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The Association of Reticular Pseudodrusen with Geographic Atrophy in Age-Related Macular Degeneration using Fundus Autofluorescence Imaging

1. Study Purpose and Rationale:

Age-related macular degeneration (AMD) is the most common cause of irreversible adult blindness in industrialized countries (Hyman, 1987). More than 8 million Americans suffer from AMD, and the overall prevalence is projected to increase by more than 50% by 2010 (Jager, Mieler, & Miller, 2008). The stages of AMD are clinically categorized as early, in which vision is not significantly affected, and late, in which patients suffer from severe loss of central vision (de Jong, 2006). Late-stage AMD is further classified into two categories: dry and wet. Dry AMD type is also known as geographic atrophy (GA), and the wet form is also known as choroidal neovascularizaton (CNV). In 10-20% of AMD patients, AMD converts to CNV, in which new blood vessels grow beneath the retina, leaking fluid and blood and causing severe central vision loss (Tielsch JM, 1995). In addition, a similar percentage of patients with early AMD convert to GA. Klein et al reported that in persons 85 years and older, the five-year incidence of pure GA (8%) is approximately four times the incidence of CNV (Klein R. , Meuer, Knudtson, & Klein, 2008). Unlike CNV patients, GA patients do not benefit from intravitreal anti-vascular endothelial growth factor treatment.

Clinically, GA starts with a sharply demarcated hypopigmented area that is often adjacent to the fovea. The initial symptoms of GA are usually indicated by visual distortions such as seeing gaps in an image (de Jong, 2006) (Klein ML, 2008). GA has the same potentially blinding visual consequences as CNV. Once present, it typically progresses linearly. The rate of GA enlargement varies among individuals. A few environmental and genetic risk factors are known to be associated with late-stage AMD, but overall our ability to predict progression rate is poor, and it is often difficult to give patients prognostic indicators based on their presentation (Klein R. , Meuer, Knudtson, & Klein, 2008).

It has been noticed by clinician-scientists at our practice and our collaborator's practice that GA often co-exists with reticular pseudodrusen (RPD). RPD is a biomarker for reticular macular disease (RMD), which can be considered as a subtype of AMD (Smith, Sohrab, Busuoic, & Barile, 2009). The prevalence of reticular pseudodrusen seemed to be much higher than previously reported (Klein R. , Meuer, Knudtson, Iyengar, & Klein, The Epidemiology of Retinal Reticular Drusen, 2008). This is perhaps due to the fact that most previous studies used colored fundus photo, which we now know is not a very sensitive method in detecting RPD. With advancements in imaging technology since RPD were first reported, reticular patterns were noted in indocyanine green angiography (ICG) (Arnold, Quaranta, Soubrane, Sarks, & Coscas, 1997) and autofluorescence (AF) (Lois N, 2002) images, leading to the definition of RMD as an entity with a systematic, uniform presentation of reticular patterns across imaging modalities, including AF, ICG, color fundus (CF), red free (RF), and infrared (IR) imaging (Smith, Sohrab, Busuoic, & Barile, 2009). The first comprehensive study of RPD found a higher incidence of RPD co-existing with the wet form of end stage AMD (Arnold, Sarks, Killingsworth, & Sarks, 1995). Several subsequent studies found further associations between RPD and CNV (Prenner JL, 2003) (Cohen SY, 2007) (Smith RT, 2006) (Hwang JC, 2006). Compared to the available

literature associating RPD with the wet form of AMD, there is a relative lack of information on dry AMD with reticular pseudodrusen. Particularly, the incidence of GA with reticular pseudodrusen as visualized by autofluorescence is unknown, and perhaps more importantly, the rate of atrophy progression when the two pathologies co-exist has not been characterized before either.

Given the prevalence of RMD and its association with late-stage AMD, increased awareness and understanding of the pathogenesis of RMD could assist with directed patient care in the future. In this study, we aim to demonstrate a stronger association of RMD with GA than previously reported. We plan to estimate the progression rate of GA, quantified as the surface area of atrophic retinal pigment epithelium (RPE), in patients with reticular finding compare to those without reticular finding.

2. Study Design and Statistical Analysis:

Hypothesis #1:

The annualized geographic atrophy (GA) progression rate, as measured by the area of GA, is higher in patients with the presence of reticular pseudodrusen (RPD) compared to those without RPD.

Hypothesis 1a:

The location of RPD affects GA progression rate.

