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Alterations of elastic fibers in genetically modified mice and human genetic diseases

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1. ABSTRACT

The two main components of elastic fibers are elastin and microfibrils. Fibrillin and microfibrillar-associated glycoproteins are the essential constituents of microfibrils. In the last ten years, the analysis of genetically modified mice and human genetic diseases has led to the observation that, besides elastin, fibrillins and microfibrillar-associated proteins, numerous other molecules play an essential role in the supramolecular organisation of the elastic fibers in the extracellular space and in the interactions between elastic fibers and cells. These recent data are summarized in this review.

2. INTRODUCTION

The proteins and glycoproteins of the extracellular matrix (ECM) play an essential role in the organisation of the tissues in multicellular organisms. They not only provide a physical scaffold for cells but also provide specific molecular and spatial information that influences cell survival, migration, and differentiation (1). Macromolecules of the ECM have been classified in four groups : collagens, proteoglycans, structural glycoproteins and elastin (2). Elastin is the main component of elastic fibers (3, 4). This protein is not detectable in invertebrates and primitive vertebrates and first appears during evolution

in vertebrates with a closed circulatory system (5). Numerous molecules are associated with elastin : the suppression of one of these molecules using knockout mice has revealed their role in elastic fiber formation and function. Elastic fibers are present in various tissues : blood vessels, skin, lungs, intervertebral disc and ear elastic cartilage where they provide elastic recoil to these tissues (6). Elastic fibers are organized as thick lamellae in the aorta whereas, according to the proportion of elastin, three types of fibers, oxytalan, elaunin and elastic fibers, are observable in the skin. The expression of the elastin gene and genes encoding other components of elastic fibers are highly regulated during development. This review will highlight some structural and functional aspects of elastic fibers and will describe their alterations in various genetically modified mice and in human genetic diseases.

3. MOLECULAR COMPONENTS OF ELASTIC FIBERS

The organisation of elastic fibers in the extracellular space varies considerably in the different elastic tissues. In the arteries, elastic fibers are mainly organized as elastic lamellae with fenestrations (7-9). At the ultrastructural level, the center of the fiber is occupied by 'amorphous' elastin whereas the periphery is composed of an outer mantle of microfibrils (3, 4). The thoracic aorta contains about 40% elastin (% dry weight) (10). In the skin, elastic fibers form a three-dimensional meshwork, which spans from the dermo-epidermal junction to the deep dermis (11, 12). In the upper dermis, the fibers are thin, perpendicular to the dermo-epidermal junction and consist mainly of microfibrils. These fibers, named oxytalan fibers, are linked together with elaunin fibers, which consist of a mixture of microfibrils and elastin. The elastic fibers in the deep dermis contain mainly elastin, like elastic fibers from the arteries. The volume density of elastin in normal human dermis is around 2-3% (13).

The two main components of elastic fibers are elastin and microfibrils (6, 14, 15). Elastin is an insoluble protein, which is synthesized as tropoelastin, a soluble precursor of ≈ 70kDa. The tropoelastin molecule consists of alternating hydrophobic and lysine-rich sequences, which are encoded by different exons (16). Hydrophobic sequences are responsible for the elastic recoil in hydrated conditions (17). Oxidative deamination of the lysine residues, catalysed by lysyl oxidases, is the first step in the formation of specific covalent cross-links, leading to insolubilisation of the protein by which it acquires its functional property, elasticity, and its resistance to numerous proteolytic enzymes (18, 19).

The elastic-fiber-associated microfibrils were first observed by electron microscopy as long, 10- to 12-nm wide microfibrils. The isolation and biochemical characterization of these microfibrils demonstrated that fibrillins and microfibril-associated glycoproteins (MAGPs) are the main constituents (4). Fibrillin-1 and Fibrillin-2 are large glycoproteins (≈ 350kDa) with highly homologous structures but distinct patterns of expression. Fibrillin-2 is expressed earlier in development than fibrillin-1 (20, 21). The two glycoproteins contain 43 calcium-binding epidermal growth factor (cbEGF)-like domains and seven Transforming Growth Factor (TGF)-beta-binding protein-like (8-cysteine) domains.

