Age-Related Macular Degeneration: Nutrition, Genes and Beyond; What have we learned from the Age-Related Eye Disease Study (AREDS) and AREDS2?

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Division of Epidemiology and Clinical Applications
June 14, 2019
I have no financial interests or relationships to disclose*

Off Label Drugs to be Discussed:
- Avastin (Intravitreous) for neovascular age-related macular degeneration (AMD)
- Age-Related Eye Disease Study (AREDS) and AREDS2 supplements (vitamins & minerals) for AMD

*NEI/NIH holds a royalty-bearing license issued to Bausch & Lomb for the AREDS Supplement
Clair Bobier Distinguished Lecture

• A fine Canadian from Peapod, Saskatchewan
• A graduate of the Ontario College of Optometry in Toronto (1948)
• a founding member of the U. of Waterloo School of Optometry
• Brought scientific rigor to the school
AMD: Disease Background

• AMD is ranked 3rd in the World Health Organization’s review of the leading causes of blindness worldwide accounting for 9% of all blindness

• In developed countries including USA, AMD is the leading cause of blindness due to the growing number of people over 70 years of age

• As populations grow and demographic shifts move towards an increase in the predominance of older age groups, the number of persons affected with AMD will increase

Eye Diseases Prevalence Research Group
Causes of Blindness in Whites in USA

Eye Diseases Prevalence Research Group

Causes of Blindness in African Americans

- Cataract: 36.8%
- Glaucoma: 26.0%
- Diabetic Retinopathy: 7.3%
- Others: 25.6%
- AMD: 4.4%

In the United States, AMD affects approximately 2 million individuals who already have vision threatening late stages of AMD.

Approximately 8 million are at high risk of developing late AMD.

Treatments are onerous both in resources and time from affected individuals and their families.

Monetary and emotional burden on family & society.
Projections for Age-Related Macular Degeneration in 2030 and in 2050 (in millions)
AMD Risk Factors

- Aging
- Genetic
- Smoking

Nutritional Risk Factors

Fundus Features
Age-Risk of increasing late AMD
Generational Differences in 5-year AMD Incidence
Beaver Dam Eye Study & Beaver Offspring Study

**AMD:**
Intermediate
Late AMD:
  - wet
  - dry

**Birth Years:**
- Greatest: 1901-1924
- Silent: 1925-1945
- Baby Boom: 1946-1964
- Generation X: 1965-1984

**Graph:**
- X-axis: Generation
- Y-axis: Incidence, %
  - Greatest: 8%
  - Silent: 4%
  - Baby Boom: 2%
  - Generation X: 1%
AMD and Genetics

Genetics may account for 60% of disease

Many genes (52 SNP/34 loci) identified to be associated with AMD
## Smoking—Dose Response: increasing risk with increasing # of cigarettes smoked

<table>
<thead>
<tr>
<th>Smoking</th>
<th>1 -19 Pack-years</th>
<th>&lt;2 X ↑ risk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-39 Pack-years</td>
<td>&gt;2 X ↑ risk</td>
</tr>
<tr>
<td>≥ 40 Pack-years</td>
<td>~5 X ↑ risk of AMD</td>
<td></td>
</tr>
</tbody>
</table>

POLA, study in French Mediterranean Sea border

Normal Eye (No AMD)
Signs of AMD: Large Drusen

Intermediate AMD
Vision Threatening late stages of AMD

Wet AMD
Neovascular AMD

Dry AMD
Central Geographic Atrophy (GA)
Timeline of Development of AMD Treatments

- **1982**: Laser Photocoagulation
- **1982**: "senile" MD
- **1999**: Photodynamic Therapy (Verteporfin)
- **2001**: Intravitreous Injections of anti-VEGF therapy
- **2001**: AREDS Supplements
- **2006**: Lucentis (ranibizumab)
- **2011**: Eyelea (aflibercept)
- **2012**: AREDS2 Supplements
History of AMD Therapies:
Intravitreal Ranibizumab: Improvement in Vision

MARINA Study

Comparison of AMD Treatment Trials

Lucentis vs. Sham

Lucentis vs. Avastin
WE ARE WHAT WE EAT

Vertemnus, by Renaissance artist Giuseppe Arcimboldo, 1591
Factors associated with age-related macular degeneration. An analysis of data from the first National Health and Nutrition Examination Survey (NHANES) survey

