

Differential molecular signature in cone vs periphery and its impact on customised crosslinking

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Introduction

Cornea

Elevated Expression of Matrix Metalloproteinase-9 and Inflammatory Cytokines in Keratoconus Patients Is Inhibited by Cyclosporine A

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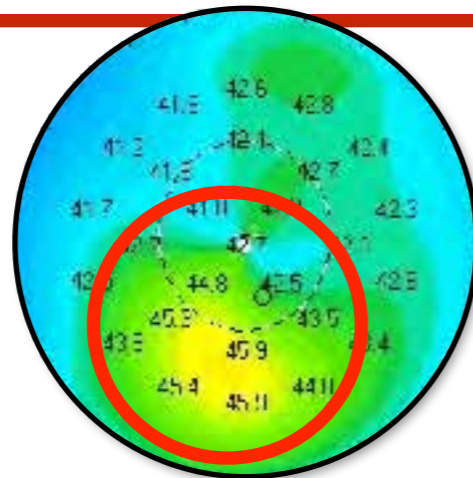
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Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity

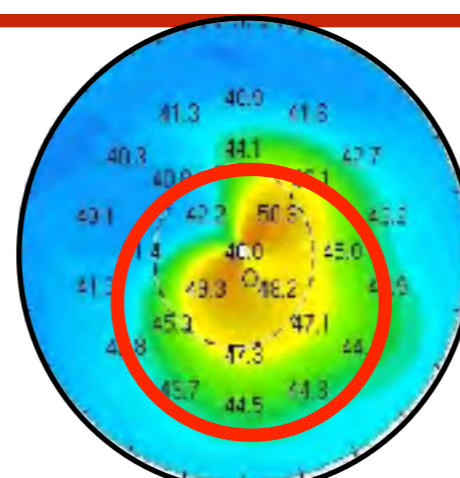
Rohit Shetty,¹ Arunapriya Sathyanarayanamoorthy,² Reshma Airody Ramachandra,² Vishal Arora,¹ Anuprita Ghosh,² Purnima Raman Srivatsa,¹ Natasha Pahuja,¹ Rudy M. M. A. Nuijts,² Abhijit Sinha-Roy,² Rajiv R. Mohan,⁴ Arkasubhra Ghosh^{2*}

Patient tear analyses as well as epithelial gene expression profiling revealed a strong correlation of increased MMP9, reduced LOX (lysyl oxidase) and elevated inflammatory cytokines and inflammation related metabolic factors in keratoconic eyes