MAGP-1 and –2 are small microfibrillar-associated glycoproteins (31 and 25 kDa, respectively) with no repetitive motifs. Human MAGP-1 is a 183-residue molecule that has an acidic N-terminal half and a C-terminal sequence that contains 13 cysteine residues (22). MAGP-2 is a 170-173-residue protein that is related to MAGP-1, mainly in the central part of the molecule that shares the precise alignment of 7 cysteine residues (23). Immunolocalisation studies suggest that these two glycoproteins are constitutive components of most microfibrils (23, 24).

Several other proteins have been localized on elastic fibers using immunohistochemistry, but little is known about them and whether they are essential components of elastic fibers. Glycosaminoglycans, decorin, biglycan and osteopontin have all been found to be associated with elastin in normal fibers and several others (osteonectin, vitronectin, clusterin, …) with pathologic elastic fibers (3, 25).

4. SYNTHESIS OF ELASTIC FIBERS

Elastic fibers are synthesized by smooth muscle cells in the aorta (26, 27), by fibroblasts in the skin (19, 28), the lung (29) and by chondrocytes in the elastic auricular cartilage (30). The formation of elastic fibers occurs during the second half of intrauterine life and during growth. Expression of fibrillin-1 and fibrillin-2 is detectable from early developmental stages whereas elastin expression begins at mid-gestation (20, 21). Synthesis of fibrillin-2 occurs mainly during *in utero* development whereas synthesis of fibrillin-1 is maintained throughout life: in adults, mRNA for fibrillin-2 is undetectable while mRNA for fibrillin-1 is only decreased (31). Elastin synthesis is the highest during childhood and decreases to a very low level in adulthood (21, 31).

The elastin gene exists as a single copy on chromosome 7 in humans, chromosome 12 in rat and chromosome 5 in mouse. The primary transcript undergoes tissue- and development-dependent alternative splicing (32). The protein product, tropoelastin, is secreted into the extracellular space after few post-translational modifications (hydroxylation of some proline residues, no glycosylation) (33). During the secretion process, tropoelastin is associated with a chaperone protein, the elastin-binding protein (EBP, a slice variant of β-galactosidase), and two other proteins, neuraminidase 1 and protective protein/cathepsin A (34).

In the extracellular space, the galactosugars of microfibrils induce the release of tropoelastin from the chaperone complex EBP-Neuraminidase-protective protein/cathepsin A and then the tropoelastin molecules aggregate *via* hydrophobic interactions. Tropoelastin molecules are covalently linked to each other through the

formation of cross-links. The specific cross-links in elastin are desmosine and isodesmosine (35). These two isomers are formed from four lysine residues. The first step in the formation of desmosine and isodesmosine is the oxidative deamination of the ε-amino groups of some lysine residues. This deamination is catalysed by one isoform of the lysyl oxidase family, LOX or LOXLs (18). This cross-linking process confers to elastin its functionality, elasticity, and its resistance to proteolytic enzymes. The formation of microfibrils, the synthesis and insolubilisation of tropoelastin molecules are essential, but not sufficient, to obtain the overall well-organized elastic fibers. The invalidation of genes encoding small proteins like fibulins (see below) demonstrates that several proteins, glycoproteins or proteoglycans interact with both cells and elastic fiber components and participate in the laying down of elastic fibers in the extracellular space. In the adult aorta, monolayers of smooth muscle cells are observed between the elastic lamellae, an organisation previously described as lamellar units by Glagov *et al* (7).

5. WHAT WE HAVE LEARNT FROM GENETICALLY MODIFIED MICE

It is not possible to live without elastin: mice that lack elastin die from arterial occlusion four to five days after birth (36). The lack of elastin is not associated with endothelial damage, thrombosis or inflammation but with an intense proliferation and reorganisation of smooth muscle cells, observed in the subendothelial space which leads to the complete occlusion of arteries. This thus demonstrates that beside its function of elasticity, "elastin is an essential determinant of arterial morphogenesis" (36). Mice hemizygous for elastin (Eln+/- mice) are viable (37). The overall appearance, behaviour and life expectancy of Eln+/- mice are similar to those of wild-type mice. The quantity of elastin in the aorta is reduced by 50% but the number of elastic lamellae is increased by 30% (37). The thickness of the aorta is not modified, in contrast to what is observed in the human aorta of patients suffering from supravalvular aortic stenosis (SVAS), a pathological state linked to loss-of-function mutations within the elastin gene (see below) (37). Eln +/- mice are hypertensive: their mean arterial pressure is increased by 30% in adult animals (38-41).