Am J Epid. 1988;128:700-10

A diet rich in fruits and vegetables with vitamins A and C, was inversely associated with AMD

Goldberg J, Flowerdew J, Smith E, Brody JA, Tso MO
The Age-Related Eye Disease Study

1992-2001-Clinical Trial of Micronutrients for AMD (n=4757)
2001-2005-Epidemiologic Follow-up Study
AREDS AMD Categories

2. Early AMD

3. Intermediate AMD

4. Late AMD
Antioxidants – Daily Oral Dose

- Vitamin C – 500 mg
- Vitamin E – 400 IU
- Beta-carotene – 15 mg
  (Equivalent to 25,000 IU Vitamin A)
- Zinc (80 mg zinc oxide) with copper 2 mg

Combination of vitamins and zinc resulted in 25% reduction in risk of progression to late AMD in 5 years

AREDS Formulation Recommended:

- patients with intermediate AMD (bilateral large drusen)
- patients with late AMD in 1 eye
- NOT for current smokers (beta-carotene increased the risk of lung cancer in smokers)
- NOT for offsprings without large drusen --does not reduce small progressing to large drusen
- NOT for general eye health

The Age-Related Eye Disease Study

Observational Data
AREDS Dietary Data (observational): Nutrients

Dietary Lutein/Zeaxanthin
- Spinach
- Kale
- Collard Greens

Dietary Omega-3 Long-chain Polyunsaturated Fatty Acids (LCPUFAs) (DHA/EPA) found in FISH
Neovascular AMD
- OR 0.65 (95% CI: 0.45-0.93)

Geographic Atrophy
- OR 0.45 (95% CI 0.24-0.86)

Large Drusen
- OR 0.73 (95% CI 0.56-0.96)

25 to 40% Less AMD with High Intake of L/Z
Wet (Neovascular) AMD-Total DHA/EPA
- OR 0.61 (95% CI: 0.41-0.90)

Neovascular AMD-Docosahexaenoic Acid
- OR 0.54 (95% CI 0.36-0.80)

Neovascular AMD-Higher fish consumption
- OR 0.61 (95% CI 0.37-1.00)

40 to 50% Less AMD with High Intake of DHA/EPA

Association of AMD progression DHA/EPA & progression to AMD in AREDS with 6.5 yr F/U

Highest intake vs. lowest intake (quintile)

Central GA AMD- Total DHA and EPA

- OR 0.45 (95% CI: 0.23-0.90)

Central GA AMD - EPA

- OR 0.44 (95% CI 0.23-0.87)

>50% reduction in developing dry AMD

Similar results found in other studies.

Arch Ophthalmol 2008;126(9):1274-1279.
Importance of Lutein/Zeaxanthin and omega-3 fatty acids

- Major component of the pigment of the macula include lutein/zeaxanthin
- Major component of retina (membranes) include omega-3

© Max Snodderly, 2002
AREDS2 Study Design

Primary Objective:

Test effects of adding

Lutein/Zeaxanthin (10mg/2 mg)

Omega-3 Long-Chain Polyunsaturated Fatty Acids (DHA & EPA=1 g total)

Adding to the AREDS Formulation to assess the Effects on AMD outcomes
Inclusion Criteria

Intermediate AMD (Bilateral Large Drusen) or Late AMD in One Eye

Large Drusen

GA

NV AMD
Primary Randomization

Randomized Participants n=4203

Control 1012
Lutein/Zeaxanthin 1044
DHA/EPA 1068
L/Z + DHA/EPA 1079

AREDS-I Type Supplements

Randomized lutein vs. beta-carotene

1° Randomization: 1° Analyses: Progression to Late AMD (neovascular or CGA)

**DHA/EPA**: neither beneficial nor harmful

**Lutein/zeaxanthin**: incremental increase in benefits (reduction of progression to late AMD)

2° analyses: 25% reduction in development of late AMD in those with the **lowest dietary intake** of L/Z

Direct comparisons with beta-carotene showed a >20% increase in beneficial effects of L/Z (Lutein/zeaxanthin favored over beta-carotene)
Results: 2º Randomization Analyses (Beta-Carotene)

2 – fold increased risk *lung cancer* in those assigned to beta-carotene vs. lutein/zeaxanthin

90% were former smokers

Safety concerns of beta-carotene led to replacement of beta-carotene with *lutein/zeaxanthin*
AREDS2 Formulation