Hypothesis 1b:

The area of reticular pattern affects GA progression rate.

Hypothesis #2:

The prevalence of RMD is higher in GA patients than the value previously reported in literature.

Study Design:

This study is a retrospective analysis of serial retinal imaging of patients at Duke University's Eye Center and Columbia's Harkness Eye Institute between 2005 and 2010. Previously, one of the co-investigators have identified 47 patients with primary GA findings on fundus autofluorescence (AF) imaging at Duke Eye Center via chart review in the aforementioned study period. Similarly, a retrospective imaging review will be conducted at Columbia University: additional patients will be identified for review by searching the Heidelberg Retina Angiograph (HRA)/HRA2 Confocal SLO Camera (Heidelberg Engineering, Inc., Dossenheim, Germany) for those with GA. The database includes all patients seen by the retinal faculty at Columbia University between January 1, 2005 and August 1, 2010. Inclusion criteria include patients with primary geographic atrophy findings, who have at least 2 fundus AF images acquired during this period, and at least one year duration between the first and last recorded AF image. Exclusion criteria are patients with other retinal pathologies, those without serial imaging data, or with less than one year between the follow-up appointments. We anticipate about 50 cases at Columbia to fit the inclusion criteria above. The following information will be recorded and reported: age, gender, dates of imaging obtained, confirmed clinical diagnosis of GA, initial visual acuity, changes in visual acuity, presence of co-morbid conditions such as hypertension or smoking, lens status, total change in the surface area of geographic atrophy on FAF images, and the presence of reticular pseudodrusen (yes/no), the area of the reticular pattern.

Imaging Analysis:

1. Image Registration:

   Using existing image data, fundus AF images will be normalized and registered, and automated measurements of the GA area will be performed using customized computer program. One of our collaborators at the Columbia University Bioengineering Department has developed an automated image analysis tool that allows retinal images to be cropped and registered for each patient. The registration tool aligns serial images taken at multiple time points by comparing the location of retinal vessels and retinal pathologies.

2. GA analysis:
   a. Calculate the area of GA: With the aid of the custom retinal image analysis tool, a user manually selects two areas: a small area of GA and a normal retinal area. The software compares relative pixel intensity of the image matrix and highlights the remaining area of GA. The user can then perform manual revision of the atrophic area. The software will calculate the size of the final highlighted area representing the GA. This measurement will be given as a percentage of the total cropped image (324 x 324 pixels) with a build-in conversion of 1% = 0.35996 mm^2. The total area of geographic atrophy will be calculated 3 times in this manner, and an average will be taken for the analysis step.
   b. Calculate an annualized GA progression rate: as described in our inclusion criteria, each patient in the study will have at least one year of follow-up period and at least two time points in the duration. For each image acquired, the GA progression rate will be calculated in the following manner:

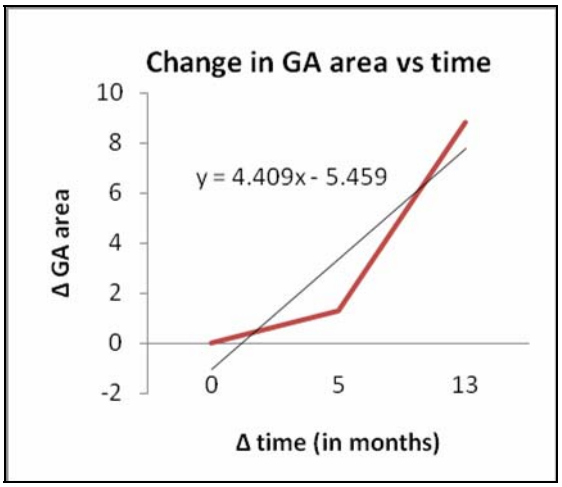
$$GA\ Progression\ Rate_n = 12\left(\frac{GA_n - GA_{n-1}}{t_n - t_{n-1}}\right)$$

where
GA_n is the size of GA at time *n,*
GA_{n-1} is size of GA at the previous image acquisition time,
and $t_n - t_{n-1}$ is the duration (in months) between the two AF images.
Multiplication by 12 annualizes the GA progression rate

If a patient has more than two serial images acquired during the duration of study, a GA progression rate will be calculate as the slope of the best fitted line with size of GA on the y axis and time on the x-axis.