The increase in elastin content in the aorta of Eln+/- mice expressing the human elastin gene in a bacterial artificial chromosome reverses hypertension and other cardiovascular changes associated with Eln+/- phenotype (42). Expression of the human elastin gene in the aorta of Eln-/- mice partially rescues the perinatal lethality associated with the null phenotype (42). These Eln -/- mice expressing the human elastin gene are hypertensive and the ascending aorta, containing 30% of the normal elastin content, is thickened (42). The cardiovascular phenotype associated with elastin insufficiency thus correlates with the elastin content in the aorta. This may explain the phenotypic variability associated with SVAS in humans (42).

Fbn1 and Fbn2 mutant mice exhibit distinct phenotypes. Four mouse strains harbouring distinct mutations in the fibrillin-1 gene have been created. The two first mutations produced were a hypomorphic in-frame deletion (mgΔ) and a hypomorphic mutation (mgR) that produces ≈ 15% of the normal amount of wild-type fibrillin-1, which in homozygosity mimics the neonatal and the adult lethal forms of Marfan syndrome, respectively (43, 44). Using homologous recombination in murine ES cells, the mutation Cys^{1039}->Gly (C1039G) was introduced into an exon of the mouse Fbn1 that encodes a cbEGF-like domain (45). Mice homozygous for this mutation die in the perinatal period but the heterozygous mice reveal impaired microfibrillar deposition, skeletal deformity and progressive alterations of the elastic lamellae in the aorta, which are comparable to the human condition (45). These heterozygous mice show a normal life span. Another mouse strain with the complete suppression of the Fbn1 gene was more recently created (mgN) (46). MgN/mgN (or Fib1-/-) mice die soon after birth. The heterozygous mgN/+ mice were crossed with Fbn2+/- mice in order to study the respective role of fibrillin-1 and fibrillin-2 in the initial steps of microfibril formation during development (46).

Mice lacking fibrillin-2 are viable, have a normal life span but present defective BMP signalling and limb patterning (syndactily) (47). Because the organisation of the arterial walls and the formation of elastic fibers are not altered, it was suggested that fibrillin-2 is either dispensable for vascular development or redundant with fibrillin-1.

The mice lacking both fibrillins die *in utero* (after E14.5) (46). Half of the mice with only one copy of the fibrillin-1 gene (Fbn1+/-; Fib2-/-) die *in utero* in contrast to Fbn1-/-;Fbn2+/- and Fbn1+/-;Fbn2+/- mice who develop to birth (or are found at the expected frequency in the newborn mice). The contribution of fibrillin-2 molecules either quantitatively or at a specific stage of development is thus essential and can only be replaced when two copies of the fibrillin-1 gene are present (46, 47). *In utero* elastogenesis is defective or delayed in Fbn1-/-; Fib2-/- and Fbn1+/-; Fib2-/- mice. A minimal amount of fibrillin molecules in the extracellular space is thus necessary for the initiation of elastic fiber formation. The complete deletion of fibrillin-2 does not impair elastogenesis like the complete deletion of fibrillin-1: the total absence of Fbn1 in the mouse aorta leads to an abnormal morphology of elastic lamellae even if the cross-linking of tropoelastin molecules is not altered (46).

On the same genetic background (C57bl/6J), it appears that the deletion of one Fbn1 allele is less deleterious than the presence of an allele with one mutation (C1039G) : compared to wild-type mice, the only anomaly detected in the aortas of adult Fbn1+/- mice was a 10% increase in length and no significant increase in the thickness or the outer diameter was observed whereas, in C1039G/+ mice, after two months of age, the diameter and thickness of the aorta progressively increased and elastic fiber fragmentation and disorganisation of vascular smooth muscle cells were observed on histological sections. The C1039G mouse model has been used to test the potential use of an angiotensin II type1 receptor antagonist for the treatment of Marfan patients (48).

The potential role of MAGP-1, one isoform of microfibrillar-associated glycoproteins, in the formation of elastic fibers is, at this time, difficult to assess as elastic tissues rich in microfibrils develop normally and show normal function in the Magp-1-deficient mice (49).