Vitamin C (500 mg)
Vitamin E (400 IU)
**Beta Carotene (15 mg)**
Lutein (10 mg)/Zeaxanthin (2 mg)
Zinc (80 mg zinc oxide)
Copper (2 mg cupric oxide)
**Omega-3 fatty acids (DHA/EPA)**
Recommendations:

Stop smoking

Consider AREDS supplements with lutein/zeaxanthin instead of beta-carotene for those with intermediate AMD (bilateral large drusen) & late AMD in one eye

What about the diet? Do we have evidence that eating certain foods or specific diets may be important?
Timeline of AREDS & AREDS Studies

1992
AREDS clinical trial

2001
AREDS follow-up

2005
AREDS2 clinical trial

2006

2012

2013
AREDS2 follow-up*

2018

*Conducted with 6 monthly telephone calls & medical records validation
Nutrition & AMD: Mediterranean Diet
Why Mediterranean Diet?

- PREvención con Dieta MEDiterránea (PREDIMED) trial-RCT
- Multicenter trial in Spain: persons at high risk for cardiovascular disease (n=7,447) randomly assigned:
  - 1. Mediterranean diet supplemented with mixed nuts
  - 2. Mediterranean diet supplemented with extra virgin olive oil (EVOO)
  - 3. Control diet
- Results showed: 0.69 (95% CI 0.53 -0.91) for Med diet & EVOO; 0.72 (95% CI 0.54 to 0.95) for Med diet & mixed nuts vs. control


Cognition & diet
Nutrition & AMD: Mediterranean Diet

Low on red meat & refined sugar

Whole grains

Moderate fish, white meat & dairy

Moderate alcohol

High consumption of fruits, vegetables, nuts, legumes,
Calculate the alternative Mediterranean Dietary Index (aMED) score:

- foods were summed for each of 9 components
- sex-specific intake quartiles (1-4) were calculated and adjusted for energy intake (separately for AREDS/AREDS2)

Genetic tests: genetic risk score (GRS) calculated for each participant

- Risk Score (GRS), a weighted risk score for late AMD, was calculated for each participant.
- 2 SNPs at 2 loci (with highest attributable risk of late AMD) selected: CFH_rs1061170 and ARMS2_rs10490924.
Statistical Analyses:

- Multivariate *proportional hazards regression*
- Progression to: late AMD, GA, and neovascular AMD according to aMED via tertiles *(Bonferroni adjustment: p=0.017)*
- Analyses repeated for genetic interaction *(Bonferroni= 0.006)*
- Analyses repeated for analyses of each of the 9 components *(Bonferonni=0.002)*
- All analyses adjusted for age, sex, smoking, caloric intake, BMI
### Nutrition & AMD: Mediterranean Diet

<table>
<thead>
<tr>
<th></th>
<th>AREDS cohort</th>
<th>AREDS2 cohort</th>
<th>Combined cohort (AREDS &amp; AREDS2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>4255</td>
<td>3611</td>
<td>7756</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>69 (SD 5.1)</td>
<td>73 (SD 7.7)</td>
<td>71 (SD 6.6)</td>
</tr>
<tr>
<td>Female: n (%)</td>
<td>2388 (56.1%)</td>
<td>2058 (57.0%)</td>
<td>4385 (56.5%)</td>
</tr>
<tr>
<td>Smoking status: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>1914 (45.0%)</td>
<td>1558 (43.1%)</td>
<td>3415 (44.0%)</td>
</tr>
<tr>
<td>Former</td>
<td>2035 (47.8%)</td>
<td>1823 (50.5%)</td>
<td>3809 (49.1%)</td>
</tr>
<tr>
<td>Current</td>
<td>306 (7.2%)</td>
<td>230 (6.4%)</td>
<td>532 (6.9%)</td>
</tr>
<tr>
<td>Alternative Mediterranean diet index tertiles: n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1349 (31.7%)</td>
<td>1224 (33.9%)</td>
<td>2542 (32.8%)</td>
</tr>
<tr>
<td>2</td>
<td>1436 (33.7%)</td>
<td>1101 (30.5%)</td>
<td>2497 (32.2%)</td>
</tr>
<tr>
<td>3</td>
<td>1470 (34.5%)</td>
<td>1286 (35.6%)</td>
<td>2717 (35.0%)</td>
</tr>
</tbody>
</table>
Genetics Association: Complement Factor H and AMD


