Objective: To examine the associations of the serum cystatin C level and chronic kidney disease with the incidence of age-related macular degeneration (AMD) over 15 years.

Methods: In this population-based cohort study of 4926 individuals aged 43 to 86 years at baseline, 3779 participated in 1 or more follow-up examinations. Age-related macular degeneration was determined by grading photographs of the macula. Individuals were defined as having mild or moderate to severe chronic kidney disease based on a value of more than 45 mL/min/1.73 m² to 60 mL/min/1.73 m² or less and 45 mL/min/1.73 m² or less, respectively, according to the Modification of Diet in Renal Disease Study equation.

Results: While controlling for age and other risk factors, the level of serum cystatin C at baseline was associated with the incidence of early AMD (odds ratio per log standard deviation [95% confidence interval], 1.16 [1.01-1.35]) and exudative AMD (1.42 [1.03-1.96]) but not geographic atrophy (0.89 [0.56-1.41]) or progression of AMD (1.02 [0.88-1.18]). Mild chronic kidney disease was associated with the 15-year cumulative incidence of early AMD (odds ratio per log standard deviation, 1.36 [95% confidence interval, 1.00-1.86]) but not the incidence of other AMD end points.

Conclusion: There is a relationship between the level of serum cystatin C and chronic kidney disease with the incidence of AMD. The underlying biological processes remain to be determined.


THE FINDING OF A RELATIONSHIP OF COMPLEMENT FACTOR H Y402H (1277T → C) GENOTYPE STATUS TO BOTH AGE-RELATED MACULAR DEGENERATION (AMD) AND KIDNEY DISEASE (MEMBRANOPROLIFERATIVE GLomerulonephritis TYPE II AND HEMOLYTIC UREMIC SYNDROME) HAS LED TO SUGGESTION THAT KIDNEY DISEASE MIGHT BE ASSOCIATED WITH AMD.1-16 FURTHERMORE, MANY FACTORS ASSOCIATED WITH CHRONIC KIDNEY DISEASE (CKD) SUCH AS OXIDATIVE STRESS, INFLAMMATION, AND ENDOTHELIAL DYSFUNCTION HAVE BEEN HYPOTHESIZED TO HAVE A ROLE IN THE PATHOGENESIS OF AMD.17-24 HOWEVER, THERE ARE FEW AND INCONSISTENT EPIDEMIOLOGICAL DATA ABOUT THE RELATIONSHIP OF CKD TO AMD.25-27

Serum cystatin C has been used to estimate glomerular filtration rate to define the presence of CKD.28,29 It is abundant in retinal pigment epithelium (RPE) cells and has been hypothesized through its effects on cathepsins to have a role in the pathogenesis of AMD.30,31 To our knowledge, no population-based epidemiologic studies have examined the association of the level of serum cystatin C with AMD. In the present study, we examined the relationship of serum blood urea nitrogen (BUN), cystatin C, gross proteinuria, and CKD to the incidence and progression of AMD during 15 years of follow-up in the Beaver Dam Eye Study.

METHODS

POPULATION

Methods used to identify and describe the population have been described previously.32-36 In brief, a private census of the population of Beaver Dam, Wisconsin, was carried out from September 15, 1987, to May 4, 1988, to identify all residents in the city or township of Beaver Dam who were 43 through 84 years of age. Of the 5924 eligible individuals, 4926 participated in the baseline examination between March 1, 1988, and September 14, 1990. Of the 3334 surviving participants in the baseline and second examinations, 2764 (82.9%) participated in the 10-year follow-up examination from March 1, 1998, through June 9, 2000.35 Of the 2480 surviving partici-
pants who were examined at the baseline and 5- and 10-year follow-up examinations, 2119 (85.4%) participated in the 15-year follow-up examination (March 31, 2003, through April 30, 2005). Comparisons between participants and nonparticipants at the baseline and 5-, 10, and 15-year follow-up examinations are reported elsewhere. Adjusting for age, sex, and other risk factors, individuals with higher levels of serum cystatin C were more likely to die (data not shown). Severity of AMD did not affect the relationship of the serum cystatin C level to participation or death (data not shown).

PROcedures AND DEFINITIONS

Similar procedures, used at both the baseline and follow-up examinations, are described in detail elsewhere. Informed consent was obtained from each participant at the beginning of the examination. Pertinent parts of the examination at both baseline and follow-up consisted of measurement of height and weight and taking of stereoscopic color fundus photographs centered on the macula (Diabetic Retinopathy Study standard field 2). The Wisconsin Age-Related Maculopathy Grading System was used to assess the presence and severity of lesions associated with AMD. Grading procedures, lesion descriptions, and detailed definitions of the presence and severity of specific AMD lesions have been reported elsewhere.

