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Cutis Laxa: Intersection of Elastic Fiber Biogenesis, TGF β Signaling, the Secretory Pathway and Metabolism

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Abstract

Cutis laxa (CL), a disease characterized by redundant and inelastic skin, displays extensive locus heterogeneity. Together with geroderma osteodysplasticum and arterial tortuosity syndrome, which show phenotypic overlap with CL, eleven CL-related genes have been identified to date, which encode proteins within 3 groups. Elastin, fibulin-4, fibulin-5 and latent transforming growth factor-beta-binding protein 4 are secreted proteins which form elastic fibers and are involved in the sequestration and subsequent activation of transforming growth factor-beta (TGF β). Proteins within the second group, localized to the secretory pathway, perform transport and membrane trafficking functions necessary for the modification and secretion of elastic fiber components. Key proteins include a subunit of the vacuolar-type proton pump, which ensures the efficient secretion of tropoelastin, the precursor of elastin. A copper transporter is required for the activity of lysyl oxidases, which crosslink collagen and elastin. A Rab6-interacting goglin recruits kinesin motors to Golgi-vesicles facilitating the transport from the Golgi to the plasma membrane. The Rab and Ras interactor 2 regulates the activity of Rab5, a small guanosine triphosphatase essential for the endocytosis of various cell surface receptors, including integrins. Proteins of the third group related to CL perform metabolic functions within the mitochondria, inhibiting the accumulation of reactive oxygen species. Two of these proteins catalyze subsequent steps in the conversion of glutamate to proline. The third transports dehydroascorbate into mitochondria. Recent studies on CL-related proteins highlight the intricate connections among membrane trafficking, metabolism, extracellular matrix assembly, and TGF β signaling.

1. Introduction

Cutis laxa (CL) is a group of disorders characterized by redundant, pendulous, prematurely wrinkled and inelastic skin. CL can either be inherited or acquired secondary to a range of

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inflammatory conditions. Considerable progress has been made in identifying the genes responsible for inherited forms of CL (Table 1). A detailed review of the clinical manifestations of the different types of inherited CL and related syndromes has recently been published (Berk et al., 2012). Recent reviews also discussed the genetic heterogeneity of CL and its implications for the complexity of elastic fiber biogenesis (Uitto et al., 2013; Urban, 2012). Complementary human genetic, biochemical, cell biological and animal model studies now suggest that many forms of inherited CL display abnormalities both of elastic fibers biogenesis and of transforming growth factor- β (TGF β) signaling, membrane trafficking and mitochondrial metabolism, highlighting the integration of these processes at multiple levels. The goal of this review is to discuss these latest insights.

2. CL genes in elastic fiber biogenesis

The skin manifestations in inherited CL are usually congenital and generalized, and are commonly associated with involvement of elastic tissues within the respiratory and cardiovascular systems (Berk et al., 2012). The phenotype is consistent with a generalized elastinopathy. However, some forms of CL are also associated with growth and developmental delay, craniofacial anomalies, reduced bone density, joint and ligament laxity, hernias and gastrointestinal and urinary tract lesions suggesting broader connective tissue involvement (Table 1). Histological and electron microscopic studies have shown a range of elastic fiber abnormalities in CL, consistent with defective assembly of these extracellular matrix (ECM) structures (Figure 1). Consistently, several genes that encode proteins associated with elastic fibers, including elastin, fibulin-4, fibulin-5 and latent TGF β -binding protein 4 (LTBP4), have been identified to harbor mutations causing CL (Table 1). The mechanisms by which mutations in these genes lead to abnormal elastic fiber assembly and altered TGF β signaling is beginning to emerge.

