Investigation of ocular biomechanical defects in mice with microfibril and elastic fiber defects

Rachel W. Kuchtey[1,2], John Kuchtey[1] , Lauren Wareham[1], Hang-Jing Wu[1], Evan Krystofiak[3], Yusheng Wu[3], Cynthia Reinhart-King[3], Vijaykrishna Reghunathan[4], Elena Pokidysheva[3], and Sergey Budko[3]

[1]Ophthalmology and Visual Sciences, [2]Molecular Physiology & Biophysics, Vanderbilt University Medical Center, [3]Vanderbilt University, [4]University of Houston

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Purpose

Lysyl oxidase-like 1 (LOXL1) and fibrillin-1 (FBN1) are abundant proteins in exfoliation material (XFM), a hallmark of exfoliation syndrome (XFS). *LOXL1* is associated with XFS and many mutations in *FBN1* cause Marfan syndrome (MFS). LOXL1 and fibrillin-1 also co-exist in normal lens zonules. Zonule weakness is a common ocular feature shared by XFS and MFS. Here we sought to uncover their involvement in ocular health and disease.

Methods

Loxl1$^{-/-}$ and *Fbn1*$^{C1039G/+}$ mice on a 129 background were gifts from Drs. Tiansen Li and Hal Dietz and were used to create a double mutant (dbm) line, *Fbn1*$^{C1039G/+}$*/Loxl1*$^{-/-}$. Survival and systemic phenotypes were monitored and anterior segment morphology was measured by SD-OCT. Immunohistochemistry (IHC), transmission electron microscopy (TEM), atomic force microscopy (AFM) and biochemistry approaches were employed.

Results

Fbn1$^{C1039G/+}$ mice survive more than 1 year, consistent with being a mild MFS mouse model. Likewise, *Loxl1*$^{-/-}$ mice survive beyond 1 year of age. However, dbm mice do not survive beyond 3 months of age due to aorta dilation. Dbm mice also exhibited more pelvic floor organ prolapse. SD-OCT demonstrated thin central corneal thickness (CCT) and increased anterior chamber depth (ACD) in both *Fbn1*$^{C1039G/+}$ and *Loxl1*$^{-/-}$ mice. Thinning of CCT and deepening of ACD were more pronounced in dbm mice with normal axial length, indicating compromised zonules, which was confirmed by IHC for MAGP-1. Compared to wt, *Loxl1*$^{-/-}$ mice had fewer and abnormally formed elastic fibers, and enlarged and less defined collagen fibrils in their peripapillary sclera (PPS) revealed by TEM. Interestingly, by AFM we observed lower Young's modulus of cornea in all 3 lines compared to wt. Conversely, *Loxl1*$^{-/-}$ mice demonstrated higher Young's modulus of PPS than wt did. To investigate such differential biomechanical effects due to LOXL1 absence in the cornea and sclera, we used acetic acid collagen extraction which revealed differential collagen solubility and post-translational modification. Lastly, we also observed the differential influence of aging on collagen crosslinking in cornea and sclera.

Conclusions

The phenotypic and mechanistic investigations of our mouse models demonstrated their potential as an invaluable resource for further understanding and treatment of ocular and systemic manifestations of connective tissue disease.

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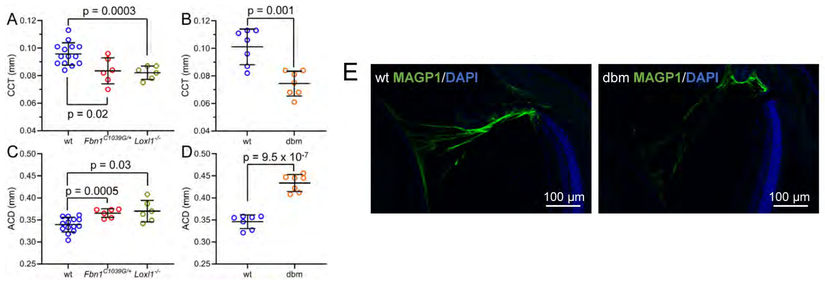

Fig. 1. Anterior segment structural changes.

