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Longitudinal Study of Age-Related Cataract Using Dynamic Light Scattering

Loss of α-Crystallin Leads to Nuclear Cataract Development

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Purpose: To conduct a longitudinal study on age-related nuclear cataracts using dynamic light scattering (DLS) to determine if cataract progression is associated with loss of the unbound form of the lens molecular chaperone protein, α-crystallin.

Design: Natural history and cohort study.

Participants: Patients 30 years of age or older of either gender seeking treatment at the Wilmer Eye Institute Cornea–Cataract Department.

Methods: All patients underwent a comprehensive dilated eye examination every 6 months, including slit-lamp grading of their lenses using the Age-Related Eye Disease Study (AREDS) clinical lens grading system and obtaining an estimate of unbound α-crystallin level in the nucleus, the α-crystallin index (ACI), using the National Aeronautics and Space Administration–National Eye Institute DLS device. We used a random effects statistical model to examine the relationship of lens opacity changes over time with ACI changes.

Main Outcome Measures: α-Crystallin Index (ACI) and AREDS nuclear cataract grade.

Results: Forty-five patients (66 eyes) 34 to 79 years of age with AREDS nuclear lens grades of 0 to 3.0 were followed up every 6 months for a mean of 19 months (range, 6–36 months). We found that lenses with the lowest baseline levels of ACI had the most rapid progression of cataracts, whereas lenses with higher ACI at baseline had no or slower cataract progression. Lenses that lost α-crystallin at the highest rates during the study also had faster progression of nuclear cataracts than lenses with a slower rate of ACI loss. Kaplan-Meier survival curves showed that lenses with the lowest initial ACI had the highest risk of undergoing cataract surgery.

Conclusions: This longitudinal study corroborates our previous cross-sectional study finding that higher levels of unbound α-crystallin as assessed by ACI are associated with lower risk of cataract formation and that loss of ACI over time is associated with cataract formation and progression. This study suggested that assessment of ACI with the DLS device could be used as a surrogate for lens opacity risk in clinical studies, and for assessing nuclear cataract events in studies where cataract development may be a side effect of a drug or device. *Ophthalmology 2015;■:1–7 © 2015 Published by Elsevier on behalf of the American Academy of Ophthalmology.*

Age-related cataract remains the main cause of blindness in the world, despite advances in its surgical treatment. With the rapid aging of the United States population, there will be an increase in the economic burden resulting from cataract in this country. Cataract surgery is performed in at least 2.5 million eyes each year in the United States and is now the most commonly reimbursed surgical procedure by Medicare.[1–4]

Nuclear cataract, which is the opacification of the nuclear region of the lens, is the most common type of age-related cataract in the United States.[5,6] Nuclear cataracts result from misfolding and aggregation of lens proteins, causing the formation of high–molecular weight protein aggregates that block, scatter, and distort light as it passes through the lens. These opacities cause progressive loss of vision that ultimately requires cataract surgery. A major cause of such protein damage in the lens is chronic oxidative stress.[7–17]

Recently, it has been found that one of the main lens proteins, α-crystallin, has protective molecular chaperone properties and can prevent the aggregation of lens proteins damaged by oxidative stress or other insults.[18–23] The α-crystallin molecule has the ability to bind to partially unfolded proteins including β- and γ-crystallins as well as to other proteins in the lens, stabilizing them and preventing uncontrolled aggregation that would produce large, light-scattering elements. For this reason, α-crystallin has been identified as an endogenous anticataract lens protein and has become an important focus of study. Many laboratories have

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been studying its properties and characteristics, using animal lenses as well as eye bank or cadaveric and surgically extracted human lenses. However, until the development of dynamic light scattering (DLS) technology, it has not been possible to study α-crystallin in the intact living human eye.

With the development of the DLS technique (also called quasielastic light scattering), α-crystallin in the lenses of animals and patients can be detected and measured in vivo, noninvasively, and safely.[8,24–36] Benedek[8] developed the first DLS device in the 1970s, followed by others, including Weiss et al,[25] Bursell et al,[29] and Thurston et al.[30] They and others studied animal and human normal and diabetic lenses.[24–30]

The discovery that α-crystallin represented endogenous molecular chaperones[18–23] led us to monitor and estimate the loss of α-crystallin in the lens in vivo using the new compact fiberoptic-based DLS technology developed for fluid physics experiments in space.[31–36] We used the DLS clinical device developed by a National Aeronautics and Space Administration–National Eye Institute team to study the early onset of cataractogenesis in model protein solutions, in live animals, and in clinical experiments.[31–34] We demonstrated its potential in helping patients to predict the fate of their lenses when exposed to cataract risks. In our earlier studies, we first confirmed in the laboratory that the DLS can detect early changes in lens crystallin during cold cataract formation in calf lenses, detecting lens protein changes much earlier than Scheimpflug slit-lamp lens imaging.[31–35] Next, in a clinical cross-sectional study[36] of 380 eyes from 235 patients 7 to 86 years of age with lens nuclear opacities ranging from clear to opaque (Age-Related Eye Disease Study [AREDS] lens grades, 0–3.8), we found that there was a corresponding loss of α-crystallin, as estimated by the α-crystallin index (ACI) obtained from the DLS device, which was associated with increasing lens nuclear opacity ($P < 0.0001$). High values of ACI, indicating high levels of unbound α-crystallin, are associated with lower risk for cataract. In the current study, we conducted a longitudinal analysis to determine whether α-crystallin levels decreases over time, whether lens opacification is associated with the decline in α-crystallin, and whether α-crystallin decrease leads to cataract surgery.