Autosomal dominant CL (ADCL) is most commonly caused by frameshift mutations within the last 5 exons of the *elastin* gene (*ELN*) (Callewaert et al., 2011; Hadj-Rabia et al., 2013). These mutations lead to the replacement of the C-terminus of the elastin precursor, tropoelastin, with a missense peptide sequence as a consequence of a stable mutant mRNA and protein (Callewaert et al., 2011; Szabo et al., 2006). ADCL-causing mutations have multiple cellular and biochemical effects. The most upstream (exon 30) mutations result in the longest missense peptide sequence, which activates the unfolded protein response (UPR) and leads to increased rates of apoptosis both in patient-derived skin fibroblasts (Callewaert et al., 2011) and in a transgenic mouse model (Hu et al., 2010). Despite activation of the UPR, the mutant tropoelastin is partially secreted by ADCL cells and alters the assembly of the elastic fibers by interfering with the binding of tropoelastin to fibulin-5 and fibrillin-1 (Sato et al., 2006). In addition, ADCL mutations increase the coacervation of tropoelastin in a dominant manner (Callewaert et al., 2011). The effects of these biochemical changes include ultrastructural disorganization and altered mechanical properties of the elastic fibers (Callewaert et al., 2011; Hu et al., 2010). In transgenic mouse models, the mutant tropoelastin was observed to contribute to lung elastic fibers, but was largely excluded from fibers in the skin and blood vessels (Hu et al., 2010; Sugitani et al., 2012). Although these findings point to tissue-specific mechanisms of elastic fiber assembly, the precise molecular determinants of such tissue specificity remain to be determined. Nevertheless, it is clear that

ADCL-related *ELN* mutations are characterized by a complex molecular disease mechanism with a gain of function in polymerization and a loss of function in elastin-microfibril interaction coupled with some non-specific toxic effects associated with protein misfolding.

The profound functional consequences of alterations at the C-terminus of tropoelastin in ADCL strengthen an existing body of evidence for the essential nature of this region for a variety of activities. For example, the last 17 amino acids of tropoelastin are key for cell attachment both via heparan sulfate proteoglycans (HSPGs) (Broekelmann et al., 2005) and through non-RGD-mediated $\alpha_v\beta_3$ integrin binding (Bax et al., 2009). Consistent with the dynamic, cell-mediated nature of elastic fiber assembly (Czirok et al., 2006; Kozel et al., 2006), antibodies against this C-terminal cell interaction domain interfere with elastin deposition (Brown-Augsburger et al., 1996). Additionally, the hydrophobic domain encoded by exon 30 is essential for tropoelastin polymerization, and hence, fiber formation (Kozel et al., 2003).

Whereas the molecular mechanisms of ADCL are quite complex, autosomal recessive CL (ARCL) involves a simpler, loss of function mechanism. Type 1 ARCL (ARCL1), characterized by severe cardiovascular or pulmonary manifestations (Table 1), can be caused by mutations in *fibulin-5* (*FBLN5*, ARCL1A) (Loeys et al., 2002), *fibulin-4* (*FBLN4/EFEMP2*, ARCL1B) (Huchtagowder et al., 2006), and *LTBP4* (ARCL1C) (Urban et al., 2009).

Loss of function mutations in *FBLN5* result in ARCL1A associated with severe, often fatal, infantile respiratory distress related to developmental emphysema. Other common manifestations include supravalvular aortic stenosis, pulmonary artery stenosis and inguinal hernias (Callewaert et al., 2013; Claus et al., 2008; Elahi et al., 2006; Loeys et al., 2002). *Fibulin-5* knockout mice show a similar phenotype with skin laxity, developmental emphysema, elongation, tortuosity and altered branching of the large arteries (Nakamura et al., 2002; Yanagisawa et al., 2002), an increased angiogenic phenotype (Sullivan et al., 2007), and pelvic prolapse (Drewes et al., 2007). ARCL1A-causing mutations affect the folding and secretion of fibulin-5 resulting in a failure of elastin to be properly integrated with microfibrils (Choi et al., 2009; Hu et al., 2006; Lotery et al., 2006). Biochemical studies have highlighted a number of activities of fibulin-5, including its ability to bind lysyl oxidase-like-1 (LOXL1) (Hirai et al., 2007b; Liu et al., 2004), LOXL2, and LOXL4 (Hirai et al., 2007b), fibrillin-1 (El-Hallous et al., 2007; Freeman et al., 2005; Ono et al., 2009), tropoelastin (Hirai et al., 2007b; Yanagisawa et al., 2002), LTBP2 (Hirai et al., 2007a) and LTBP4 (Noda et al., 2013). The binding of fibulin-5 increases the coacervation and crosslinking of tropoelastin, facilitating elastic fiber formation (Hirai et al., 2007b; Wachi et al., 2008). Furthermore, an RGD motif in fibulin-5 binds $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_4\beta_1$, $\alpha_5\beta_1$ and $\alpha_9\beta_1$ integrins (Lomas et al., 2007; Nakamura et al., 2002). In vascular smooth muscle cells (VSMC), the main integrins responsible for attachment to fibulin-5 are $\alpha_4\beta_1$ and $\alpha_5\beta_1$, however, binding to fibulin-5 does not result in integrin activation (Lomas et al., 2007). Additionally, *in vivo* evidence suggests that fibulin-5-integrin interactions are not necessary for elastic fiber formation, as mice carrying homozygous RGD>RGE mutations in *fibulin-5* are normal (Budatha et al., 2011).