For example, in a hypothetical patient case we have three imaging points in the study duration:

YEAR	GA AREA (%)	Δ time (mo)	Δ time (yr)	Δ GA area
2005-09	15.3273	0	0.00	0
2006-02	16.5866	5	0.42	1.2593
2007-03	25.4049	13	1.08	8.8183



The average annual GA progression rate for this hypothetical case will be the slope of the best fitted line, 4.4092 %/year. The % change per year will then be converted to mm^2/yr using the conversion factor 1%=0.35996 mm^2. Thus for the above case, the annual GA progression rate is $4.4092\,\%\frac{}{yr} \times 0.35996\, mm^2/_{\%} = 1.59\, mm^2/_{yr}$

3. RPD Analysis:
   a. Presence of RPD: Each fundus AF retinal image will be screened for the presence of RPD. A junior grader will screen all images, and a senior grader will confirm all positive cases, as well as for which cases the junior grader was uncertain. Images will be subdivided into +RPD group (GA patients with the presence of reticular pattern) and –RPD group (GA patients without the presence of reticular pattern) subsequently.
   b. Location of RPD: The location of RPD will be recorded as one of the four categories: the superior arcades, the inferior arcades, the temporal field, and the nasal field as defined in other studies (Klein R. , Meuer, Knudtson, Iyengar, & Klein, The Epidemiology of Retinal Reticular Drusen, 2008).
   c. Size of the reticular pattern: The registered AF image will be divided into a 16 grid template and the size of the RPD will be measured roughly as how many grids it occupies. We realize there are limitations of this method. As the GA area enlarges, it can overlay the reticular pattern area thus an otherwise larger area would not be detected. However, there is no existing literature on how to quantify the area of reticular pattern. We will continue to search ways to make size quantification more accurate and representative.

Statistical Analysis

1. GA progression rate
   a. Literature Reviews

   With color fundus photography, Klein et al reported a 5 year GA progression rate of 6.4 mm^2 by examining 53 eyes of multiple visits (Klein R. , Meuer, Knudtson, & Klein, 2008). Converting this to an annualized rate, it gives 1.28 mm^2/yr. Another paper reported a median enlargement of 1.52 mm^2/yr using fundus AF pictures of 195 eyes. The author reported Interquartile Range (IQR) of 0.81 to 2.33 mm^2/yr. This paper also mentioned

eyes with “diffuse trickling pattern” showed higher GA progression rate with median of 3.02 mm^2/yr (Holz FG, 2007). The image illustrating “diffuse trickling pattern” appeared to be similar to the reticular pattern, but it is unclear how the authors define “diffuse trickling,” and this classification has not been used other literature. We use 3.02 mm^2/yr an estimate of the GA progression rate in the reticular group in the power analysis below. Klein et al reported right eyes with reticular pattern at baseline has 21% 15-year cumulative incidence of GA, which we will use as the prevalence estimate.

b. Power analysis

By hypothesis #1, we expect a higher GA progression rate in the eyes with RPD compared to the eyes without RPD. In the Imaging Analysis section above, we divide our GA patients into *With Reticular Findings (+)* and *Without reticular findings (-).* We use 21% as the estimate of + reticular group in the total GA patients.

Our best estimate for average GA progression rate using fundus AF imaging would be 1.52 mm^2/yr in the general GA patients based on literature. Assuming normal distribution, IQR of 0.81 to 2.33 mm^2/yr gives a standard deviation of 1.125.

Using the Power Analysis tool “Find Sample Size” tab of two sample unpaired t-test on the biomath.info/power website, with the following input:

Group 1 (+reticular) mean = 3.02
Group 2 (- reticular) mean = 1.52
Standard Deviation: 1.125
Alpha = 0.05 Power = 0.8

Assume unequal group size: Group2/Group1 = 4 (Based on the estimate of 21% GA patients have reticular findings)

We find the sample size to be 29 in Group 2 (- retic) and 8 in Group 1 (+ retic) with the assumptions/estimates above.

The power calculation is repeated for a few more iterations of various mean effect sizes and the tabulated results of the total number of study subjects needed to detect a statistically significant mean difference in GA progression rate in the + RPD group compare to the – RPD group are shown below:

Mean difference mm^2/yr	# RPD subjects needed in study	# total subjects needed in study
0.3	140	559
0.5	52	226
1	15	57
1.5	8	29
2	<6	19

With the 47 patients from the Duke study and the anticipated 50 additional cases from Columbia (total N = 97; + reticular = 97*0.21 = 21), this project is powered to detect a mean difference of about 0.78 mm^2/yr in GA progression rate.