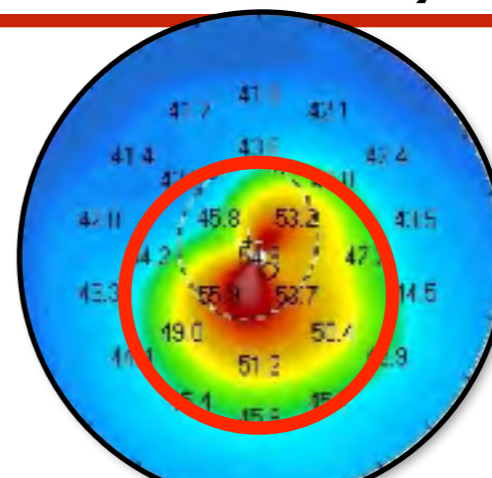
Observations in the clinic



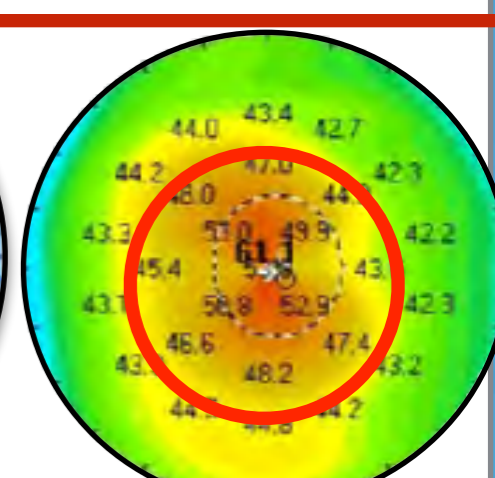
KC Grade I



KC Grade II



KC Grade III



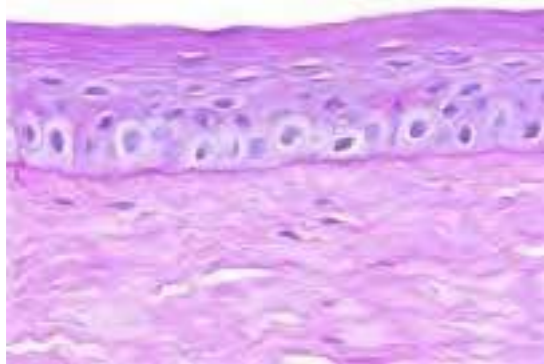
KC Grade IV

The disease is characterized by the focal thinning and protrusion.- Localised

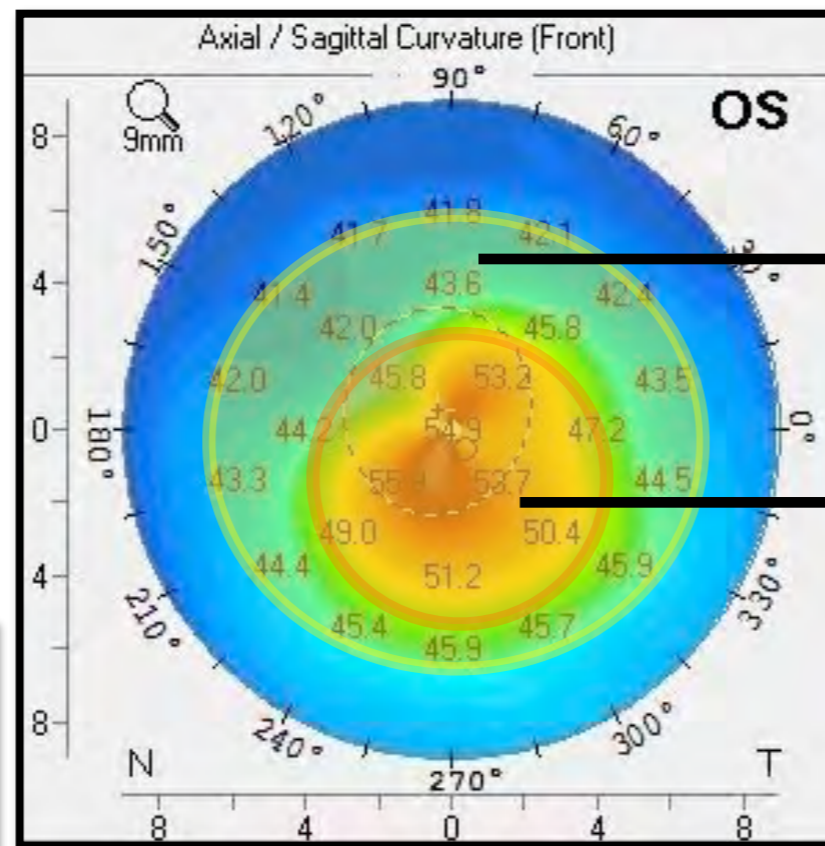
Research question ?

To evaluate if the gene expression profile of corneal epithelium from the cone area in Keratoconus (KC) differs from the peripheral non-ectatic areas

