GENETIC TESTING IN AMD: CRITICAL.......USEFUL.......OR INNAPROPRIATE?

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GENETIC TESTING IN AMD

- DETERMINES RISK OF PROGRESSION TO ADVANCED AMD (GEOGRAPHIC ATROPHY OR CNV) BASED UPON GENETICS AND OTHER FACTORS
- 5 LEVELS OF RISK PROJECTED OUT OVER 2-10 YEARS
## The Genetic Players

- **A Very Large Number, but Two Main Players**
  - CFH (Complement Factor H)
  - ARMS II (Age Related Maculopathy Sensitivity II)

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<th>CFH Binds to Zinc</th>
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<td>ARMS II Localizes to Mitochondria</td>
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<td>Patients can carry 0, 1, or 2 Alleles for both CFH and ARMS II</td>
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• UTILIZES BUCCAL SWAB FROM EACH CHEEK AND DEMOGRAPHIC FACTORS
• TEST KITS KEPT IN OFFICE
• NO BILLING BY COLLECTING DOCTOR, BILLING BY THE LAB (OUT OF POCKET COST HAS VARIED OVER TIME, OFTEN $0.00-$50.00)
• RESULTS IN ABOUT TWO WEEKS
• HIGH RISK PATIENTS CAN BE FOLLOWED MORE CLOSELY, UTILIZE FORSEE AT HOME, ETC.

• NEWLY APPROVED TEST FROM VISIBLE GENOMICS. JUST BECAME COMMERCIALLY AVAILABLE, SOME DIFFERENCES COMPARED TO ARTIC DX TESTING
BUT WHAT ABOUT GENETIC TESTING IN AMD AS IT RELATES TO ZINC? CONTROVERSIAL!

• ZINC IS AN ESSENTIAL MINERAL, SO WE NEED IT (IMMUNE SYSTEM, CELL GROWTH, ETC.)

• RDA OF ABOUT 10MG FOR ADULTS, UPPER TOLERABLE LIMIT OF 40MG (80 MG IN AREDS / AREDS II FORMULA)

• IN EXCESS............CAN LEAD TO NAUSEA, DIARRHEA, HEADACHES, GENITOURINARY TRACT PROBLEMS AND PERHAPS EVEN ALZHEIMER’S (CONTROVERSIAL)
THE CAMPS

• DR. CARL AWH, ET AL
  • GENETICS PLAY A MAJOR ROLE IN THE BENEFIT....... OR DETRIMENT....... OF ZINC SUPPLEMENTATION IN PATIENTS WITH AMD

• DR. EMILY CHEW, ET AL
  • GENETICS PLAY NO ROLE IN THE BENEFIT OF ZINC IN AMD
REFRESHER: ORIGINAL AREDS

- BOTH GROUPS ANALYZED DATA FROM AREDS I (2001) PATIENTS WHO HAD AVAILABLE DNA
- IN AREDS, AMD CLASSIFIED INTO 4 CATEGORIES, WITH CATEGORY 4 BEING ADVANCED
- BASIC FINDING WAS THAT THE AREDS FORMULA DECREASED THE 5 YEAR PROGRESSION RATE OF CATEGORY 3 INTERMEDIATE DISEASE TO CATEGORY 4 ADVANCED DISEASE BY 25%
- NO BENEFIT IN SLOWING PROGRESSION OF EARLY DISEASE TO INTERMEDIATE DISEASE
- 15 MG BETA CAROTENE
- 500 MG VITAMIN C
- 400 IU VITAMIN E
- 80 MG ZINC
- 2 MG COPPER
- AREDS II REMOVED BETA CAROTENE (POSSIBLE INCREASED RISK OF LUNG CANCER IN SMOKERS), BUT ADDED 10MG OF LUTEIN AND 2MG OF ZEAXANTHIN
AREDS REFRESHER

• FOUR GROUPS
  • PLACEBO
  • ANTIOXIDANTS
  • ZINC
  • ANTIOXIDANTS PLUS ZINC (FULL ORIGINAL AREDS FORMULA)

• COULD ALSO TAKE CENTRUM (66% CHOSE TO), SO THESE PATIENTS HAD MORE ZINC, WITH 1.5 EXTRA MILLIGRAMS, AND VERY FEW TRUE “PLACEBO” PATIENTS
AWH STUDY #1 IN 2013
(OPHTHALMOLOGY 120;11; NOV. 2013)