Fibulin-5-deficient tissues show increased angiogenesis and vascular invasion, with elevated VEGF and angiopoietin expression playing a role (Sullivan et al., 2007). Conversely, transplanted tumors show decreased growth in fibulin-5 knockout mice associated with increased reactive oxygen species (ROS) production dependent on β_1 -integrin and fibronectin (Schluter et al., 2010). Fibulin-5 also binds the extracellular superoxide dismutase, Sod3. In the absence of fibulin-5, the localization of Sod3 to the ECM is lost, leading to an elevated extracellular superoxide levels in vessels (Nguyen et al., 2004). However, it remains unclear if any of these physiological changes contribute to disease in human patients with *fibulin-5* mutations.

Mutations in *FBLN4* cause ARCL1B, a disease associated with widespread systemic involvement, including arterial tortuosity, aortic aneurysm, pulmonary hypertension, developmental emphysema, bone fragility, arachnodactyly, joint laxity and diaphragmatic and inguinal hernias (Dasouki et al., 2007; Hoyer et al., 2009; Huchtagowder et al., 2006; Renard et al., 2010). Knockout and hypomorphic mice replicate many of these phenotypes (Hanada et al., 2007; Horiguchi et al., 2009; Huang et al., 2010; McLaughlin et al., 2006). Fibulin-4 binds tropoelastin (McLaughlin et al., 2006), fibrillin-1 (Choudhury et al., 2009; El-Hallous et al., 2007; Ono et al., 2009) and LTBP1 (Massam-Wu et al., 2010). A somewhat different set of ligands, distinct binding affinities for shared ligands and cell type- and developmental stage-specific expression of fibulin-5 and fibulin-4 ensure that these proteins perform non-overlapping functions in elastic fiber assembly. For example, fibulin-5 has higher affinity for tropoelastin than fibrillin-1, whereas fibulin-4 preferentially binds fibrillin-1 over tropoelastin. Fibulin-4 can form a ternary complex with tropoelastin and LOX, facilitating the crosslinking of elastin (Choudhury et al., 2009; Horiguchi et al., 2009). In addition, fibulin-5 enhances tropoelastin coacervation (Hirai et al., 2007b; Wachi et al., 2008). Both fibulin-4 and fibulin-5 limit tropoelastin droplet size during the maturation of coacervates (Cirulis et al., 2008). In both human mutations and animal models, the absence of fibulin-5 leads to elastin deposits that are large and not integrated with the microfibril scaffold (Hu et al., 2006; Nakamura et al., 2002; Yanagisawa et al., 2002). In contrast, *fibulin-4* mutant patients and mice show greatly reduced amounts of elastic fibers, a decrease in elastin-specific desmosine crosslinks and disorganization of the elastic fiber ultrastructure (Horiguchi et al., 2009; Huchtagowder et al., 2006; McLaughlin et al., 2006).