Gene expression in corneal epithelium



35 KC patients
9 control subjects



Non-ectatic area

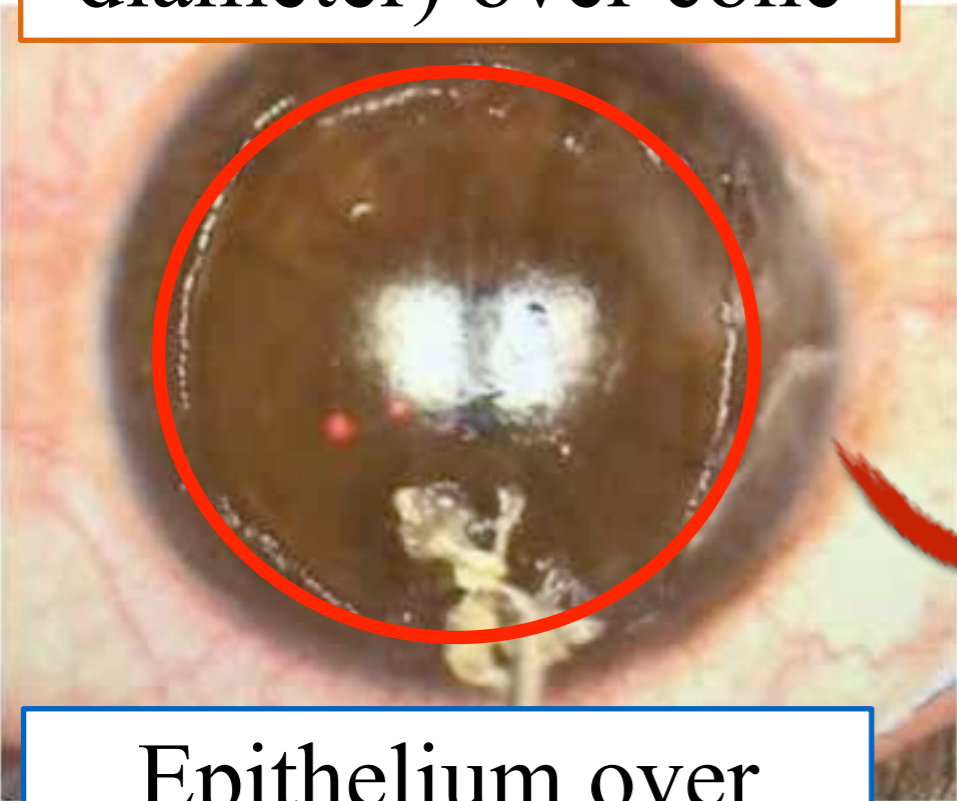
Cone

Is there a difference ?