Hageman G. PNAS 2005, May 17, 102(20);7227-32
Complement Factor H in the Eye

CFH (red), RPE (yellow), Bruch’s membrane (green)

J Tsai at NEI Biological Imaging Core
Josephine Hoh’s GWAS Results: CFH

- Genotyped 146 AREDS specimens (96 cases, 50 controls) using a gene chip- Genome-Wide Association (GWAS) study
- Found 2 SNPs associated with AMD
- Both located in complement factor H gene
  
<table>
<thead>
<tr>
<th>SNP</th>
<th>Odds Ratio (95% CI)</th>
</tr>
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<tbody>
<tr>
<td>rs380390</td>
<td>7.4 (2.9-19)</td>
</tr>
<tr>
<td>rs1329428</td>
<td>6.2 (2.9-13)</td>
</tr>
</tbody>
</table>

Science. 2005 Apr 15;308(5720):385-9
Complement Factor H (CFH)
A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants

Advanced age-related macular degeneration (AMD) is the leading cause of blindness in the elderly, with limited therapeutic options. Here we report on a study of >12 million variants, including 163,714 directly genotyped, mostly rare, protein-altering variants. Analyzing 16,144 patients and 17,832 controls, we identify 52 independently associated common and rare variants ($P < 5 \times 10^{-8}$) distributed across 34 loci. Although wet and dry AMD subtypes exhibit predominantly shared genetics, we identify the first genetic association signal specific to wet AMD, near MMP9 (difference $P$ value $= 8.1 \times 10^{-10}$). Very rare coding variants (frequency <0.1%) in CTH, C1H and TIMP3 suggest causal roles for these genes, as does a splice variant in SIC16AB. Our results support the hypothesis that rare coding variants can pinpoint causal genes within known genetic loci and illustrate that applying the approach systematically to detect new loci requires extremely large sample sizes.

"we analyze >12 million variants.....16,144 patients and 17,832 controls....26 research centers"

Case-control methodology
The AMD Gene Consortium

52 independent variants
34 genetic loci

Chromosomes 1 (CFH) and 10 (ARMS2)

## Insight into mechanisms of disease

<table>
<thead>
<tr>
<th>Pathways</th>
<th># of Genes in Gene Set</th>
<th>#AMD loci in Gene Set</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Complement Pathways</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regulation of Complement Cascade</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>Complement Cascade</td>
<td>36</td>
<td>5</td>
</tr>
<tr>
<td><strong>Collagen Pathways</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Assembly of Collagen Fibrils &amp; other Multimeric Structure</td>
<td>54</td>
<td>3</td>
</tr>
<tr>
<td>Collagen Formation</td>
<td>84</td>
<td>3</td>
</tr>
<tr>
<td><strong>Lipid Pathways</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipoprotein Metabolism</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>HDL-Mediated Lipid Transport</td>
<td>15</td>
<td>2</td>
</tr>
<tr>
<td>Lipid Digestion, Mobilization &amp; transport</td>
<td>50</td>
<td>3</td>
</tr>
</tbody>
</table>
AMD Gene Consortium: Variants associated with the 2 late AMD sub-types

Increased Likelihood of 2 subtypes of late AMD

ARMS2/HTRA1
CETP
MMP9
SYN3/TIMP3

AMD Gene Consortium
MMP-9 Increased Neovascular AMD in AREDS

Baseline Severity 7–8 (rs142450006)

Survival probability

Year after randomization

Neovascular AMD

Baseline Severity 7–8 (rs142450006)

Survival probability

Year after randomization

Central Geographic Atrophy

• **Wet and dry AMD subtypes** exhibit predominantly shared genetics, we identify: the first genetic association signal specific to wet AMD, near **MMP9** (difference $P$ value $= 4.1 \times 10^{-10}$).

• Very rare coding variants (frequency <0.1%) in **CFH, CFI** and **TIMP3** suggest causal roles for these genes, as does a splice variant in **SLC16A8**.

• Our results support the hypothesis that rare coding variants can pinpoint causal genes within known genetic loci and illustrate that applying the approach systematically to detect new loci requires extremely large sample sizes.
AREDS/AREDS2 Genetic Analyses: AMD Progression using Genetic Risk Score

Primary Goal: assess genetic effects on AMD progression, including the baseline AMD severity scores & the effects of estimates of genetic variants (Genetic Risk Score[GRS]).