In a VSMC conditional knockout of fibulin-4, the VSMC were shown to be undifferentiated, together with enhanced angiotensin production, increased ERK signaling and cell proliferation (Huang et al., 2010; Huang et al., 2013). Aortic aneurysms caused by fibulin-4 deficiency can be prevented by treatment with an angiotensin converting enzyme inhibitor (captopril) or an angiotensin II type 1 receptor inhibitor (losartan) (Huang et al., 2013; Moller et al., 2005), indicating a role for fibulin-4 in the regulation of angiotensin signaling and VSMC homeostasis and identifying a potential approach for the treatment of patients with *fibulin-4* mutations.

Mutations in *LTBP4* cause ARCL1C, also known as Urban-Rifkin-Davis syndrome, characterized by severe developmental emphysema, severe diverticulosis, tortuosity, enlargement and stenosis of the gastrointestinal tract, diverticulosis of the bladder and diaphragmatic and inguinal hernias (Callewaert et al., 2013; Urban et al., 2009). Respiratory

failure and, less frequently, intestinal perforation are causes of premature death. *LTBP4* utilizes 3 alternative promoters producing one small (LTBP4S) and 2 large (LTBP4L) isoforms. Human mutations identified to date affect all isoforms. A knockout mouse has only been reported for *Ltbp4S* (Sternier-Kock et al., 2002), which nevertheless replicates the elastic fiber phenotypes seen in humans, such as developmental emphysema and the abnormal morphology of elastic fibers with large elastin deposits with a smooth surface devoid of microfibrils (Dabovic et al., 2009; Urban et al., 2009). However, the structural anomalies of the gastrointestinal and urinary systems observed in humans with *LTBP4* mutations are not present in *Ltbp4S*^{−/−} mice, suggesting that normal development of the digestive system and bladder primarily requires LTBP4L.

An increasing body of evidence shows that LTBP4 has at least two functions, one related to TGFβ sequestration, and the other for facilitating elastic fiber assembly. Indeed, the developmental emphysema observed in *Ltbp4S*^{−/−} mice could be partially reversed by reducing the TGFβ2 dose, but the elastic fiber abnormality was not corrected (Dabovic et al., 2009). LTBP4 isoforms are functionally specialized, with LTBP4L preferentially binding TGFβ, and LTBP4S associated with the ECM (Kantola et al., 2010). Consistent with phenotypic similarities between ARCL1A and ARCL1C and between *Fbln5*^{−/−} and *Ltbp4S*^{−/−} mice, LTBP4 binds fibulin-5 (Noda et al., 2013) and fibrillin-1 (Isogai et al., 2003), facilitating the incorporation of fibulin-5/elastin complexes onto microfibrils. LTBP4 is also known to interact with fibronectin and HSPGs and is capable of supporting cell adhesion (Kantola et al., 2008). Although the importance of these functions for elastic fiber formation or TGFβ signaling remains unclear, a human mutation eliminating the C-terminal cell-attachment region results in microfibrillar bundles of abnormally thick and wavy morphology (Callewaert et al., 2013) suggesting that a balanced binding of cells and ECM molecules is necessary for LTBP4 to contribute to normal deposition of elastic fibers.

4. CL and TGFβ signaling

TGFβs are a family of cytokines involved in reciprocal interactions with the ECM. They are secreted in a latent form strongly but non-covalently bound to their propeptides, also known as latency-associated peptides, which in turn interact with latent TGFβ-binding proteins (LTBPs). LTBPs target latent TGFβs to the ECM by binding to fibrillin-1, fibronectin and HSPGs. Activation of latent TGFβs can occur by integrin-mediated force generation, proteolytic degradation, exposure to ROS or interaction with matricellular proteins, such as thrombospondin-1 (Horiguchi et al., 2012).

