The pathogenesis of Keratoconus

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Characteristics

- Bilateral asymmetric thinning
- Ectasia causes keratometric myopia
- Irregular astigmatism
- Delayed onset until teens
- Eventual stabilization in older age
- Natural ‘cross-linking’

Environmental and genetic factors

Different phenotypes

A major cause for corneal transplantation in the developed world
31% grafts for keratoconus (Australian Corneal Graft Registry)

Is there a common mechanism?

Also: keratoconus suspect and forme fruste disease

Diagnosis + clinical signs

Tomography, hysteresis.

Prevalence in North Europe

National Patient Registry using ICD10 code
4600 cases in Denmark (50,000 in UK, cf. 9 x 10⁶ myopes)***
1995-2005 Hospital based (excludes optometrist managed)

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence (10⁵)</td>
<td>86 (1:1620)</td>
<td></td>
</tr>
<tr>
<td>Incidence (10⁵) yr</td>
<td>1.3</td>
<td>1.0-1.7</td>
</tr>
</tbody>
</table>

*In susceptible population 14-78 years

**Acute cornea hydrops**

Significant cause of ocular morbidity and occasional blindness

<table>
<thead>
<tr>
<th>Condition</th>
<th>Adjusted Odds</th>
<th>P value</th>
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<tbody>
<tr>
<td>VKC</td>
<td>15, CI 1.30-173.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Asthma</td>
<td>4.92, CI 1.22-19.78</td>
<td>0.03</td>
</tr>
<tr>
<td>VA</td>
<td>4.11, CI 1.18, 14.32</td>
<td>0.026</td>
</tr>
<tr>
<td>Keratometry</td>
<td>4.44, CI 0.85-23.18</td>
<td>0.077</td>
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**Multiple potential mechanisms**

- Biomechanics
- Ultrastructure
- Enzymology
- Proteomics
- Molecular genetics

DNA $\rightarrow$ RNA $\rightarrow$ Protein $\rightarrow$ Structure

**Environmental factors**

- Atopy (up to 26% in VKC)
- Eye rubbing

**Structural changes**

- No deposits – not a dystrophy

**X-ray scattering**

- Collagen fibril
- Detectors

**Effect of race**

Catchment 900,000, ages 10-44 years

87% white, 11% Asian

<table>
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<tr>
<th>Race</th>
<th>Prevalence ($10^5$)</th>
<th>Incidence (10^5/yr)</th>
<th>Presentation</th>
<th>PK</th>
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</thead>
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<tr>
<td>Asian</td>
<td>229</td>
<td>19.6</td>
<td>22.3</td>
<td>19.1</td>
</tr>
<tr>
<td>White</td>
<td>57</td>
<td>4.5</td>
<td>26.5</td>
<td>25.7</td>
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**Environmental factors**

- Stretching of collagen, release of cytokines?

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Collagen Orientation
Peripheral migration of collagen by sliding

Collagen and proteoglycan

Enzymology – cause or effect?

Extracellular matrix genes
TIMP3  No change

Collagens
COL8A1, COL8A2, COL4A3, COL4A4

Apoptosis
FLG

Enzymology – an example of a false dawn

SOD1 is an antioxidant
1. SOD1 (superoxide dismutase)
2. Located on Chr 21 (Down syndrome)
3. Higher expression in KC tissue
4. Segregation of mutations in 2 families
5. But only in 0.9% of 430 subsequent

Tears and cornea: Biochemistry & Proteomics

Tears
abnormal levels of TH1, TH2 and TH17 cytokines

Biochemistry
A2M (alpha-2 macroglobulin) decreased by 0.74 fold
SOD1 (superoxide dismutase 1)↑

Proteomics
932 and 1157 proteins in the epithelium and the stroma

Chaerkadi et al. J Proteomics 2013;87:122

Collagen Orientation
Anchoring fibres


Fibres have slipped but collagen spacing and diameter are unaltered
Comparative RNA transcriptome

87 genes differentially expressed (69↓ and 18↑)
Apoptosis, differentiation, and proliferation pathways

Is this the cause or the effect or corneal distortion?

Network biology analysis

(Myce et al. IOVS 2011;52:6181)

A genetic effect

Family history (autosomal dominant) 6 – 31%
Concordance in twins monozygotic > dizygotic

Prevalence in 1st degree relatives 15 to 67x increased

Association studies with large patient populations

Myopia

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<tr>
<th>Axial length</th>
<th>Mean (mm)</th>
<th>SD</th>
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<tr>
<td>Normal (25)</td>
<td>23.46</td>
<td>0.78</td>
</tr>
<tr>
<td>Keratoconus PK (60)</td>
<td>24.84</td>
<td>1.65</td>
</tr>
<tr>
<td>Keratoconus (70)</td>
<td>24.47</td>
<td>1.65</td>
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A global distension of the eye


Candidate gene approach

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<tr>
<th>Systemic</th>
<th>Ocular</th>
<th>Corneal</th>
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<tbody>
<tr>
<td>Apert</td>
<td>Blue sclera</td>
<td>Axenfeld</td>
</tr>
<tr>
<td>Bardet-Biedl</td>
<td>Flat eye lid</td>
<td>Chandler</td>
</tr>
<tr>
<td>Ehlers-Danlos</td>
<td>Gynat atrophy</td>
<td>ICE syndrome</td>
</tr>
<tr>
<td>Marfan</td>
<td>Ichthyosis</td>
<td>Fuchs</td>
</tr>
<tr>
<td>Neurofibroma</td>
<td>Lenticonus</td>
<td>Lattice</td>
</tr>
<tr>
<td>Turner</td>
<td>ROP</td>
<td>PPCD 1</td>
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Genetic studies

Linkage analysis
Candidate gene studies
Genome-wide association studies
Segregation studies
Exome sequencing

GWAS corneal thickness

CCT 20,000 white & Asian – 6 loci
874 KC vs 6,085 controls

Suggestive effect of:
ZNF469, FOXO1, RXRA-COL5A1, FNDC3B, MPDZ-NF1B, COL5A1

rs9938149 SNP in ZNF469 had unexpected effect direction - more likely to have thicker cornea

(Lu et al. Nature Genetics 2013;45)
Brittle cornea syndrome (AR)

Type 1 - ZNF469
Type 2 – PRDM5

One allele affected causing keratoconus
Two alleles affected causing BCS?

Corneal hysteresis (CH)

GWAS in the EPIC-Norfolk Eye Study – 8623 participants
Khawaja: ARVO 2016

Corneal resistance factor (CRF)

GWAS in the EPIC-Norfolk Eye Study
Khawaja: ARVO 2016

Where is this going?

Spectacles
Contact lenses
Surgery

Better detection, early intervention, new treatments

Where is this going?

Spectacles
Contact lenses
Surgery

CXL

Detection, early intervention, new treatments
Conclusions

- A complex disease with multiple susceptibility loci
- Environmental and genetic contribution (spectrum)
- Polygenic disorder
- Resources
- Pathway to disease in uncertain
- Research may open doors to more targeted treatment

Funding

Special Trustees of Moorfields Eye Hospital
Moorfields Eye Hospital Development Fund