An essential step in the formation and stabilisation of elastin is the cross-linking process. The crosslinks are essential for the mechanical stability and the resistance to proteolytic enzymes of elastic fibers as well as collagen fibers. Lysyl oxidases are enzymes that initiate the formation of the lysine and hydroxylysine-derived crosslinks in collagens and lysine-derived cross-links in elastin. Five human lysyl oxidase isoenzymes (LOX and four LOX-like, LOXL-1 to 4) have been described but little was known about their specific functions before the creation of the knockout mice. The isoform LOX plays an essential role in the development and function of the cardiovascular system as inactivation of the Lox gene causes structural alterations in the arterial walls and rupture of aortic aneurysms, leading to the perinatal death of Lox-/- mice (50-52). At the microscopic level, elastic fibers are fragmented in the aorta. The amount of desmosine was decreased by ≈60% in the aorta of Lox-/- mice (52). However, the detection of desmosine in the aorta and lung of homozygous mutant null mice demonstrated that other isoform(s) of lysyl oxidase participate in the formation of elastin cross-links (52). The essential role of LOX during development and the formation of elastin fibers is in agreement with its high expression during the perinatal period (31). Inactivation of the mouse Loxl1 gene is not lethal but Loxl1-/- mice developed enlarged airspaces of the lung, increased laxity of the skin, defective post-partum retraction of the uterus and, as shown by electron microscopy, the elastic lamellae in the aorta had a reduced amorphous elastin polymer content (53). The desmosine content was reduced in all elastic tissues. Beside LOX, LOXL1 thus plays a role in the formation of elastin cross-links. The expression of LOXL1 is maintained throughout life and may thus participate in the process of tissue remodelling (31). In addition to its enzymatic activity, LOXL-1 has a structural role through its interactions with fibulin. 5.

Together with the main components of elastic fibers, numerous other proteins are associated with elastic fibers : Fibulins, EMILINs, etc.

EMILIN-1 (Elastin-Microfibril Interface Located Protein-1) is one member of the newly defined EMILIN/Multimerin family (54). Its role in elastic fiber assembly is not yet clear but it can act as a bridge between tropoelastin and fibulin-5 (55).

Fibulins are a family of seven extracellular glycoproteins with tandem cbEGF-like modules and a fibulin-type C-terminal domain (56). Fibulin-1 to -5 are found in elastic tissues (57). Fibulin-1 deficiency in mice causes extensive haemorrhaging due to abnormal endothelial integrity and perinatal death (58, 59). Inactivation of the fibulin-2 (Fbln2-/-) and –3 (Fbln3-/-) genes has no effect on elastic fiber formation (60, 61) whereas mice lacking fibulin-4 (Fbln4-/-) or fibulin-5 (Fbln5-/-) have highly disrupted and disorganized elastic fibers, leading to defects in skin, arterial blood vessels, and lungs (62-66).

Some other glycoproteins like latent-TGF-beta binding proteins (LTBPs) do not interact with elastin but, *via* their interactions with fibrillins, play a role in elastogenesis (67-73).

Elastic fibers are thus composed of the protein elastin together with multiple glycoproteins. The deletion of one of these glycoproteins leads minimally to morphologically altered elastic fibers and maximally to functionally defective elastic fibers and deficient aorta and lung organogenesis.

6. ALTERATIONS OF ELASTIC FIBERS IN HUMAN GENETIC DISEASES

Several genetic diseases in humans are due to deficiency of one elastic fiber component. Mutations in other proteins, not related to ECM, may also lead to severe alterations of elastic fibers (Table).

Supravalvular aortic stenosis (SVAS) is a congenital narrowing of the ascending aorta, which can occur sporadically as an autosomal dominant disorder or as one component of the Williams-Beuren syndrome (74, 75). Williams-Beuren syndrome (WBS) is a multisystem disorder caused by deletion of 26 to 28 genes of chromosome 7q11.23, including the elastin gene. Functional hemizygosity for elastin is known to be the cause of SVAS (76-79). Mutations in the elastin gene associated with this vascular disease are located in the 5'-end and middle region of ELN; they include nonsense, frameshift, translation initiation, and splice site mutations (80). The reduced elastin mRNA levels, tropoelastin synthesis and deposition of insoluble elastin in the aorta of patients with SVAS leads to the increased proliferation of arterial smooth muscle cells (81, 82). This results in the increased number of elastic lamellae and lamellar units as well as increased aortic wall thickness (37). The addition of exogenous elastin reversed this increased proliferation of SVAS smooth muscle cells. Similarly, the addition of elastin to vascular smooth muscle cells from Eln -/- mice inhibits proliferation, regulates migration and induces actin stress fiber organisation (81). All these results indicate that elastin stabilizes arterial structure by inducing a quiescent contractile state in smooth muscle cells.