TGFβs, in turn, up-regulate the expression of many genes necessary for the production of elastic fibers including *fibronectin* (Ignatz et al., 1987), *LTBPs* (Ahmed et al., 1998; Weikkolainen et al., 2003), *ELN* (Kahari et al., 1992; Kucich et al., 1997), *LOXs* (Boak et al., 1994; Kim et al., 2008) and *FBLN5* (Kuang et al., 2006). This regulation occurs at the transcriptional (Ahmed et al., 1998; Ignatz et al., 1987; Kim et al., 2008; Kuang et al., 2006) or posttranscriptional level depending on the gene (Kahari et al., 1992; Kucich et al., 1997). Posttranscriptional regulation of *ELN* and *fibrillin-1* (*FBNI*) is likely achieved in part by TGFβ-mediated suppression of the miR29 family of micro-RNAs (van Rooij et al., 2008), which have binding sites in several mRNAs encoding elastic fiber proteins (Table 2).

During the course of normal development or injury, sequestration of TGF β by elastic fibers serves as a negative feedback signal indicating that sufficient amounts and quality of ECM has been produced. Consistently, loss of function mutations in genes responsible for TGF β sequestration, including *FBN1* and *LTBP4*, result in elevated TGF β signaling (Dabovic et al., 2009; Neptune et al., 2003; Urban et al., 2009). However, recent studies have shown elevated TGF β in several types of CL caused by mutations in genes not directly involved in TGF β sequestration, but rather required for the biogenesis of elastic fibers including *ELN* (Callewaert et al., 2011; Hu et al., 2010), *fibulin-4* (Hanada et al., 2007; Renard et al., 2010) and *ATP6V0A2* (Fischer et al., 2012). Thus, impaired elastic fiber function sensed by cells, in turn up-regulates TGF β activity. Sensing of ECM dysfunction may involve integrin-mediated activation of TGF β itself, which is dependent on the mechanical properties of the ECM, as well as on force generation by the cells (Hinz, 2009). Consistently, mutations in actin (*ACTA2*) and myosin (*MYH11*) genes, required for the contractility of VSMC, are mutated in familial thoracic aortic aneurysms and are associated with increased TGF β signaling (Renard et al., 2013).

5. The CL proteins and TGF β in evolution

In humans, there are three fibrillins and four LTBP. These proteins contain multiple TGF β -binding (TB) domains interspersed with numerous calcium-binding epidermal growth factor domains. The TB domain, which is found in no other proteins, emerged over 600 million years ago in the Eumetazoa (Peterson and Butterfield, 2005) within a single fibrillin gene (Piha-Gossack et al., 2012). Diversification of the TB domain led to the emergence of the first LTBP-like protein in sea urchins (Robertson et al., 2011). TGF β also appeared at this time suggesting co-evolution of their functions (Robertson et al., 2011). Elastin appeared later still, with the divergence of jawless fish (agnatha) and jawed vertebrates, known as gnathostomes within the phylum Chordata (Keeley, 2013). This time period coincided with a duplication event leading to two fibrillins (Piha-Gossack et al., 2012), and the appearance of a closed circulatory system (Faury, 2001). The single fibrillin had also acquired a unique hybrid domain and RGD-intergrin binding sites in two of the TB domains by this time (Piha-Gossack et al., 2012). Like fibrillin, fibulin-like proteins also existed in the Eumetazoa, much earlier than the emergence of elastin supporting a functional role for fibulins independent of elastic fiber assembly (Segade, 2010). Interestingly, diversification of the fibulins also occurred at the same time as the evolution of elastin. The appearance of specialized domains and the occurrence of duplication events around the time of the evolution of elastin underscores the potential functional significance of these specialized regions and duplicated proteins in elastic fiber assembly.

6. CL and the secretory pathway

Several CL-related genes are required for intracellular protein trafficking, highlighting the importance of the secretory pathway in elastic fiber biogenesis. Loss of function mutations in *ATP7A*, a copper transporter localized to the Golgi apparatus, cause Menkes disease or a milder disease, occipital horn syndrome (OHS) (Das et al., 1995). OHS, also known as X-linked CL displays LOX deficiency associated with impaired crosslinking of elastin and collagen (Byers et al., 1980). Recessive mutations in the gene for the A2 subunit of the

