**28693-SD - Solving the Puzzle Of Keratoconus**

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**Description:** This course will present the intertwined puzzle of the genetics of keratoconus. Past research in this area is presented along with the limitations of current studies. Corneal topographic diagnosis is covered as a way to screen for keratoconus in normal population and vice versa.

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Instructor: **Loretta Szczotka-Flynn O.D.**
Co-instructors: 
Adjunct/Assistant Instructors:

**Learning Objectives:**
1. Review genetic theories
2. Learn of the genetic hits found for keratoconus
3. Understand how to screen for normals and keratoconus in genetic case control studies.
Solving the Genetic Puzzle of Keratoconus

Loretta Szczotka-Flynn OD, PhD, FAAO
Professor
Case Western Reserve University Department of Ophthalmology
University Hospitals of Cleveland

Diagnosis/Early Signs
Keratoconus Signs

Epidemiology of Keratoconus
Incidence & Prevalance
- 2/100,000/year (I)
- 55/100,000 (P)
- 1 in 2,000

Virtually All Diseases (Except Maybe Trauma) Have a Genetic Component
Keratoconus as a Genetic Disorder is Suggested by:

- Familial occurrence (17.8% in CLEK year 7)
- 15-67X greater in 1st degree relatives than in the general population
- Twin Studies
- Bilaterality
- Association with other genetic disorders

Keratoconus is a Complex Disease

- Keratoconus is likely a complex disorder characterized by multiple genes and environment contributing to the etiology
  - Linked to multiple chromosomal regions
  - Multiple-susceptibility gene model, all having a small or moderate effect in determining the pathological phenotype

Some reminders about genes and heredity

[Genetic map with chromosomes labeled 1 to 22, X, and Y]
What is gene mapping?
- Identification of specific genes for a trait or disease
- Estimated 30,000 - 40,000 genes in the human genome
  - Location of genes determined through the Human Genome project
- Function of all genes – yet unknown

Broad Genetic Epidemiology Design Studies
- **Linkage Analysis**
  - Follows meiotic events through families for co-segregation of disease and particular genetic variants
  - Large Families
  - Sibling Pairs (or other family pairs)
  - Works **VERY** well for 'Mendelian' diseases

- **Association Studies**
  - Detect association between genetic variants and disease across families
  - Exploits linkage disequilibrium
  - Case-Control designs
  - Cohort designs
  - Parents – affected child trios (TDT)
  - May be more appropriate for complex diseases
Extended families or affected sib pairs

Case-control

Properties of DNA variants that have an impact on gene discovery

- Common variant with large effect (rarely occurs)
- Rare variant, small effect

Linkage studies

Association studies

Possible KC loci (Linkage Studies)

- 16q 22.3 – q23.1 Finland
- 20q 12 Tasmania
- 3p14-q13 Italy
- 6p 25 Canada
- 5q21.2 Italy
- 2p24 France, Spain, W. Indies
- 5q14.3-q21.1 U.S. (Caucasian)
- 13q32 Ecuador
The first genome-wide linkage analysis was performed by Tyynismaa et al. on 20 Finnish families with KC in 2002. The authors reported linkage to chromosome 16q22.3-q23.1 (LOD=4.10; KTCN2;608932).

In 2004, Brancati et al. performed a genome-wide analysis to a 7 family member pedigree with 2 generations. They mapped the disorder in this family to chromosome 3p14-q13 (LOD=3.09; KTCN3;608586).

In 2005, Hutchings et al. performed genome-wide linkage analysis in 28 families with KC recruited in France, Spain, and Guadeloupe (West Indies). They found evidence of linkage on chromosome 2p24 (LOD= 3.26; KTCN4;609271).
In 2005, Tang, Rabinowitz et al performed a genome scan in a four-generation pedigree with 27 family members.

Evidence of suggestive linkage was observed on chromosome 5q14.1-q21.3 (LOD=3.8).

Fine-mapping has further narrowed this candidate region to 6 cM.

In 2003, Hughes et al. examined a large 3-generation family affected with early onset autosomal dominant anterior polar cataract and clinically severe KC.

Mapped a novel locus to chromosome 15q22.33-24.2.

In 2006, Li, Rabinowitz et al. reported a linkage study using 107 (mostly Caucasian) KC affected sibpairs.

The most significant evidence of linkage was observed on chromosome 9q (LOD=4.5).

Other suggestive linkages were identified on chromosomes 4q, 5q, 6p, 10p, 12p, and 14q.

This 5q peak is distal to the previously reported 5q peak.

However, even though these peaks do not completely overlap, they have a high probability of being related to the same KC susceptibility locus.
Candidate Genes

- VSX1 gene on Chromosome 20
  - In 2005, Bisceglia et al. evaluated the role of the VSX1 gene in 80 KC Italian patients.
    - They found a total of 4 missense mutations (3 that were previously identified and 1 that was novel) in 7 out of the 80 patients.
- SOD1 gene on Chromosome 21

What is the role of VSX1 in the general keratoconus population?