Materials & methods



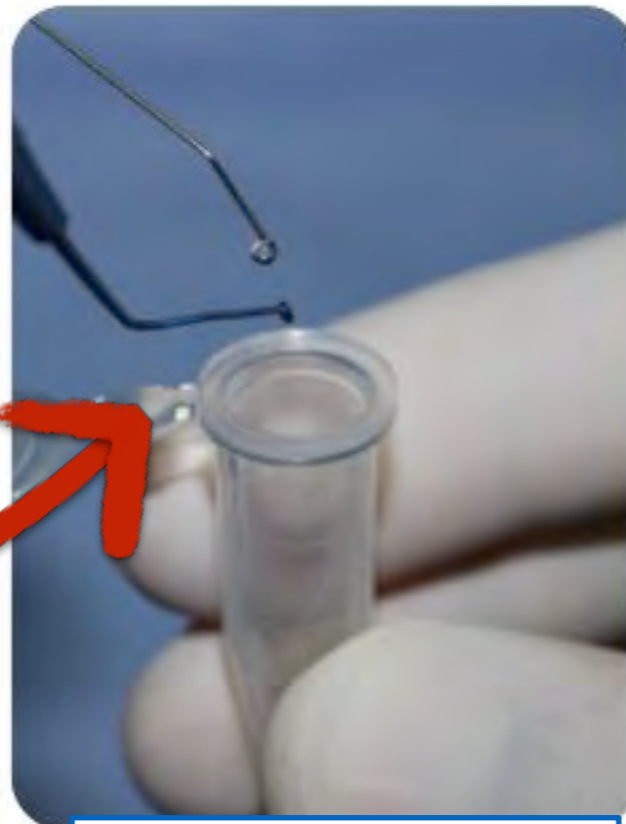
Epithelium (4.5 mm diameter) over cone



Epithelium over non-ectatic area



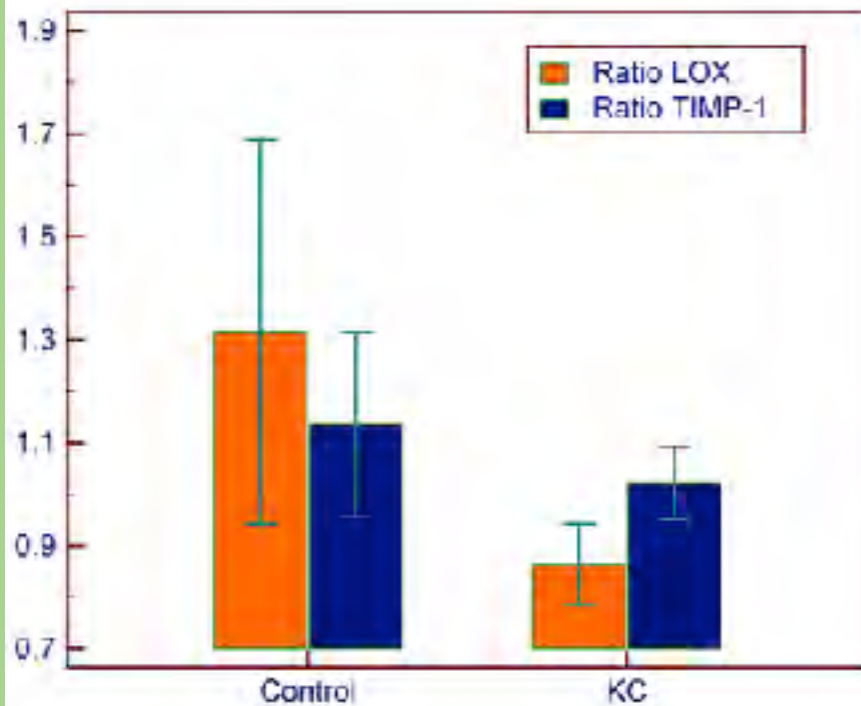
Cone



Periphery

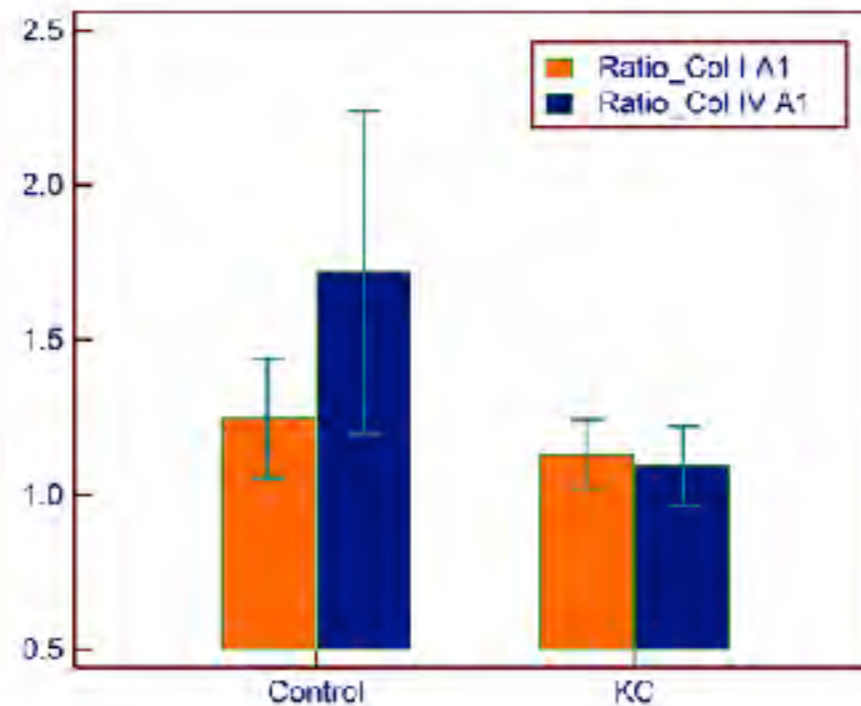
The cone vs periphery distinction is based on keratometry and location of the cone based on elevation map. Using a 4.5 mm trephine centered on the cone, epithelium was scraped separately for cone and rest as periphery. . In non-ectatic controls, the central 4.5 mm area was taken as cone.