vacuolar proton pump, *ATP6V0A2*, cause ARCL2A (Kornak et al., 2008). *ATP6V0A2*-deficient cells show accumulation of tropoelastin in the Golgi, Golgi fragmentation and an accumulation of lamellar bodies, leading to impaired secretion of tropoelastin, but relatively preserved production of fibrillin-1 and LOXs (Huchtagowder et al., 2009). Macrocephaly alopecia CL scoliosis (MACS) syndrome is caused by a recessive mutation in *RIN2* (Ras and Rab interactor 2) (Basel-Vanagaite et al., 2009). Subsequently, a recessive *RIN2* mutation was also described in an Ehlers-Danlos syndrome-like condition (Syx et al., 2010). Tissues and cells from individuals with *RIN2* mutations show abnormal endoplasmic reticulum, Golgi apparatus, elastic and collagen fibers (Basel-Vanagaite et al., 2009; Syx et al., 2010). Geroderma osteodysplasticum, a disease related to CL, is caused by recessive mutations in the *GORAB* (goglin, Rab6 interacting) (Hennies et al., 2008). *GORAB* is localized to the Golgi apparatus where it interacts with Rab6 G-proteins, which recruit kinesin motor proteins required for the trafficking of secretory vesicles to the plasma membrane (Grigoriev et al., 2007).

7. CL and metabolism

Three conditions related to CL are caused by mutations in molecules required for cellular metabolism. ARCL2B, is caused by recessive mutations in gene for pyrroline-5-carboxylate reductase 1 (*PYCR1*) (Guernsey et al., 2009; Reversade et al., 2009), a mitochondrial enzyme that catalyzes the final step of proline biosynthesis (De Ingeniis et al., 2012). *PYCR1* mutant cells do not show overt proline deficiency, but are sensitive to oxidative stress, suggesting that *PYCR1* is required for the maintenance of mitochondrial antioxidant balance (Reversade et al., 2009). De Barsy syndrome, also known as ARCL3, can be caused by mutations in *ALDH18A1* (Bicknell et al., 2008; Skidmore et al., 2011), the gene for another mitochondrial enzyme in the proline biosynthetic pathway, δ^1 -pyrroline-5-carboxylate synthase (*P5CS*). In *ALDH18A1* mutant cells, the assembly of collagen type I and elastin into ECM fibers is diminished (Bicknell et al., 2008; Skidmore et al., 2011). Arterial tortuosity syndrome (ATS), related to CL, is caused by mutations in the *SLC2A10* gene (Coucke et al., 2006), which encodes the facilitative glucose transporter family member 10 (GLUT10). Patients show disorganized elastic fibers in the arterial wall and elevated TGF β signaling. Surprisingly, mice with inactivating missense mutations in *Slc2a10* do not show phenotypes characteristic of ATS (Callewaert et al., 2008; Cheng et al., 2009). In contrast, *slc2a10* knockdown in zebrafish produces disorganization of the vasculature, wavy notochord and cardiac edema, as well as mitochondrial dysfunction and reduced TGF β signaling (Willaert et al., 2012). *SLC2A10* was shown to be transport dehydroascorbate (oxidized vitamin C) into mitochondria to limit the production of ROS (Lee et al., 2010). Thus, together with *PYCR1*, *SLC2A10* is also required for the maintenance of mitochondrial redox balance.

8. Concluding remarks

Human genetic studies on CL patients have revealed a network of genes required for elastic fiber assembly, TGF β sequestration and activation, vesicular trafficking in the Golgi apparatus and metabolic function of the mitochondria. The connections between secreted proteins (elastin, fibulin-4, fibulin-5, LTBP4) and their interactions with other elastic fiber-

related proteins (fibronectin, lysyl oxidases, fibrillins, LTBP) are well known and often involve direct binding. However, the temporal and spatial hierarchy of these interactions has not been defined yet. A combination of developmental and live imaging investigations will be necessary to have a comprehensive mechanistic view of elastic fiber assembly. The involvement of the posttranslational and sorting mechanisms that occur in the secretory pathway will also need to be investigated, as they may define the timing, order and precise biochemical milieu in which the components can interact. Finally, the role of small molecule metabolites (ROS, proline) as well as signaling molecules (angiotensin, TGF β) in elastic fiber biogenesis and dysfunction will need to be elucidated, as these molecules are more amenable to therapeutic modification than structural elastic fiber proteins.