- PURCHASED APPLICABLE DNA FROM PATIENTS IN AREDS I
- USED WHITE PATIENTS WITH CATEGORY 3 (INTERMEDIATE) DISEASE IN AT LEAST ONE EYE, BUT COULD BE CATEGORY 3 OR LESS IN THE FELLOW EYE (COULD NOT HAVE CATEGORY 4 IN EITHER EYE)
- 4757 IN STUDY.........2258 CAUCASIANS WITH CATEGORY 3 IN AT LEAST ONE EYE AND NOT CATEGORY 4 IN EITHER......995 WITH DNA. SO 995 EVALUATED
AWH STUDY # 1

• The 995 were compared to the 2258 and were not statistically different in sex, smoking, BMI, education, treatment category, or progression percentage.
• .6 year difference in average age.
• CFH 1, CFH 2 had no benefit from any zinc containing formula.
• CFH 2, ARMS II 0 showed 43% greater progression rate with any zinc than with placebo.
• With antioxidant therapy alone, more ARMS II alleles = greater progression.
• CFH 2, ARMS II 2 = 75% progression rate no matter what they took, with no benefit from anything.
AWH STUDY #1

- Authors' Conclusion: Zinc potentially harmful in CFH patients, but Zinc potentially helpful in ARMS II patients
- This study started the controversy
- Projected estimated 10 year progression rate:
  - Placebo: 47%
  - AREDS: 40.5%
  - If targeted: 31.5%
CHEW RESPONSE ANALYSIS (OPHTHALMOLOGY 2014)

• Used the AREDS patients with the same criteria as AWH, but also included patients with Category 4 in one eye and less than Category 3 in the fellow eye.

• Used sex, age, smoking, etc. as variables along with CFH and ARMS II, so 27 separate categories studied.

• Concluded that genetics had no role in the protective value of zinc or antioxidants, and that all groups showed a benefit from the AREDS formula.
AWH STUDY #2  
(OPHTHALMOLOGY 2014)

- Looked at same category groups as before, but also included those patients with category 4 in one eye
- No statistical difference from AREDS white, DNA available population regarding age, sex, smoking, BMI, etc.
- Had 9 total groups, based upon CFH 0-2 and ARMS II 0-2
- Looked at actual 7 year progression rate (not projected) in each group

- Samples:
  - CFH 2, ARMS II 0: Placebo 17% progression, any zinc 43% progression
  - CFH 0 or 1 ARMS II 1 or 2: Placebo 43% progression, any zinc 25% progression
  - CFH 2, ARMS II 1 or 2: Placebo 48% progression, nothing else any better
AWH STUDY # 2

• SO THINK IN TERMS OF 4 GROUPS

• ZINC **INCREASES** THE DELETERIOUS EFFECTS OF CFH AND ZINC **DIMINISHES** THE DELETERIOUS EFFECTS OF ARMS II

• LOW CFH, LOW ARMS II (28% OF STUDY GROUP): ZINC DOES NOT HELP OR HURT

• HIGH CFH, LOW ARMS II (13%): ZINC IS HARMFUL AND AT LEAST DOUBLES THE RISK OF PROGRESSION

• LOW CFH, HIGH ARMS II (35%): ZINC HELPS

• HIGH CFH, HIGH ARMS II (23%): NOTHING HELPS
INDEPENDENT STATISTICAL ANALYSIS

• 2015
• RAFAL KAFSTRA, PHD
• BIOSTATISTICS, UNIVERSITY OF TORONTO
• BERNARD ROSNER, PHD
• BIOSTATISTICS, HARVARD MEDICAL SCHOOL

• BOTH ANALYZED THE DATA USED BY AWH AND CHEW, AS WELL AS THEIR CONCLUSIONS
  DETERMINED THAT GENETICS PLAY A ROLE IN THE RESPONSE TO ZINC, AND THAT ZINC IS HARMFUL TO SOME
INDEPENDENT STATISTICAL ANALYSIS

- Seddon, Silver, and Rosner
- July, 2016 in British Journal of Ophthalmology
- Use the individual eye, not the patient, as the endpoint. This increased the statistical power
- Looked at 2317 people, 4124 eyes
- Assessed CFH and ARMS 2 (0=low, 1 or 2 = high)
- Low/low, low/high, high/low, high/high
- Average follow-up of 6.6 years
- 882 progressed to advanced disease (GA or NV)
- Conclusion: The effectiveness of antioxidants and zinc do differ by genotypes
TWO MORE IN LATE 2017

• ASSEL, ET. AL IN OPHTHALMOLOGY
• THREE INDEPENDENT GROUPS OF STATISTICIANS WORKING SEPARATELY
• DETERMINED ZINC PLAYS NO ROLE