Other elastin mutations can lead to a completely different phenotype, the extremely rare autosomal cutis laxa (83). The presence of redundant, sagging skin, already apparent at birth or in early childhood, leads to early diagnosis. Histological studies are frequently pathognomonic, demonstrating fragmented elastic fibers in the skin. The mutations described are located in the exons 30 and 32, in the 3' end of the coding region of ELN (84-86). In comparison to the mutations detected in SVAS, which are consistent with loss of function and result in haploinsufficiency, mutations in autosomal dominant cutis

laxa are consistent with a dominant negative mechanism (80). Mutations in fibulin-5 and fibulin-4 may also result in either autosomal dominant or autosomal recessive cutis laxa (87-90).

Patients with an X-linked cutis laxa syndrome have distinct clinical features : besides generalized cutis laxa, they have skeletal anomalies and cardiovascular defects. X-linked cutis laxa syndrome is allelic to Menkes disease and is caused by mutation in the copper-transporting P-type ATPase ATP7A. Copper being an essential co-factor of lysyl oxydases, a defect in the transport of copper leads to deficient cross-linking in elastin and collagens (18).

Marfan syndrome is an autosomal dominant, multi-system disease characterized by long bone overgrowth, dislocation of the ocular lens, and dilatation of the aortic root (91). The tissue distribution, severity, and the age of onset of clinical features are variable among patients. Mutations in the FBN1 gene cause classic Marfan syndrome. However, besides classic Marfan syndrome, mutations in FBN1 have been associated with a broad spectrum of phenotypes : neonatal Marfan syndrome, isolated ectopia lentis, isolated ascending aortic aneurysm and dissection, and isolated skeletal features (91). Nevertheless, it has been discovered that i) mutations in exons 24-32 of FBN1 are associated with a more severe and complete Marfan phenotype, ii) a higher probability of ectopia lentis was found for patients with a missense mutation substituting or producing a cysteine residue, when compared with other missense mutations, and iii) premature termination codons are associated with severe skeletal and skin phenotypes (92). The difficulty in investigating clear genotype-phenotype correlations is illustrated by the clinical heterogeneity among individuals with the same mutation, within and among families (92). This complexity has recently increased by the demonstration that mutations within the N-terminal part of the fourth TGF-beta-binding protein-like (TB4) domain of fibrillin-1, which contains the unique RGD motif within the molecule, are responsible for the stiff skin syndrome (93). The stiff skin syndrome, an autosomal dominant congenital form of scleroderma, is characterized by excessive microfibrillar, elastin and collagen deposition, and impaired elastogenesis in the dermis. The 10 affected individuals had no signs of the caracteristic skeletal, ocular or cardiovascular alterations of Marfan patients (93). The three mutations detected in the four families analyzed create or substitute a cysteine residue in the TB4 domain. Interestingly, mutations affecting the corresponding amino acid residues in the TB6 domain result in typical Marfan syndrome. The increased concentrations of LTBP-4 (Latent TGF-beta Binding Protein-4), phosphorylated Smad 2 and CTGF (Connective Tissue Growth Factor) demonstrate an activated TGF-beta signalling in the dermis of affected patients relative to controls. This activated TBF-beta signalling can stimulate the observed epithelial-to-mesenchymal transition and the fibrotic process (93).

The demonstration that fibrillin-1 regulates the bioavailability of TGF-beta (94) and the overlap of pathological phenotypes associated with mutations in either fibrillin-1 or TGFBR1/2 led to the hypothesis that up-regulation of TGF-beta signalling is the predominant mechanism in these pathologies. In fact, the treatment of Fbn1 C1039G/+ mice with TGF-beta-neutralizing antibody reduces the elastic fiber fragmentation, the enlargement of the aortic root and the increase in aorta thickness (95). Similarly, Losartan, an angiotensin II type 1 receptor antagonist known to decrease TGF-beta signalling, reduces the rate of aortic dilation both in C1039G/+ mice (95) and in children with Marfan syndrome who had severe aortic root enlargement (96). However, the recent demonstration of a similarly increased TGF-beta content and phosphorylated Smad 2 in the aneurysmal wall of patients suffering from thoracic aortic aneurysm of different etiologies : Marfan syndrome, bicuspid aortic valve or the degenerative form, maintains the controversy over the link between the defective fibrillin-1 molecule in Marfan syndrome and the increase in TGF-beta signalling (97, 98).