Secondary Goal: develop prediction models of progression
AREDS/AREDS2 Genetic Analyses: AMD Progression using Genetic Risk Score

• Trained prediction model on the AREDS data
• Evaluated the accuracy within the AREDS cohort (5X cross-validation or bootstrap resampling)
• Evaluated accuracy independently on AREDS2 cohort
• 5 models were evaluated using the following covariates:
  – Baseline age
  – Sex
  – Smoking
  – Genetic Risk Score (GRS)
  --Education
  --Baseline AMD severity of study eye
  --Baseline AMD severity of fellow eye
### Predictors for each Prediction Model A-E

<table>
<thead>
<tr>
<th>Model Index</th>
<th>Model Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Baseline Age, Education, &amp; Smoking</td>
</tr>
<tr>
<td>B</td>
<td>Baseline Age, Education, &amp; Smoking &amp; GRS</td>
</tr>
<tr>
<td>C</td>
<td>Baseline AMD severity score (study eye) &amp; Baseline severity score (fellow eye)</td>
</tr>
<tr>
<td>D</td>
<td>Baseline Age, Education, Smoking, Baseline Severity Score (study eye) &amp; Baseline Severity Score (fellow eye)</td>
</tr>
<tr>
<td>E</td>
<td>Baseline Age, Education, Smoking, Baseline Severity Score (study eye), Baseline Severity Score (fellow eye) &amp; GRS</td>
</tr>
</tbody>
</table>
### AREDS/AREDS2 AMD Progression using Genetic Risk Scores: Multivariable Cox Model

<table>
<thead>
<tr>
<th>Variables</th>
<th>AREDS</th>
<th>AREDS2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR (95% CI)</td>
<td>P-value</td>
</tr>
<tr>
<td>Age (year)</td>
<td>1.05 (1.03, 1.06)</td>
<td>6.4 X 10^{-10}</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>never</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>former</td>
<td>1.14 (0.98, 1.33)</td>
<td>0.06</td>
</tr>
<tr>
<td>current</td>
<td>1.98 (1.51, 2.58)</td>
<td>5.7 X 10^{-7}</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;high school</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>&gt;high school</td>
<td>0.87 (0.75, 1.01)</td>
<td>0.006</td>
</tr>
<tr>
<td>Baseline Study Eye AMD severity</td>
<td>1.85 (1.78, 1.92)</td>
<td>4.3 X 10^{-226}</td>
</tr>
<tr>
<td>Genetic Risk Score (GRS)*</td>
<td>1.34 (1.26, 1.42)</td>
<td>1.6 X 10^{-22}</td>
</tr>
</tbody>
</table>

*Genetics 2017;206:119-113
AREDS/AREDS2 AMD Progression using Genetic Risk Scores: Multivariable Cox Model

**Figure 1** KM plots on progression to advanced AMD by GRS groups. (A) AREDS, (B) AREDS2. Eyes were categorized into three groups according to their GRS: low: 0–25% quartile; medium: 25–75% quartile; high: >75% quartile.
## Predictors for each Prediction Model A-E

<table>
<thead>
<tr>
<th>Model Index</th>
<th>Model Predictors</th>
<th>C-index</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Baseline Age, Education, &amp; Smoking</td>
<td>0.62</td>
</tr>
<tr>
<td>B</td>
<td>Baseline Age, Education, &amp; Smoking &amp; <strong>GRS</strong></td>
<td>0.75</td>
</tr>
<tr>
<td>C</td>
<td>Baseline AMD severity score (study eye) &amp; Baseline severity score (fellow eye)</td>
<td>0.88</td>
</tr>
<tr>
<td>D</td>
<td>Baseline Age, Education, Smoking, Baseline Severity Score (study eye) &amp; Baseline Severity Score (fellow eye)</td>
<td>0.89</td>
</tr>
<tr>
<td>E</td>
<td>Baseline Age, Education, Smoking, Baseline Severity Score (study eye), Baseline Severity Score (fellow eye) &amp; <strong>GRS</strong></td>
<td>0.89</td>
</tr>
</tbody>
</table>

*Genetics 2017;206:119-113*
Model C would be reasonable

Models D and E are very similar

Although the Genetic Risk Score (GRS) only provided minimal improvement to the prediction performance when added to a model containing baseline severity score of both the study and fellow eyes, it was still a significant predictor for AMD progression.

