Current and Near-Term Impact of Biomarkers for Retinal Neurodegenerations
Retinal Neurodegenerative Disease - Clinical Targets

Glaucoma 2 million
Diabetic Retinopathy 5 million
Retinal Dystrophies 200,000 *
AMD - Age-Related
  Macular Degeneration 8 million
    Atrophic (“dry” form) 85%
    Neovascular (“wet” form) 15%

“Vision Problems in the U.S.”
National Eye Institute and Prevent Blindness America
Glaucoma

Optic nerve “cupping” in advanced

- Ganglion cell neurodegenerative retinopathy / optic neuropathy
- Elevated intra-ocular pressure is triggering event
- Lowering IOP slows clinical progression
- Novel treatment strategies - neuroprotection by trophic factors


Biomarkers

- TGFβ
- α-fodrin
  [ a.k.a. spectrin (Alzheimer)]
- Heat shock proteins (HSPs)
- γ–Enolase
- Glutathione S-transferase
- Anti-phosphatidylserine
- Glycosaminoglycans

BDNF neuro-protection in rat glaucoma model

Rod & Cone Photoreceptors

120 million rods in the retina. Concentrated in mid-periphery. Single photon detectors that provide dim-light vision.

6 million cones discriminate color. Concentrated in macula. Provide high spatial resolution for reading acuity tasks.

Scanning EM of human rod photoreceptors
Testing for Three Chromatic Types of Cone Photoreceptors

Visual psychophysics provides rapid, precise & specific screening for cells in outer retina.

Did you pass?
Retinal Degenerative Diseases

Retinitis Pigmentosa

Hereditary, pan-retinal rod photoreceptor degeneration

“Night-blindness” - early symptom of rod dysfunction and loss.
“Tunnel vision” - loss of peripheral vision.
Total blindness ensues when cones die secondarily.

Numerous genetic causes:
Photo-transduction cascade mutations, including:
rhodopsin, transducin, PDE, arrestin, recoverin,
G-gated cation membrane channel, ….
Retinitis Pigmentosa ("RP")

73 y/o woman
Totally “night-blind”
Severely constricted visual field
20/60 acuity, but cannot drive

P23H rhodopsin mutation.
No rod photoreceptors remaining.
Some macular cone photoreceptors persist.
- 60% cone cell loss.
- 80% shortening of outer-segments.

Retinal fundus photograph

1 hour post-mortem hemisected eyecup
Human Retinitis Pigmentosa - P23H rhodopsin

Only cones remain.
60% loss of cone photoreceptors.
Severely truncated inner & outer segments.

Still has 20/60 acuity
Normal human parafovea

P23H parafoveal section

Outer & Inner Segment

Cone Nuclei
Retinal Neurodegenerative Disease Genes

Genes – disease pathophysiology correlates to develop new risk biomarkers

Approaching 200 cloned or mapped retinal disease genes

Mapped and Identified Retinal Disease Genes 1980 - 2007

http://www.sph.uth.tmc.edu/Retnet/sum-dis.htm#D-graph
Ocular Disease Genes - Too many successes?

<table>
<thead>
<tr>
<th>Eye Region</th>
<th>Genes/Conditions</th>
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<tbody>
<tr>
<td>Cornea</td>
<td>ARSC1, CHST6, COL8A2, GLA, KRT3, and KRT12</td>
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<tr>
<td></td>
<td>lattice corneal dystrophies associated with amyloid deposition (GSN, M1S1, TGFBI [BIGH3]).</td>
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<tr>
<td>Lens</td>
<td>Zonular pulverulent cataract (CZP3), Congenital cerulean (CCA1), Crystallins (CRYAA, CRYAB..), Aniridia AN2, Forkhead, FOXE3.</td>
</tr>
<tr>
<td>Retina</td>
<td>Retinitis pigmentosa (RHO, RPGR, RPE65, RDS, PRPF8); Macular degeneration (ARMD1, EFEMP1, ELOVL4, RDS, TIMP3, VMD2, ABCA4, RDH4, RPGR); Usher Syndrome (MYO7A, PCDH15, USH3A, USH2A); Bardet Biedl Syndrome (BBS1-4, MKKS, TTC8).</td>
</tr>
<tr>
<td>Glaucoma</td>
<td>(MYOC, OPTN, CYP1B1); Open angle (GLC1D, GLC1F, GLC3B..).</td>
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<tr>
<td>Optic nerve atrophy</td>
<td>(OPA1, TIMM8A);</td>
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<tr>
<td>Ocular muscle strabismus</td>
<td>Kinesin (CFEOM1, CFEOM3).</td>
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Age-Related Macular Degeneration

- Leading cause of legal blindness
- 8 M Americans have AMD
- 1.8 M are already legally blind
- Aging baby-boomers on the cusp
- Two forms: “wet” & “dry”
AMD Genes

Complement Factor H polymorphism
April, 2005

CFH molecule in “inflammation cascade”
About 40% of AMD genetic risk
Immediately replicated by 5 groups

Three additional genes implicated:
Complement component 2 (C2)
Complement Factor B (BF)
HTRA1 (serine protease – in mast cells)

Together these account for 74% of AMD risk
Complement System: Protection from Pathogens

CLASSICAL PATHWAY
Antigen-antibody complexes

LECTIN PATHWAY
Microbial surfaces

ALTERNATIVE PATHWAY
Microbial surfaces (nonspecific activators)

Bind
Mannose-binding lectin

Factor H
(on cells & in circulation)