- Other published abstracts and our group suggest the mutations in VSX1 are rare in KC patients
  - 2006 Tang & Rabinowitz
  - 2006 Aldave IOVS
  - 2007 Liskova Mol. Vision
  - 2008 Hosseini Mol. Vision
  - 2009 Gajacka IOVS
  - Also excluded SOD1

- Unlikely to be the cause of the common forms of KC

Candidate Genes

- RAB3GAP1 gene on Chromosome 2
  - In 2012, Li et al. (part of Rabinowitz Group) reported a novel locus on 2p21.3.
  - First GWAS in KC
  - Rab4 GTPase activating protein
  - Previously implicated in Warburg Micro Syndrome including corneal malformation, congenital cataracts and optic atrophy
Genome-wide scan approach

- Positive linkage signals in the vicinity of susceptibility loci

- HGF gene

- In 2010 and 2011, 2 groups suggested between the HGF gene and keratoconus:
  - Burdon et al (ARVO 2010) in Adelaide, Australia reported a GWAS study using 110 cases and 216 controls, and a replication sample of 100 KC patients.
  - Bykhovskaya, Li, et al (Rabinowitz group - American Society of Human Genetics Nov 2010) reported on a GWAS study of 222 Caucasian patients and 3324 Caucasian controls (OR 1.5 CI 1.2-1.9)

- HGF on 7q21.1

- Hepatocyte growth factor is secreted by mesenchymal cells
- HGF acts as a multi-functional cytokine on cells of mainly epithelial origin
- It stimulates mitogenesis, cell motility, and matrix invasion
- Central role in angiogenesis, tumorogenesis, and tissue regeneration.
**HGF gene**

- In 2011, Combined GWAS between the groups
  - IOVS 2011 Burdon et al
  - OR 1.5-2.2: A large effect for a common disease
  - HGF gene has been associated with refractive error, myopia in Chinese and myopia and hyperopia in Caucasians
  - Found in cornea and upregulated in response to corneal injury
  - Role in KC is probably through inflammatory pathways

**Candidate Genes**

- **Lysyl Oxidase (LOX) gene on Chromosome 5**
  - In 2012, Bykhavskaya et al. (part of the Rabinowitz group) evaluated the role of the LOX gene in 222 Caucasian KC patients and 5201 controls
  - Another 304 cases and 518 controls used for the replication cohort (including UHCMC patients)
  - LOX gene under a previous peak located at 5q23.2
  - LOX initiates the crosslinking of collagens and elastin

**Candidate Genes:**

- **Lysyl Oxidase (LOX) gene**
  - Mutations in LOX can potentially lead to the reduction of natural crosslinking of collagen fibers in the corneal stroma, biomechanically weakening the cornea and making it easier for carriers to develop KC
Gene Expression Profile Studies/Microarrays

- Nielsen et al. investigated differential gene expression in corneal epithelium from samples with and without KC.
- Rabinowitz et al. demonstrated that aquaporin 5 (AQP5) a gene known to be expressed in the corneal epithelium is differentially expressed in KC and may be a biomarker for a particular stage of the disease.

Other genes that have been excluded

- BigH3 (TGFBI)
- COL6A1
- COL8A1
- MMP-9

More candidate loci on chromosomes that have been excluded

- Ferrini et al
  - 1, 15, 16, 20
- Gajecka et al
  - 3, 15, 16, 20 (linkage analysis)
Strategies for Human Genetic Epidemiology Studies

Collect families

Perform genome scan:
- Linkage
- Association (?)

Follow-up linked candidate genes in dense marker set

Sequence/genotype candidate genes in linked region to look for mutations/variants

Genotype候选人 candidate genes (candidates from previous information)

If gene found:
- Characterize variant risk in population
- Penetrance
- Attributable risk
- Interactions, etc.

Candidate Genes:
Lysyl Oxidase (LOX) gene

- Mutations in LOX can potentially lead to the reduction of natural crosslinking of collagen fibers in the corneal stroma, biomechanically weakening the cornea and making it easier to carriers to develop KC

History of Corneal Cross-Linking (CXL)

- Basic research 1993-97 by Seiler & Spoerl in Germany
- First patients Txd in 1998
- Today nearly 500 centers
- Standard of care for KCN

CXL Slides courtesy of Shamie Bajna MD
Corneal Collagen Cross-linking (CXL) with Riboflavin

- ↑ R rigidity
  - 329% increase
- In Europe since 1998
- KCN, pellucid, ectasia, post-RK

The link between Corneal Cross-Linking (CXL) and Genetics

- May fulfill the promise of individualized medicine
- Incorporation of genetic testing for LOX polymorphisms will ensure that only "genotypically suitable patients" will undergo treatment

