and intronic variants curated from published case reports, ClinVar and HGMD. Variants resulting in SVAS (black), ADCL (red), COPD (light blue), and pathogenic/likely pathogenic variants as classified by a clinical laboratory and submitted to ClinVar, but without phenotype available (gray). Green font denotes variant in exon 25 that has been described in individuals with features of SVAS as well as one individual with possible features of ADCL (for full details refer to text) and orange depicts a patient with proposed autosomal recessive cutis laxa. All intronic variants to date have been described as causing SVAS, whereas exonic variants can cause either SVAS or ADCL. Most ADCL-causing variants are located in the C-terminal domain exons 30–34, with few exceptions. Alternatively spliced exons are shown (asterisk), as well as commonly spliced exon 22 (red asterisk). Lines point to intron/exon where variants are located, not specific mutation site.

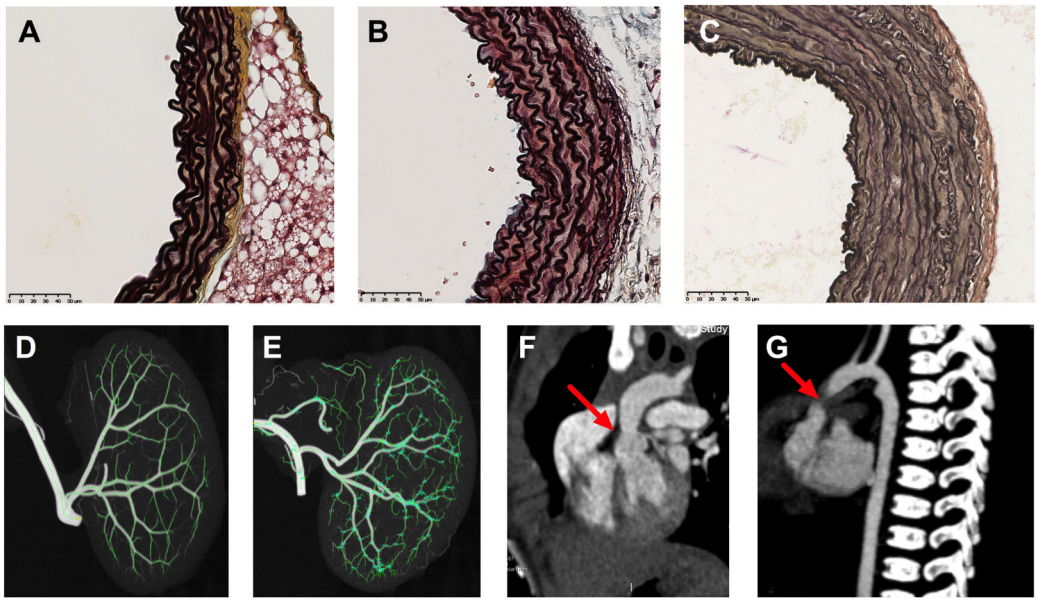

Figure 3. Elastin insufficiency mediated vascular disease.

Images of aorta taken from WT (A), *Eln*$^{+/-}$ (B), and *mEln*$^{-/-}$*; hBAC-ELN* (C) mice and stained with Verhoeff Van Gieson stain. Full scale bar = 50 μm. Elastin is black with the vessel lumen to the left. Vessels were fixed with a flow rate of 1.5ml/min. Note the thicker wall and increased number of elastic lamellae as Eln dosage decreases. The next two panels are microCT images of left kidneys from WT (D) and *Eln*$^{+/-}$ (E) mice. Latex containing a radio-opaque dye (Microfil, Flow Tech, Inc. USA) was injected into the left ventricle of the mice and the arteries were back-perfused until the contrast reached the capillary phase of the kidneys. After hardening, kidneys were removed and imaged. Tracing was done in Analyze software (AnalyzeDirect, USA) to highlight the vessels. Note the smooth arteries in the WT mice and the tortuosity of the *Eln*$^{+/-}$ vessels. Panels F and G are CT angiograms of patients with Williams Beuren syndrome. Both images show SVAS (arrow) in different orientations. Photo credit for A-C to Angela Troia, for D and E to Russell Knutsen and Dr. Delong Liu and to Dr. Shabana Shahanavaz and for F and G.