Clearly, the **baseline retinal AMD severity** is an important risk factor for progression.
BACKGROUND:
-C3, COL8A1, CFB and RAD51B have also been associated with AMD progression in both uni- and multi-variable studies
-C2 and C9 are associated with AMD progression based on uni-variable studies
-previous studies investigated only a small number of variants.

We used the **GWAS data** from **AREDS** for discovery of risk variants for **AMD progression**, using Cox Proportional Hazards model.
Genome-wide analysis of disease progression in AMD in AREDS, FINDINGS:

- Replicated previously reported susceptibility loci showing genome-wide significance with AMD progression:
  - ARMS2/HTRA1 ($P = 8.1 \times 10^{-43}$),
  - CFH ($P = 3.5 \times 10^{-37}$),
  - C2-CFB-SKIV2L ($P = 8.1 \times 10^{-10}$)
  - C3 ($P = 1.2 \times 10^{-9}$).

- Associations with progression to wet (neovascular AMD):
  - rs58978565 near TNR ($P = 2.3 \times 10^{-8}$)
  - rs28368872 near ATF7IP2 ($P = 2.9 \times 10^{-8}$)
  - rs142450006 near MMP9 ($P = 0.0006$)

- Secondary analyses of 34 reported risk variants:
  - LIPC & CTRB2-CTRB1 ($P < 0.0015$)

Genome-wide analysis of disease progression in AMD in AREDS

Manhattan plots of GWAS results of AMD progression. (A) GWAS results with adjustment for baseline severity score, and Summary of genome-wide association results using Cox model with robust variance for paired eyes. The red horizontal line is the conservative significance level ($P = 5 \times 10^{-8}$) and the blue horizontal line is the suggestive significance level ($P = 1 \times 10^{-5}$).

Genome-wide analysis of disease progression in AMD in AREDS-adjusted for baseline severity

CFH

Overall

Baseline severity 1-3

Baseline severity 4-6

Baseline severity 7-8

Genome-wide analysis of disease progression in AMD in AREDS-Stratified by Neovascular AMD (CNV) and geographic atrophy (GA)

Genetic Testing in AMD?

- Should this be done to predict those individuals who will progress to AMD?—after all 23and ME offers AMD genes
- Should this be done to predict response to therapies for AMD?
  - Anti-VEGF therapies (injections) for wet neovascular AMD?
  - AREDS2 supplements for AMD?
  - For dietary recommendations?

- No evidence-based data to support such testing for prediction of progression or for response to such therapies.
Genetics of AMD

• AMD is a **Complex** genetic disease
  – Associated with multiple genes
• AMD associated with environmental factors
  – Smoking and diet
• Certain genetic variants (risk alleles or polymorphisms) are associated with increased risk of AMD while some are protective
• Our previous prediction model was not improved markedly by the genetic information
Results of AMD Risk Models

A Risk Score for the Prediction of Advanced Age-Related Macular Degeneration

Development and Validation in 2 Prospective Cohorts

Chung-Jung Chiu, DDS, PhD,1,2 Paul Mitchell, MD, PhD,3 Ronald Klein, MD, MPH,4 Barbara E. Klein, MD, MPH,4 Min-Lee Chang, MS,1 Gary Gensler, MS,5 Allen Taylor, PhD1,2

• AREDS data-validated with Blue Mountain Eye Study
• **No genetic risk** factors evaluated
• Age, sex, education, smoking, RPE pigmentary changes, large drusen
• AUC=0.88 for AREDS

• Validation Sample= 0.91

• No genetic markers included in the model
Results of AMD Risk Models

Risk Assessment Model for Development of Advanced Age-Related Macular Degeneration

Michael L. Klein, MD; Peter J. Francis, MD, PhD; Frederick L. Ferris III, MD; Sara C. Hamon, PhD; Traci E. Clemons, PhD

- AREDS data, validated by CAPT (Complications of AMD Prevention Trial) data
- Evaluated genetic factors: CFH and ARMS2
- Age, sex, education, smoking, RPE pigmentary changes, large drusen

Arch Ophthal 2011:129:1543-1550
Three Risk Models Evaluated

- Model A: phenotypic, demographics and **genetic** factors
- Model B: phenotypic & demographic factors
- Model C: **Genetic** & demographic factors

<table>
<thead>
<tr>
<th>C Statistic (AUC)</th>
<th>Model A</th>
<th>Model B</th>
<th>Model C</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.872</td>
<td>0.865</td>
<td>0.727</td>
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</tbody>
</table>