Compared with wt (blue), *Fbn1*$^{C1039G/+}$ (red) and *loxl1*$^{-/-}$ (green) mice have thinner CCT (A) and deeper ACD (C). Similarly, double mutant (dbm) *Fbn1*$^{C1039G/+}$*/Loxl1*$^{-/-}$ (orange) have even thinner CCT (B) and deeper ACD (D) compared with wt mice. The deeper ACD in dbm mice indicates more severe compromised zonules, which can be seen by IHC staining of MAGP1 antibody (E).

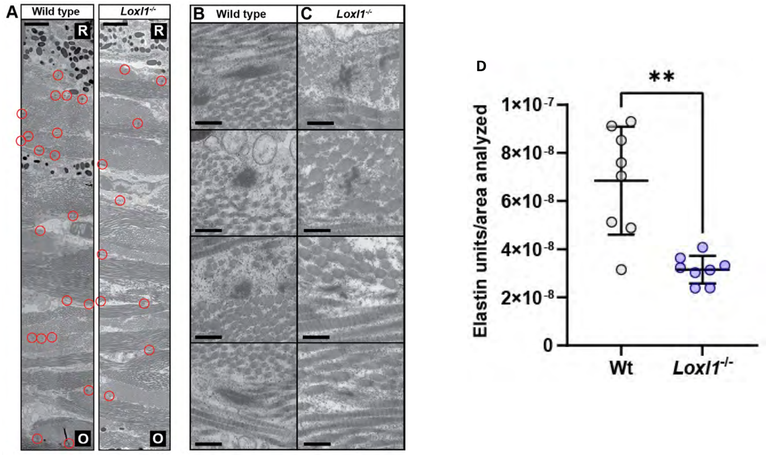

Fig. 3. Elastic fibers in PPS.

Representative TEM of PPS of wt and *Loxl1*$^{-/-}$ mice: red circles indicate elastic fibers units (A); Higher magnification images of elastic fibers in the PPS of wt (B) and Loxl1-/- mice (C) (Scale = 325 nm). Quantification of elastin units is higher in wt than *Loxl1*$^{-/-}$ mice (D).

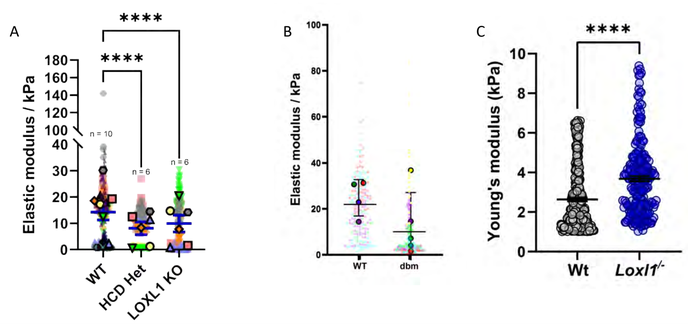

Fig. 4. Elastic modulus measured by AFM in cornea and PPS.

Young's modulus of cornea in all 3 lines (*Loxl1*$^{-/-}$, *Fbn1*$^{C1039G/+}$ and *Fbn1*$^{C1039G/+}$*/Loxl1*$^{-/-}$) were lower compared to wt (A and B). Conversely, *Loxl1*$^{-/-}$ mice demonstrated higher Young's modulus of PPS than wt did (C).

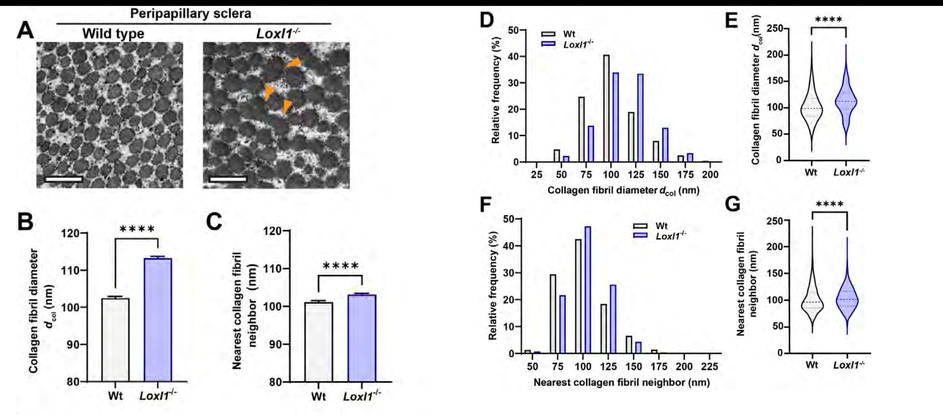

Fig. 2. Collagen fibrils in PPS.