The link between Corneal Cross-Linking (CXL) and Genetics

- LOX genetic testing could further improve the effectiveness of CXL in KC by eliminating non-responders (those with no LOX polymorphisms) by identifying them prior to treatment
CXL Technique
- Vitamin-Light Therapy
- Anesthetic drops, painless
- Prepare cornea: epi-off versus epi-on
- Riboflavin drops for 30 to 60 mins
- UV light for 30 mins
- Bandage contact lens

Defining Cases and Controls
- Keratoconus Cases
  - KSS scale
- Controls
  - Posterior corneal elevation data may identify forme fruste KC at an earlier stage
    - Scheimpflug camera-based Pentacam
    - Slit-based Orbscan II system

Defining Cases: KSS Score
KSS Score

KSS 0
- Unaffected normal topography
- Required features:
  - No corneal scarring consistent with keratoconus
  - No slit-lamp signs for keratoconus
  - Typical axial pattern
  - Average corneal power (ACP) ≤47.75 D
  - Higher-order RMS error ≤0.65

KSS 1
- Unaffected atypical topography
- Required features:
  - No corneal scarring consistent with keratoconus
  - No slit-lamp signs for keratoconus
  - Atypical axial pattern
    - Irregular pattern
    - Inferior or superior steepening no more than 3.00 D steeper than ACP
      or
    - Asymmetric superior bowtie
      or
    - Asymmetric inferior bowtie
  - ACP ≤48.00 D
  - Higher-order RMS error ≤1.00
KSS 2
- Suspect topography
- Required features:
  - No corneal scarring consistent with keratoconus
  - No slit-lamp signs for keratoconus
  - Axial pattern with isolated area of steepening
  - Inferior steep pattern
  - Central steep pattern
- Additional features:
  - ACP <=49.00 D
  - Higher-order RMS error >1.00, <=1.50

KSS 3
- Affected mild disease
- Required features:
  - Axial pattern consistent with KCN
  - May have positive slit-lamp signs
  - No corneal scarring consistent with keratoconus
- Additional features:
  - ACP <=52.00 D
  - Higher-order RMS error >1.50, <=3.50

KSS 4
- Affected moderate disease
- Required features:
  - Axial pattern consistent with KCN
  - Must have positive slit-lamp signs
- Additional features:
  - ACP >52.00 D, <=56.00 D
  - Higher-order RMS error >3.50, <=5.75
  - Corneal scarring and overall CLEK grade up to 3.0
**KSS 5**

- Affected severe disease
- Required features:
  - Axial pattern consistent with KCN
  - Must have positive slit-lamp signs
  - Additional features:
    - Corneal scarring CLEK grade 3.5 or greater overall
    - ACP >56.00 D
    - Higher-order RMS error >5.75

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**Defining Controls: Orbscan II**

- In non-diseased eyes, the average amount of maximum posterior elevation using Orbscan II default settings compared to the best fit sphere is about 21-28 μm
  - Rao 2002
  - Wei 2006
  - Lim 2007
  - Sonmez 2007

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**Elevation Topology: Central Hill**

- The normal cornea is prolate, meaning that meridional prolateness of the normal cornea causes it to rise centrally.
- Immediately surrounding the central hill is an annular sea.
- In the far periphery, the prolate cornea again rises above the reference surface, producing peripheral highlands.
Defining Controls: Orbscan II

- One study of 140 normal eyes documented the maximum posterior elevation was never greater than 46 μm
  - Wei 2006
- Another study of 50 normal eyes the posterior elevation was never greater than 40 μm
  - Rao 2002

Defining Controls: Orbscan II

Posterior Elevation on Orbscan II >50 microns indicative of Keratoconus

Post LASIK Orbscan v Pentacam
Michael Belin MD
Defining Controls: Pentacam

- Posterior elevation ranges from -6 µm to +18 µm in normals
- 15.5-32 µm of elevation on the posterior corneal surface can delineate forme fruste KC from normals


Post LASIK Orbscan v Pentacam
Michael Belin MD

OrbScan 41 µ thinner

OrbScan 20 µ thinner
Post LASIK Orbscan v Pentacam
Michael Belin MD

Posterior Elevation

OrbScan 37 μm thinner

Posterior Elevation

OrbScan 13 μm thinner

Posterior Elevation >15-18 microns on Pentacam suggests keratoconus
Summary

- There is a wealth of data suggesting that KC pathogenesis is mediated by genetic causes.
- However, very few investigations have led to causative genes for KC, other than rare mutations in a few families, which have been difficult to substantiate, and are unlikely to be the cause of the common forms of the disorder.

Big Picture

- Disease with genetic component
- Map the disease gene
- Clone the disease gene
- Diagnostics
- Gene therapy
- Drug or interventional Therapy (CXL)

Summary

- Genetic Epidemiology Case-Control Studies are an excellent way to determine the genes involved in complex diseases such as keratoconus
  - Each gene may have a moderate or small effect size but collectively will be representative of the common form(s) of the disease
  - Impact on personalized medicine is near
Ultimate Goal

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