Results



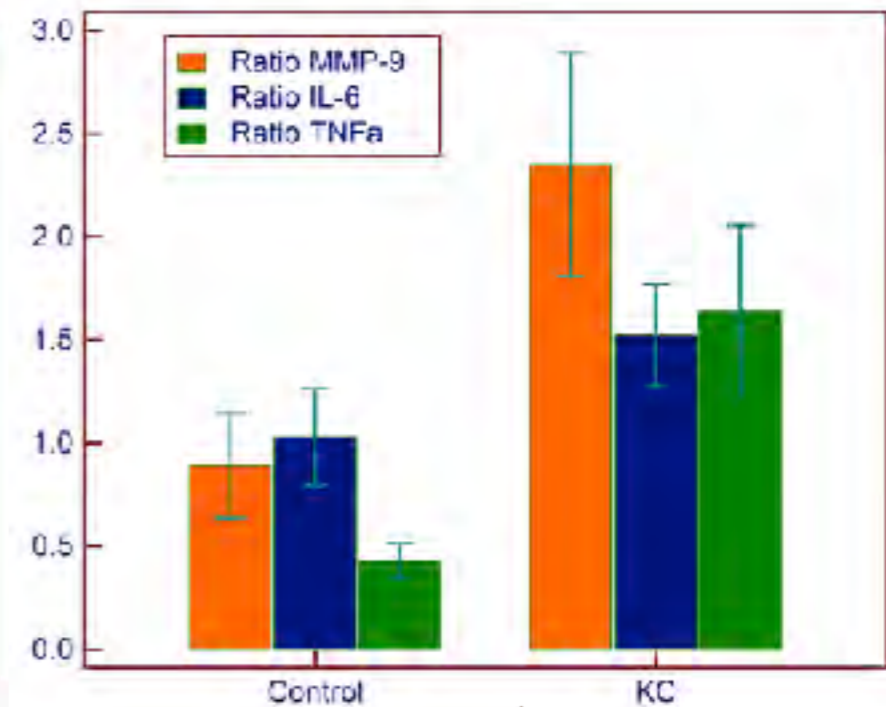
- LOX ↓
- TIMP-1 ↓

- Lysyl oxidase levels were significantly reduced in the cone of KC patients ($p=0.002$).
- TIMP1 showed a reducing trend that was not significant ($p=.09$)



- Col I A1 ↓
- Col IV A1 ↓

- Structure related genes COL1 ($p=0.01$) and COL4 ($p=0.008$) were also reduced significantly in KC patient cones.



- MMP9 ↑
- IL6 ↑
- TNFα ↑

- The cytokines IL6 and TNFα did show an increased trend.
- Matrix remodeler MMP9 showed an increasing trend at the cone