• VAVVAS, AWH, ET. AL
• ONLY LOOKED AT PROGRESSION TO NV, AS AREDS FORMULA NOT SHOWN TO PROTECT AGAINST GEOGRAPHIC ATROPHY
• USED “BOOTSTRAPPING” TECHNIQUE
• FOUND AN EVEN STRONGER ASSOCIATION BETWEEN GENETIC TYPES AND HARM FROM ZINC OR BENEFIT FROM AREDS FORMULA
• USED A NEVER BEFORE STUDIED GROUP OF 299 AREDS STUDY PATIENTS
GAIN STUDY: **GENETICS & AREDS FORMULA INTERACTION IN NEOVASCULAR AMD**

- Conducted at multiple retinal practices around the country (Ohio, Pennsylvania, California)
- Stephen Kaufman, MD & Pradeepa Yoganathan, MD with others
- Started with a group of 1000 patients who had recently converted to neovascular AMD (important: not speculative)
- Inclusion: Reliable history of greater than 5 years of AREDS formula use (either one or two pills per day) or no history of AREDS formula use (less than 30 days total use ever)
- Exclusion: Any genetic testing prior to wet AMD diagnosis, macular laser, vitrectomy, history of non-AMD induced CNV
• MASKED GENOTYPING: GENOTYPE GROUPS 1, 2, 3, 4, BASED UPON HIGH OR LOW CFH AND ARMS II
• 266 PATIENTS MET THE CRITERIA: 46 AREDS USERS (5 OR MORE YEARS) AND 219 NON-USERS

• OF THESE, 27 AREDS USERS WITH GENOTYPE 2 (HIGH CFH, LOW ARMS II) OR GENOTYPE 3 (LOW CFH, HIGH ARMS II), AND 140 NON-USERS WITH GENOTYPES 2 OR 3

• ALSO COLLECTED AGE, SEX, SMOKING STATUS, AND BMI. (ALL PATIENTS WERE CAUCASIAN)
GAIN STUDY

• If there is no interaction with genetics, then the ratio of AREDS users to non-users will be the same in genotype group 2 and genotype group 3.

• If there is an interaction with genetics, then there will be an increased proportion of AREDS users in genotype group 2 (because zinc harms them), and an increased proportion of non-AREDS users in genotype group 3 (because zinc helps them).
**GAIN STUDY RESULTS**

- Odds ratio for AREDS use in genotype group 2 vs genotype group 3............ **4.18 (4.81 when adjusted for confounders)**

- High dose zinc appeared to harm genotype group 2, and help genotype group 3 (remember that patients were included if they took one or two pills per day, so either 40 mg or 80 mg of zinc)

- Things to consider.....
  - Real world patients, not from the AREDS study population
  - Only included patients who had already converted to wet AMD
  - Showed “harm” and “help” as predicted if there is an interaction
  - Relatively small total patient numbers in group 2 (47) and group 3 (120)
  - AREDS formula use history collected by an independent data coordinating center (relied on patient reporting), that also collated genetic testing results
PREDICTORS OF PROGRESSION TO ADVANCED DISEASE IN AMD
ARTICLE

• “DEVELOPING PROGNOSTIC BIOMARKERS IN INTERMEDIATE AGE RELATED MACULAR DEGENERATION: THEIR CLINICAL USE IN PREDICTING PROGRESSION”

• CLINICAL AND EXPERIMENTAL OPTOMETRY 2018;101:172-181

• FROM AUSTRALIA: INTENSIVE LITERATURE SEARCH

• LOOKED AT CONVERSION OF INTERMEDIATE AMD TO GEOGRAPHIC OR EXUDATIVE DISEASE
PREDICTORS OF PROGRESSION

• USED SD-OCT FINDINGS
• LOOKED AT EYES WITH INTERMEDIATE AMD PROGRESSING TO ADVANCED DISEASE
• MANY, IF NOT MOST, OD’S HAVE OCT CAPABILITY, SO VERY VALUABLE AND PRACTICAL INFORMATION.
• MANY DIFFERENT PREDICTORS IDENTIFIED

• HYPER-REFLECTIVE FOCI
• RETICULAR PSEUDODRUSEN
• NASCENT GEOGRAPHIC ATROPHY
• SUB-RPE HYPER-REFLECTIVE COLUMNS
• DRUSEN WITH SUBRETINAL FLUID
• DRUSEN SUBSTRUCTURES
• DRUSEN LOAD
• DRUSEN REGRESSION
1) HYPER-REFLECTIVE FOCI