Alternatively, we do a power calculation using the hypothetical situation that we only study the 47 available cases from Duke University's GA pool (images already collected). Using the Unpaired t-test to find the effect size, we enter the following:

N for Group 1 (+RPD) = 10 (Using 21% as the estimate of RPD prevalence in the 47 GA patients we have, that gives an estimated 10 patients with + RPD findings)
Standard deviation of 1.125
Group 2/ Group 1 = 4

We need an effect size of 1.1 mm^2/yr in the GA progression difference to reach statistical significance of 5% with 80% power with 47 patients (of which 10 is estimated to have the reticular pattern).

Study of how location of RPD and size of RPD affects GA progression rate
The study is not currently powered to detect statistically significant result based on RPD location and size. We know that RPD tends to occur in the superior arcades and the temporal retina, but the location and size of RPD has not been described to affect GA progression in literature. Our study result would be provide useful estimated progression rate to power future larger study to detect statistical significance of RPD location and size.

2. The prevalence rate of RPD in patients with GA
   a. Literature Reviews:

The Beaver Dam Population Eye study reported a 15-year incidence rate of RPD varied with age, from 0.4% in those 43 to 54 years old, to 6.6% in those 75 years or older at baseline. The authors also found Reticular drusen has a 15-year incidence of 21.1% in the right eye with GA and 36.4% with left eye with GA (Klein R. , Meuer, Knudtson, Iyengar, & Klein, The Epidemiology of Retinal Reticular Drusen, 2008). The Beaver Dam study was done using standard color fundus photographs. In another study, reticular findings were present in 24% of CNV patients using color fundus photo (Cohen, Dubois, Tadayoni, Mazzaa, Debibie, & Quentel, 2007). There is no available estimate of RPD prevalence rate using autofluorescence imaging.

   b. Power analysis

By hypothesis #2, we expect the prevalence of RMD in GA, as seen in autofluorescence imaging, to be higher than previously reported using color fundus photos. We will use the average 15-year incidence rate of the right eye (21.1%) and the left eye (36.4%) as reported in the Beaver Dam study to be the estimate of RMD prevalence rate (result = 28.75%). Literature search did not find a standard deviation of the reported prevalence rate of RPD, thus in the sample calculation below, the square root of 28.75% (equals 5.36%) is used as a rough estimate of the standard deviation.

With one-sample Chi-square test of proportion, with the following inputs:

Alpha	0.05
Power	0.8

Group proportion: 45% (clinical investigator's conservative estimate of the true reticular with GA prevalence rate)
Comparison proportion: 28.75%

Result: we need at least 75 patients.

With the 47 patients in the Duke study, and the anticipated 50 patients from Columbia chart review, our study is sufficiently powered to detect a statistical significance of the reticular prevalence rate in geographic atrophy.

3. Predicting GA progression:
    a. Use a linear regression model to predict GA progression as a function of age (continuous variable), RPD (1=presence, 0=none), location (1=superior or temporal fields, 0 = other), gender (male =1), smoking (=1), Hypertension (=1). Aim to see what factors are statistically significant in predicting GA progression.

3. Study Procedures:

N/A. This is a retrospective review of retinal images and charts and involves no intervention.

4. Study Drugs or Devices:

N/A. This is a retrospective review of retinal images and charts and involves no drugs or devices.

5. Study Questionnaires:

N/A. This study does not plan to employ questionnaires.

6. Study Subjects

We will be using images that were already taken at one of the principle investigator's practice at the Duke University Eye Center. The dataset include fundus AF pictures of 47 patients with geographic atrophy (GA) and at least two follow up appointments in the study period. In addition, fundus AF images of patients seen at the Columbia University Harkness Eye Institute retinal practice between 2005 and 2010 will be reviewed for GA findings. We anticipate about 50 patients in the Columbia group.

7. Recruitment:

N/A. This is a retrospective review of retinal images and charts and involves no additional patient recruitment.

8. Confidentiality of Study Data:

Data obtained from chart review will be given unique code numbers and stored in an Excel file on the investigator's password – protected computer and accessed only by the investigator. The key to the code will be kept in a locked file in the investigator's office. The key to the code will be destroyed once all data is recorded and analyzed.

9. Potential Risks:

This is a retrospective review and will pose no additional risk to any patient.

10. Potential Benefits:

This study will not directly benefit patients enrolled; however, results may possibly benefit other patients with this problem.

11. Alternatives: Not applicable.

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