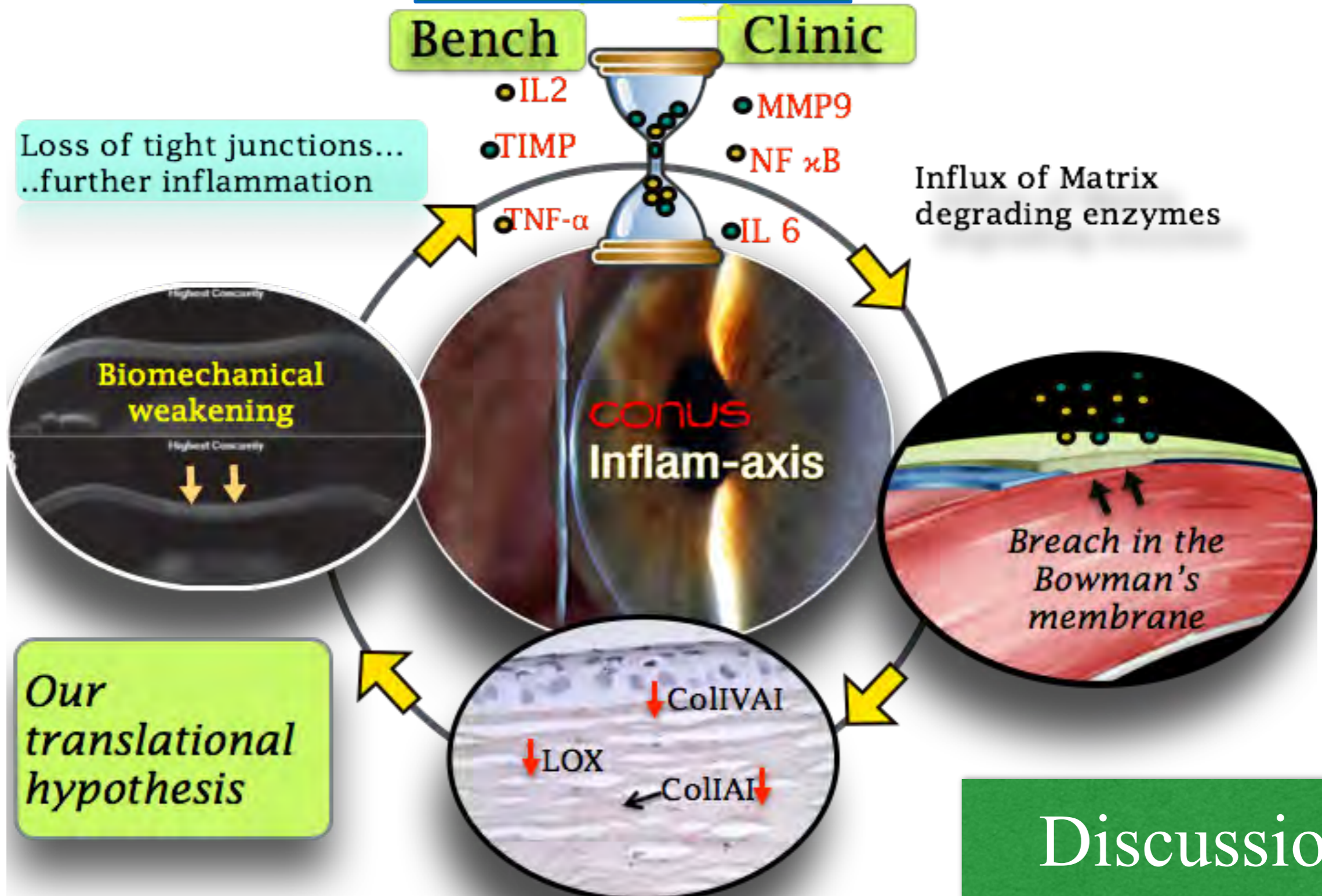
Connecting...

Elevated Expression of Matrix Metalloproteinase-9 and Inflammatory Cytokines in Keratoconus Patients Is Inhibited by Cyclosporine A



Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity

Our current study



Conclusion

- Ectasia in KC may be driven by local molecular factors at the cone that possibly spreads to other parts of cornea as disease progresses

Implications

- Localized treatment in the form of customized corneal collagen crosslinking with focal irradiation can be justified.
- Therapeutic drugs targeting matrix degrading enzymes for treating KC

References

Shetty R, Ghosh A, Lim RR, Subramani M, Mihir K, Reshma AR, Ranganath A, Nagaraj S, Nuijts RM, Beuerman R, Shetty R, Das D, Chaurasia SS, Sinha-Roy A, Ghosh A. Elevated expression of matrix metalloproteinase-9 and inflammatory cytokines in keratoconus patients is inhibited by cyclosporine A. Invest Ophthalmol Vis Sci. 2015 Feb

Shetty R, Sathyanarayanamoorthy A, Ramachandra RA, Arora V, Ghosh A, Srivatsa PR, Pahuja N, Nuijts RM, Sinha-Roy A, Mohan RR, Ghosh A. Attenuation of lysyl oxidase and collagen gene expression in keratoconus patient corneal epithelium corresponds to disease severity. Mol Vis. 2015 Jan 12