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Figure 1.

Electron microscopic analyses of elastic fibers in skin punch biopsies from unaffected and CL patients. (A) An unaffected individual; (B) A patient with a heterozygous mutation in the *elastin* gene; (C) A patient with a homozygous mutation in the *LTBP4* gene; (D) Dermal elastic fibers from a patient with CL of an unknown cause. Bar = 500 nm.

Table 1

Cutis laxa and related genes

Disease *	Distinguishing Clinical Features	Mutated Genes	Gene function	References
ADCL	Pulmonary and cardiovascular manifestations absent, milder or later onset	ELN	Structural protein of the elastic fibers	(Callewaert et al., 2011; Hu et al., 2010)
ARCL1A	Supravalvular aortic stenosis, lethal developmental emphysema	FBLN5	Accessory protein of the elastic fibers, binds ELN, FBN1, LOXL1, LTBP2 and LTBP4	(Loeys et al., 2002)
ARCL1B	Arterial tortuosity, lethal pulmonary hypertension, bone fragility	FBLN4 EFEMP2	Accessory protein of the elastic fibers, binds LOX and FBN1	(Dasouki et al., 2007; Huchtagowder et al., 2006; Renard et al., 2010)
ARCL1C/URDS	Severe gastrointestinal and urinary malformations, lethal developmental emphysema mild cardiovascular involvement	LTBP4	Accessory protein of the elastic fibers, binds TGF β 1, FN, FBN1, and FBLN5	(Urban et al., 2009)
ARCL2A	Growth and developmental delay, abnormal glycosylation of serum proteins	ATP6V0A2	A subunit of the vacuolar-type proton pump, acidifies vesicles in the secretory pathway	(Huchtagowder et al., 2009; Kornak et al., 2008)
ARCL2B	Growth and developmental delay, triangular face, normal glycosylation	PYCR1	Mitochondrial enzyme in the proline-biosynthetic pathway	(Guernsey et al., 2009; Reversade et al., 2009)
XLCL	Occipital exostoses, pili torti	ATP7A	Copper transporter required for lysyl oxidase activity	(Byers et al., 1980; Kennerson et al., 2010; Moller et al., 2005)
DBS/ARCL3	Corneal clouding, athetoid movements	ALDH18A1	Mitochondrial enzyme in the proline-biosynthetic pathway	(Bicknell et al., 2008; Skidmore et al., 2011)
GO	Bone fragility, short stature	GORAB	Localized to the Golgi, binds RAB6, a G-protein involved in vesicle trafficking	(Hennies et al., 2008)
MACS	Macrocephaly, alopecia, scoliosis	RIN2	Localized to the Golgi, is a guanine nucleotide exchange factor of RAB5	(Basel-Vanagaite et al., 2009)
ATS	Triangular face, arterial tortuosity, normal lungs	SLC2A10	Transports dehydroascorbate into mitochondria	(Coucke et al., 2006; Lee et al., 2010)

* ADCL, autosomal dominant cutis laxa; ARCL, autosomal recessive cutis laxa; URDS, Urban-Rifkin-Davis syndrome; XLCL, X-linked cutis laxa; DBS, DeBary syndrome; GO, geroderma osteodysplasticum; MACS, macrocephaly alopecia cutis laxa scoliosis syndrome; ATS

Table 2Elastic fiber genes as targets of miR29 regulation^{*}

Gene	Number of binding sites (target score)			
	miR29a-3p	miR29b-3p	miR29c-3p	miR29b-5p
ELN	3 (78)	3 (77)	3 (78)	0
FBN1	2 (86)	2 (85)	2 (85)	1 (78)
LOX	3 (81)	3 (81)	3 (80)	0
LTBP1	0	0	0	1 (53)

^{*}Data based on miRDB searches (<http://mirdb.org/miRDB/>)