Arch Ophthal 2011:129:1543-1550
Results of AMD Risk Models

• Genetics markers added little to the predictive value of the risk models
• Overwhelming factor important in predictive power are the fundus findings: the presence of large drusen and RPE pigmentary changes
• Most important predictive test is a dilated fundus exam or a color fundus photograph

Arch Ophthal 2011:129:1543-1550
CFH and LOC387715/ARMS2 Genotypes and Treatment with Antioxidants and Zinc for Age-Related Macular Degeneration

Michael L. Klein, MD,1 Peter J. Francis, MD, PhD,1 Bernard Rosner, PhD,2 Robyn Reynolds, MPH,3 Sara C. Hamon, PhD,4 Dennis W. Schultz, PhD,1 Jurg Ott, PhD,4,5 Johanna M. Seddon, MD, ScM3
• Treatment response to AREDS supplements may be affected by CFH genotype
• However, all genotypes receive benefit, and no alternative interventions are yet available
• Results do not justify routine genetic screening at this time
• Corroboration of findings needed

CFH and ARMS2 Genetic Polymorphisms Predict Response to Antioxidants and Zinc in Age-Related Macular Degeneration

Suggested that those with CFH had a harmful interaction with Zinc therapy: therefore everyone should have genetic testing prior to taking AREDS supplements. AREDS investigators disagreed.

Scientific disagreement reported to central NIH Leadership from the non-AREDS investigators

AREDS Report Number 38
Genetic Testing & Treatment Responses to AREDS supplements: 3 Independent statistical groups

• NIH Research Integrity Officer requested 3 independent statistical groups
  – Duke University
  – Memorial Sloan Kettering Cancer Center
  – MD Anderson Cancer Center

• Unpaid replication was provided by the 3 institutes
Genetic Testing & Treatment Responses to AREDS supplements: 3 independent statistical groups

Genetic Polymorphisms of CFH and ARMS2 Do Not Predict Response to Antioxidants and Zinc in Patients with Age-Related Macular Degeneration

Independent Statistical Evaluations of Data from the Age-Related Eye Disease Study

- Received data from the two different teams
- 3 separate statistical teams to reanalyze the data
- 1st to assess data concordance
- 2nd to replicate the key claim of interaction of genetics with treatment response
- 3rd to evaluate baseline predictors of treatment response

_Ophthalmology_, 2018 Mar;125(3):391-397
• “…..found errors in the data (in the challengers)…..”
• “..found evidence of high-risk patients had more to gain from treatment”
• “….unable to replicate any genotype-treatment interactions after adjusting for multiple testing.”
• “Patients who meet criteria ..should be offered zinc and antioxidants without consideration of genotype.”

Ophthalmology. 2018 Mar;125(3):391-397
Conclusions for Genetic Testing

- Genetic testing remains important in research.
- Hopefully, genetic testing in the near future will help to predict progression of disease and guide current and future treatments for AMD.
- We are not ready to change the recommendations of the AAO TASK FORCE on genetic testing: AVOID genetic testing of AMD...
  .......for now

*AAO: American Academy of Ophthalmology
<table>
<thead>
<tr>
<th></th>
<th>AMD Genetic Risk Score</th>
<th>CFH (rs1061170)</th>
<th>ARMS2 (rs10490924)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Late AMD</td>
<td></td>
<td>0.29</td>
<td>0.39</td>
</tr>
<tr>
<td>Geographic atrophy</td>
<td></td>
<td>0.29</td>
<td>0.17</td>
</tr>
<tr>
<td>Neovascular AMD</td>
<td>0.27</td>
<td>0.35</td>
<td>0.88</td>
</tr>
</tbody>
</table>

No genetic testing required for dietary recommendations
### Interaction of Genetics and Mediterranean diet

**AREDS Population**

<table>
<thead>
<tr>
<th></th>
<th>AMD Genetic Risk Score</th>
<th>( CFH ) (rs1061170)</th>
<th>( ARMS2 ) (rs10490924)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Late AMD</strong></td>
<td>0.07</td>
<td>0.03</td>
<td>0.18</td>
</tr>
<tr>
<td><strong>Geographic atrophy</strong></td>
<td>0.91</td>
<td>0.006</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Neovascular AMD</strong></td>
<td>0.007</td>
<td>0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>
# Nutrition & AMD: Interaction of Genetics and Fish Consumption (AREDS)