Representative TEM of collagen fibrils in PPS of wt and Loxl1-/- mice. Collagen fibrils in Loxl1-/- mice are not well defined and fusion of fibrils (arrows) is apparent (A). Collagen fibril diameter measurements indicate that fibrils are larger in Loxl1-/- mice (102.5 ± 0.48 nm vs. 113.3 ± 0.48 nm; wt n = 3168, Loxl1-/- n = 2884; ****p<0.0001, Mann-Whitney test) (B). Nearest collagen neighbor distance is significantly larger in Loxl1-/- mice (101.1 ± 0.37 nm vs. 103.1 ± 0.30 nm; wt n = 3947, Loxl1-/- n = 4532; ****p<0.0001, Mann-Whitney test) (C). Frequency distribution of collagen fibril diameters in wt and Loxl1-/- mice show a shift towards larger fibrils in Loxl1-/- mice (D). Violin plots showing the median (darker dotted line) and upper/lower quartiles (lighter dotted lines) for collagen fibril diameters (wt median = 98.86 nm vs. 112.5 nm, upper quartile = 117.5 nm vs. 128.7 nm, lower quartile = 84.53 vs. 97.69 nm; ****p<0.0001, Mann-Whitney test) (E). Frequency distribution of nearest collagen fibril neighbor distances in show a shift towards larger interfibrillar distances in Loxl1-/- mice (F). Violin plots for nearest neighbor distances (wt median = 96.19 nm vs. 101.5 nm, upper quartile = 114.2 nm vs. 116.4 nm, lower quartile = 85.19 vs. 88.85 nm; ****p<0.0001, Mann-Whitney test) (G).

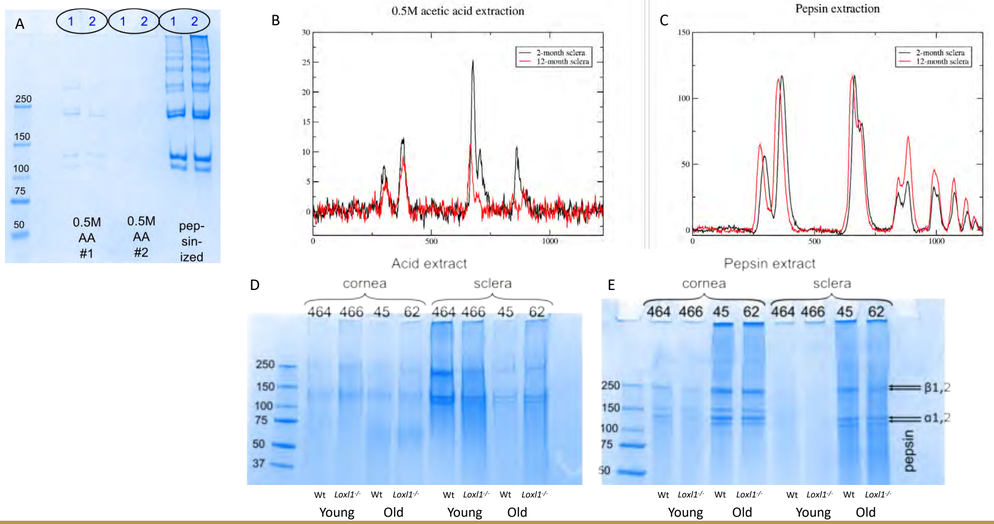

Fig. 5. Scleral and corneal collagens in young and old mice.

Scleras of both 1-month old (1) and 12-month old (2) mice are solubilized minimally by acetic acid, and sclera of 12-month old mouse is less digested by pepsin than that of 1-month old mice as demonstrated by more intense multimer bands in sample 2 than in sample 1 (A). Densitometry plots further demonstrate the age effect (B and C). Although sample size is limited, solubility with acetic acid treatment and pepsin digestion of cornea and sclera tissues appear affected by age and lack of LOXL1 differently (D and E).