- DOT SHAPED INTRARETINAL LESIONS AT THE APEX OF DRUSEN
- OFTEN CORRESPOND TO FOCAL HYPERPIGMENTATION
- START IN THE OUTER RETINA AND MIGRATE INWARD
- LIKELY REPRESENT PIGMENT GRANULES
- ANCILLARY AREDS II OCT STUDY SHOWED THEM TO BE ASSOCIATED WITH A 5X RISK OF GEOGRAPHIC AMD IN TWO YEARS. NO EXTRA RISK OF CNV
HYPER-REFLECTIVE FOCI
HYPER-REFLECTIVE FOCI
2) RETICULAR PSEUDODRUSEN

- SUBRETINAL DRUSENOID DEPOSITS ON OCT (BELOW THE RETINA BUT ABOVE THE RPE)
- SHOW UP WELL ON FAF ALSO
- YELLOWISH INTERCONNECTED DEPOSITS
- MOST FREQUENT IN THE SUPERIOR MACULA AND SUPEROTEMPORAL ARCADE (ODDLY, BIGGER RISK)
- SHOW UP POORLY IN PHOTOGRAPHS
- 2-6 X INCREASED RISK OF PROGRESSION TO ADVANCED DISEASE; MORE GA THAN CNV

FAF better than photo
TRADITIONAL DRUSEN: PHOTO SHOWS MUCH BETTER THAN FAF
3) NASCENT GEOGRAPHIC ATROPHY

- THINNING OF THE OPL AND INL WITH A HYPOREFLECTIVE WEDGE
- NO PHOTORECEPTOR OR RPE LOSS
- 90% OF THE TIME WITHIN CENTRAL 1500 MICRONS OF THE MACULA
- STRONGLY ASSOCIATED WITH IMPENDING GA
- NO EXTRA RISK OF CNV
4) SUB-RPE HYPER-REFLECTIVE COLUMNS

- INCREASED TRANSMISSION OF SIGNAL COLUMNS BENEATH THE RPE (HYPER-REFLECTIVE)
- OVERLYING RPE APPEARS INTACT
- MAY REPRESENT FINE CRACKS IN IN THE RPE
- OPPOSITE APPEARANCE OF SHADOWS CAST BY RETINAL BLOOD VESSELS
- EXTRA RISK OF GEOGRAPHIC DISEASE AND CNV
5) DRUSEN WITH SUBRETINAL FLUID WITHOUT EVIDENT CNV

- SUBRETINAL FLUID POCKETS ABOVE DRUSEN
- FLUID DOES NOT EXTEND HIGHER THAN THE PEAKS OF THE DRUSEN
- NO CNV ON ADVANCED TESTING (IVFA, ICG)
- MAY BE SUBCLINICAL CNV OR MECHANICAL STRAIN
- INCREASED RISK OF CNV
6) DRUSEN SUBSTRUCTURES

- Non-homogeneous internal reflectivity of soft drusen
- All look the same on examination/photos, but have differing OCT reflectivity
- May precede drusen regression
- Increased risk of GA but not CNV
THREE IN ONE!

2019
ERM too

2016
7) DRUSEN LOAD AND DRUSEN REGRESSION

- Central Drusen Volume Important
- Drusen Volume Greater Than .03 Cubic MM in the Central 3 MM Macular Diameter = 4 x Risk of Progression to Advanced Disease
- Regression of Drusen Can Occur in Up to 50% of Intermediate AMD Eyes Over 2 Years
- Increased Risk of Geographic Atrophy or CNV. Often a Direct Precursor Event
DRUSEN REGRESSION OD 2015-2019 WITH GA
DRUSEN REGRESSION OS 2015-2019 WITH GA
DRUSEN REGRESSION GA OU FAF
8) OTHER RISKS SPECIFICALLY FOR CNV

- 2019 JAMA OPHTHALMOLOGY ARTICLE PUBLISHED 4-25 ON-LINE
- SECONDARY ANALYSIS OF THE FELLOW EYES IN THE HARBOUR TRIAL
- INCREASED CNV RISK WITH..................
- INCREASED CENTRAL DRUSEN VOLUME, CONFIRMING PREVIOUS FINDINGS
- INCREASED REFLECTIVITY OF DRUSEN
- FEMALE
- AGE (OF COURSE!)
- PRESENCE OF THE GENE VARIANT RS61941274 @ THE ACAD10 LOCUS