<table>
<thead>
<tr>
<th>CFH (rs1061170)</th>
<th>Fish intake</th>
<th>HR (95% CL)</th>
<th>P</th>
<th>P-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.50 (0.30, 0.84)</td>
<td>0.009</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.92 (0.66, 1.30)</td>
<td>0.65</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.86 (0.60, 1.24)</td>
<td>0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.73 (0.49, 1.07)</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.35 (0.19, 0.64)</td>
<td>0.0007</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>0.83 (0.57, 1.22)</td>
<td>0.34</td>
<td>0.60</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1.14 (0.79, 1.64)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.03 (0.68, 1.56)</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

No genetic testing required for dietary recommendations.
Conclusions:

• Genetic testing important for research

AMD Gene Consortium has made great advances

  Functional biology and pathway analyses are important in the next steps

  Avoid AMD genetic testing prior to AREDS supplementation

Conclusion
Artificial Intelligence/Machine Learning/Deep Learning
DeepSeeNet: A Deep Learning Model for Automated Classification of Patient-based Age-related Macular Degeneration Severity from Color Fundus Photographs

Yifan Peng, PhD, 1,*, Shazia Dharssi, BS, 1,2,*, Qingyu Chen, PhD, 1 Tiarman D. Keenan, BM BCh, PhD, 2 Elvira Agravón, MA, 2 Wai T. Wong, MD, 2 Emily Y. Chew, MD, 2 Zhiyong Lu, PhD 2

National Eye Institute | National Center for Biotechnology Information
Deep Learning/Purpose

• To evaluate the machine learning/deep learning technique of assessing the severity of AMD, using the AREDS Simplified Severity Scale to predict the risk of progression to late AMD.

• Although several automated deep learning systems have been developed for classifying color fundus photographs (CFP) of individual eyes by AREDS severity score, none to date has used a patient-based scoring system that uses images from both eyes to assign a severity score.
AREDS simple scale of classifying AMD

- **Right Eye**
  - Late AMD
    - No
      - Large Drusen
        - No = 0
        - Yes = 1
    - Yes
      - Pigment Changes
        - No = 0
        - Yes = 1

- **Left Eye**
  - Late AMD
    - No
      - Large Drusen
        - No = 0
        - Yes = 1
    - Yes
      - Pigment Changes
        - No = 0
        - Yes = 1

**Scores**
- 5
- 4 to 0 (no. of yes)
DeepSeeNet was trained on 58,402 and tested on 900 images from the longitudinal follow-up of 4549 participants from AREDS.

Gold standard labels were obtained using reading center grades.

Methods: DeepSeeNet simulates the human grading process by first detecting individual AMD risk factors (drusen size, pigmentary abnormalities) for each eye and then calculating a patient-based AMD severity score using the AREDS Simplified Severity Scale.
Deep Learning of AREDS simple scale: Methodology

- Two images of both eyes are input to the model.
- CNN (D-Net) for Drusen size.
- CNN (P-Net) for Pigmentary abnormality.
- CNN (A-Net) for Advanced AMD.
- Risk factor scoring for patient output.
- Simplified score output.
- Advanced AMD output.
Main Outcome Measures: Overall accuracy, specificity, sensitivity, Cohen’s kappa, and area under the curve (AUC).

The performance of DeepSeeNet was compared with that of retinal specialists.
DeepSeeNet performed better on patient-based classification (accuracy = 0.671; kappa = 0.558) than retinal specialists (accuracy = 0.599; kappa = 0.467) with high AUC in the detection of large drusen (0.94), pigmentary abnormalities (0.93), and late AMD (0.97).

DeepSeeNet also outperformed retinal specialists in the detection of large drusen (accuracy 0.742 vs. 0.696; kappa 0.601 vs. 0.517) and pigmentary abnormalities (accuracy 0.890 vs. 0.813; kappa 0.723 vs. 0.535) but showed lower performance in the detection of late AMD (accuracy 0.967 vs. 0.973; kappa 0.663 vs. 0.754).
By simulating the human grading process, DeepSeeNet demonstrated high accuracy with increased transparency in the automated assignment of individual patients to AMD risk categories based on the AREDS Simplified Severity Scale.

These results highlight the potential of deep learning to assist and enhance clinical decision-making in patients with AMD, such as early AMD detection and risk prediction for developing late AMD.

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