Methods

We conducted a natural history and cohort study of patients 30 years of age and older seeking treatment at the Stark-Mosher Center for Cataract and Corneal Disease of the Wilmer Eye Institute of Johns Hopkins Hospital in Baltimore, Maryland. Excluded were patients who had tear film disorders, corneal opacities or disorders, uveitis, or glaucoma or those who had difficulty fixating, any adverse reaction to dilating drops, or an inability to return for follow-up visits. The study was approved by the Johns Hopkins Medical Institutions Intramural Research Board and complied with the Health Insurance Portability and Accountability Act. All tenets of the Declaration of Helsinki were followed, and all patients gave written informed consent.

All patients underwent a comprehensive dilated eye examination at baseline and then every 6 months, including slit-lamp grading of lenses using the AREDS clinical lens grading system (using a Haag Streit BM 900 slit lamp; Haag Streit, Koeniz, Switzerland) and measurement of the ACI using the National Aeronautics and Space Administration–National Eye Institute DLS device as described previously.[36] The ACI is an estimate of unbound α-crystallin present in the lens nucleus as reported earlier.[36] Unbound α-crystallin is the native molecule (molecular mass, approximately 800 000 daltons) not bound to other partially unfolded proteins. It is computed as the sum of intensities from the first 6 particle intervals of the DLS output (the first peak, representing unbound α-crystallin) divided by the sum of the intensities of all 18 particle size intervals, expressed as a percentage (explained in detail in a previous study[36]).

For statistical analysis, we used SAS software version 9.2 (SAS Inc, Cary, NC) to compare the ACI change over time between subgroups. We first compared the baseline characteristics across ACI categories. P values were obtained from analyses of variance for continuous variables and from chi-square tests for categorical variables. A growth curve model (also known as random effects or mixed model) then was used to compare the nuclear opacity or cataract progression rate between subgroups. To evaluate the relationship between ACI categories and incident cataract surgery, Kaplan-Meier survival analyses was performed. Significance level was $P < 0.05$.

Results

We studied 45 patients (66 eyes) 34 to 79 years of age, 50% of whom were women, with AREDS nuclear lens grades of 0 to 3.0 at baseline (full scale, 0–4). Subjects underwent complete dilated eye examinations including AREDS clinical lens nuclear grading and DLS measurements to obtain the ACI every 6 months for a mean of 19 months (range, 6–36 months). Table 1 shows the demographic data of this patient population.

We divided the population into tertiles based on baseline ACI level: group 1 included lenses with ACI of more than 10.8, group 2 included lenses with ACI between 10.8 and 6.1, and group 3 included lenses with ACI less than 6.1. For each ACI group, we computed the slope of the cataract grade over time.

Figure 1 shows the nuclear cataract progression rate for each of the 3 baseline ACI groups. The rate of lens opacity progression increased with decreasing baseline ACI. The rate of increase in nuclear grade per year was 0.1, 0.2, and 0.4, respectively, for groups 1, 2, and 3 ($P < 0.0001$ for trend). Based on model estimates, for each 10-unit decrease in ACI, there is an associated AREDS scale 1-grade increase in nuclear opacity in 4 years.

Figure 2 shows the Kaplan-Meier survival curves of the cumulative probability of not having cataract surgery, stratified by baseline ACI status. Those with the lowest baseline ACI (group 3 as above) had the highest risk for cataract surgery. Group 3 was statistically significant compared with either groups 1 or 2 ($P = 0.03$ and $P = 0.05$, respectively). There was no significant difference between groups 1 and 2.

Figure 3 shows the changes in ACI related to aging during follow-up studies after dividing the patients by age groups (<50 years, 50–60 years, 60–70 years, and >70 years). Rates of decline in ACI were similar in all age groups, similar to the age-related decrease in ACI reported in our previous cross-sectional study.[36] Slopes were −0.028, −0.991, −0.068, and −0.102 for the group younger than 50 years, 50 to 60 years, 60 to 70 years, and older than 70 years, respectively. Baseline age was associated highly with ACI level ($P < 0.001$).

Figure 4 shows slit-lamp photographs from a 43-year-old woman followed up for 20 months. The right eye initially had an early nuclear cataract, AREDS nuclear grade of 2.2, and ACI of 13.5, and by 20 months, a clinically significant nuclear cataract had