Pseudoxanthoma elasticum (PXE) is a genetic disorder characterized by ectopic mineralization of connective tissues, especially within elastic fibers, with primary clinical manifestations in the skin, cardiovascular system, and eyes (99). The classic forms of PXE are due to loss-of-function mutations, multi-exon or single-exon deletions in the ATP-binding cassette transporter ABCC6 gene (100, 101). The ABCC6 protein is a transmembrane efflux transporter expressed in the liver and the kidneys. The physiological function of ABCC6 as well as its relation to ectopic mineralization and fragmentation of elastic fibers are still unknown. The description of a PXE-like phenotype in patients with missense mutations in the gamma-glutamyl carboxylase (GGCX) gene led to the hypothesis that defective gamma carboxylation of glutamyl residues in the inhibitors of calcification, matrix gla protein (MGP) and osteocalcin, results in ectopic calcification and elastic fiber mineralization; the defective carboxylation could also be due to a lower concentration of vitamin K, an essential co-factor the gla-protein carboxylation (102). Whether vitamin K is the substrate of the ABCC6 transporter requires additional experiments to establish.

Other elastinopathies could be due to mutations in other proteins. Neuraminidase-1 (Neu1), cathepsin A/protective protein (CathA) and beta-galactosidase (beta-Gal) are integral components of a lysosomal multiprotein complex. The enzymatically inactive spliced variant of beta-galactosidase, S-Gal, also binds to Neu1 and CathA. S-Gal, also named elastin-binding protein (EBP), acts as a chaperone for tropoelastin in the secretion process. The heterotrimeric complex, EBP-Neu1-CathA, also acts as a non-integrin cell surface elastin receptor that recognizes elastin peptides. The essential role of EBP in the formation of elastic fibers is supported by the observation of altered elastic fibers in GM1-gangliosidosis and Morquio B patients who have mutations in the GAL gene (103). Mutations in CathA and Neu1 lead to galactosialidosis and

sialidosis, respectively. Defects in elastic fibers are also observed in these two multiorgan diseases (104).

7. CONCLUSIONS

Whatever the initial mutated gene, any defect in the synthesis of elastic fibers or in the laying down of elastic fibers in the extracellular space leads to altered function of elastic tissues. However, the genetic defect in an elastic fiber component is not equally expressed in all elastic tissues : the skin, the lungs or the arteries and aorta may be mainly affected, depending on the defective protein or glycoprotein.

Other alterations of elastic fibers occur during aging (2). These alterations are described in the chapter entitled "Aging of the extracellular matrix". They result from the binding of calcium and lipids to elastin and from the enzymatic degradation of elastin and microfibrils which is due to the imbalance between proteases and their inhibitors through the induction of protease expression, the activation of zymogens or the secretion of enzymes by inflammatory cells. The degradation of elastin and the biological functions of the elastin peptides released is dealt with in the chapter "The cell-elastin-elastase(s) interacting triade directs elastolysis"**.**

Given that elastin synthesis occurs essentially during development and growth and that elastin synthesis is very low in adult tissues, everything possible should be done to preserve this stock of elastin.

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Abbreviations : ABCC6, adenosine triphosphate (ATP)-binding cassette transporter C6 ; beta-gal, beta-galactosidase : BMP, bone morphogenic protein ; CathA,

cathepsin A/protective protein ; cbEGF, calcium-binding epidermal growth factor ; CTGF, connective tissue growth factor ; EBP, elastin-binding protein ; ECM, extracellular matrix ; EMILIN, elastin-microfibril interface located protein-1 ; GGCX, gamma-glutamyl carboxylase ; LOX, lysyl oxidase ; LOXL, lysyl oxidase-like LTBP, latent-TGF-beta binding protein ; MAGP, microfibril-associated glycoprotein ; MGP, matrix gla protein ; Neu1, Neuraminidase –1 ; PXE, *Pseudoxanthoma elasticum* ; SVAS, supravalvular aortic stenosis ; TB, TGF-beta-binding protein-like domain ; TGF, transforming growth factor ; TGFBR, TGF-beta receptor

Key Words : Elastin, Fibrillin-1, Fibrillin-2, Fibulin-5, Lysyl Oxidase, Lysyl Oxidase-Like, Supravalvular Aortic Stenosis, Marfan Syndrome, *Pseudoxanthoma elasticum*, Review

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