C3(H2O) + Factor B
Factor D

C3b, Bb (C3 convertase)
C3

C3b, Bb, C3b (C5 convertase)
C5

C5a + C5b

C5b, 6, 7
C6

C5b, 6, 7, 8, 9 (membrane attack complex)
C7
C8
C9

Lysis, Cytotoxicity

Modified Donoso et al.  
## Ocular Imaging - History

<table>
<thead>
<tr>
<th>Year</th>
<th>Inventor/Technique</th>
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<tbody>
<tr>
<td>1850</td>
<td>Helmholtz: Ophthalmoscope invented</td>
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<tr>
<td>1925</td>
<td>Nordeson: Retina fundus camera invented</td>
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<tr>
<td>1960</td>
<td>Novotny &amp; Alvis: FA - fluorescein angiography by IV dye administration</td>
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<tr>
<td>1990’s</td>
<td>OCT - Optical Coherence Tomography: 3D image</td>
</tr>
<tr>
<td>2000</td>
<td>Adaptive Optics applied to retina fundus imaging</td>
</tr>
</tbody>
</table>
Structural Markers

Fluorescein Angiogram: patient complains of “blurry vision”

Retina color photograph  FA shows fluid leakage within the macula

Photograph from http://www.stlukeseye.com/eyeq/FluoresceinAngiogram.asp
• High resolution & 3D image reconstruction for volumes.
• Capture microstructure internal to biological tissues.
• Utility for ocular diagnostics and therapeutic tracking:
  - retinal ganglion cell (RGC) axon loss in glaucoma
  - retinal nerve fiber layer (R-NFL) thinning in multiple sclerosis
  - photoreceptor imaging in neuro-degenerative disease
Structural Markers

OCT of Congenital X-Linked Retinoschisis

Foveal schisis
OPL schisis
NFL-GC-IPL schisis
Deep-retina photoreceptor layer schisis

Scanned using OCT-3, Carl Zeiss Meditec, Inc.
OCT Image as Biomarker in Multiple Sclerosis

- 80% of MS patients develop some visual loss to low contrast letter testing.
- Demyelinating disease causes axonal loss and nerve fiber layer thinning.
- R-NFL thickness declines with increasing neurologic impairment and correlates with disease duration.

Each 4µ R-NFL thinning correlates with one line decrease in low-contrast letter acuity

Structural Markers

Adaptive Optics: noninvasive imaging of cone photoreceptors (5 µ diameter) in the living human fovea

Adaptive Optics Ophthalmoscope University of Rochester

Human foveal cones
2 µm resolution

Courtesy of David R. Williams, PhD
Adaptive Optics: High-Resolution Retinal Imaging

Ocular media aberrations

- Lens: Low order semi-static
- Cornea: Low order static
- Lacrimal tear film: High order dynamic
- Eye + head movement: Low order dynamic

Courtesy of Jose-Alain Sahal, MD, PhD
Structural Markers

Adaptive Optics Imaging of the normal human trichromatic cone mosaic.
A. Roorda & D.R. Williams; Nature (1999)

Thomas Young postulated in the early 1800’s that human color vision depends on 3 color fundamentals.

Adaptive Optics provides the first images of the 3 cone classes in the living human eye.

Courtesy of David R. Williams, PhD
**Metabolic Biomarkers**

**AMD disease markers**

- Elevated homocysteine
  
  AMD = 9.5-11.7 μmol/l; control = 8.81-8.88 μmol/l

- Markers of systemic inflammation
  
  Interleukin-6
  
  C-reactive protein
  
  AMD = 3.42 mg/l (n=79); control = 2.30 mg/l (n=77) \((p=0.03)\)

- Carboxyethylpyrrole protein (CEP) adducts from DHA fragments of photoreceptor membrane after phagocytosis by RPE.

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**Note:** Some of these risk factors cross-react with cardiovascular disease
Metabolic Biomarkers

CEP Immunoreactivity in AMD:
Diagnostic Utility for Predicting Susceptibility

Omega-(2-carboxyethyl)pyrrole (CEP) protein adducts from oxidative fragments of DHA-Ω3 liberated from photoreceptor membranes after phagocytosis by RPE

CEP immunoreactivity in serum plasma
AMD sera titer ~1.5-fold higher than control (p<0.004)

AMD donors (83 yr mean age, n=19),
Older healthy donors (82 yr mean age, n=19)
Younger healthy donors (27 yr mean age, n=9)

Gu et al.
**Metabolic Molecular/Structural /Dynamic Biomarkers**

Parameters for developing high-impact biomarkers for retinal neurodegenerative disease

- Rapid assessment with high sensitivity and specificity.
- Disease-related molecular cellular tag.
- Structural localization at high resolution.
- Track function & pathophysiology dynamically *in situ*.

Monkey retina ganglion cells labeled with rhodamine dextran by retrograde transport following injection into the lateral geniculate nucleus. 

Courtesy of David R. Williams, PhD
Summary: Biomarkers for retinal degenerations

- Neurodegenerative retinopathies present a variety of targets highly suitable for biomarker development at the cellular level.

- Blinding diseases with great human toll:
  - Socially isolating, economically devastating.
  - Incidence increasing as population ages.

- Retina provides a window into CNS neurodegenerative disease.

- Science is poised to develop dynamic functional markers:
  - Genetic insights into pathophysiology.
  - Ultra-high resolution imaging tools.
  - Excellent animal disease models.

- Sensory organ system provides sensitive and specific vision outcome measures for clinical trials endpoints.