Early AMD was defined by the presence or absence of soft indistinct drusen or any type of drusen associated with RPE depigmentation or increased retinal pigment. Incidence of early and late AMD and component lesions was defined by their presence at follow-up in either eye when absent at the previous examination. For each eye, a 6-level severity scale for AMD has been defined elsewhere. In brief, level 1 is equivalent to having no AMD, levels 2 through 4 indicate lesions defining early AMD of increasing severity (by type, size, area of drusen, and presence of pigmentary abnormalities), and levels 5 and 6 indicate lesions defining late AMD. Progression of AMD was defined as an increase in severity in either eye by 2 steps or more from levels 1 through 3 and 1 step or more from level 4 or 5 on the following AMD severity scale:

- Level 1: no drusen or only hard drusen or small soft drusen (<125 µm in diameter) regardless of the area of involvement, and no pigmentary abnormality (increased retinal pigment or RPE depigmentation)
- Level 2: hard drusen or small soft drusen (<125 µm in diameter) regardless of the area of involvement, with increased retinal pigment but no RPE depigmentation; or soft drusen (≥125 µm in diameter), with drusen area smaller than 196 350 µm² (equivalent to a circle with a diameter of 300 µm) and no pigmentary abnormalities
- Level 3: soft drusen (≥125 µm in diameter), with drusen area smaller than 196 350 µm² and RPE depigmentation present; or soft drusen (≥125 µm in diameter), with drusen area 196 350 µm² or larger with or without increased retinal pigment but no RPE depigmentation
- Level 4: soft drusen (≥125 µm in diameter), with drusen area 196 350 µm² or larger and RPE depigmentation with or without increased retinal pigment
- Level 5: geographic atrophy in the absence of exudative macular degeneration
- Level 6: exudative macular degeneration with or without geographic atrophy

Casual blood specimens were obtained at each examination. An aliquot of blood was used immediately for determination of the white blood cell count. Serum BUN was measured using a colorimetric method (the Berthelot reaction) on an automatic analyzer (RA-1000 AutoAnalyzer; Technicon Instruments Corp, Tarrytown, New York). Gross proteinuria concentration was determined using a dipstick on a casual urine sample and defined as 1+ or greater. Remaining serum was stored without preservative at −80°C in cryogenic vials with O-rings for up to 17 years until the vials were shipped on dry ice to the University of Minnesota Fairview Laboratory for the analyses reported herein. Serum creatinine concentration was measured using reflectance spectrophotometry on a chemistry analyzer (Vitros; Ortho Clinical Diagnostics, Inc, Rochester, New York). The laboratory coefficient of variability was 2.2%. The level of serum cystatin C was determined using a nephelometer (BN100; Dade Behring, Inc, Deerfield, Illinois). The interassay precision was determined at 2 control levels: 1.72 mg/L (coefficient of variability, 6.4%) and 0.78 mg/L (coefficient of variability, 9.2%). The glomerular filtration rate from serum creatinine was estimated using the validated MDRD (Modification of Diet in Renal Disease [Study]) prediction equation, which included age, sex, and race/ethnicity. Mild CKD was defined as more than 45 mL/min/1.73 m² to 60 mL/min/1.73 m² or less, and moderate to severe CKD as 45 mL/min/1.73 m² or more using the MDRD equation.

Cigarette smoking status was determined as follows: individuals were classified as nonsmokers if they had smoked fewer than 100 cigarettes in their lifetime, as ex-smokers if they had smoked more than 100 cigarettes in their lifetime but had stopped smoking before the examination, and as current smokers if they had not stopped smoking. Hypertension was defined as a mean systolic blood pressure of 140 mm Hg or higher, a mean diastolic blood pressure of 90 mm Hg or higher, having a history of hypertension, and use of antihypertensive medication at the time of the baseline examination. Individuals were classified as having diabetes if they had a history of diabetes mellitus and were being treated with insulin, oral hypoglycemic agents, or diet modification or had newly diagnosed diabetes at the baseline examination. Body mass index was defined as weight in kilograms divided by height in meters. Current vitamin use was defined at 3 levels: no use, use of multivitamins, or use of other single or combinations of vitamins (eg, vitamin B complex).

STATISTICAL ANALYSES

For statistical analyses, we examined the relationships between serum cystatin C, creatinine, and BUN levels, the presence of gross proteinuria, and the presence of CKD to the incidence of early AMD, exudative AMD, geographic atrophy, and the progression of AMD. Commercially available software (SAS, version 9; SAS Institute, Inc, Cary, North Carolina) was used to analyze the data. Multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated from discrete linear logistic hazard models. These analytical approaches enabled those individuals who were right censored (not seen after the 5- or 10-year examination because of death or nonparticipation) to contribute information to the estimates. Models using time-varying covariates updated at each follow-up examination (ie, for each interval in which an individual participated), the values of the mild CKD at the beginning of the interval were used. Analyses first controlled for age in 4 categories of approximately 10-year bands and for sex. We considered factors related to both the renal risk factors and AMD end points (eg, smoking history, white blood cell count, body mass index, vitamin use, and sedentary lifestyle) as potential confounders at multivariate analyses. Characteristics such as stroke, coronary heart disease, poor general health, and biochemical end points that were not related to both independent renal risk factors and incident AMD end points in our study were excluded as confounders. The distributions of cystatin C, creatinine, and BUN levels were highly skewed; therefore, we modeled these after log-transforming the variables. We present ORs as a deviation increase in the risk factor for continuous measures. Interactions were tested only for cystatin C level and AMD status with CKD status (none or mild to severe).
RESULTS

BASELINE CHARACTERISTICS

Baseline characteristics of participants included and not included in analyses are given in Table 1. Individuals without AMD incidence data were more likely to be older and, while controlling for age, were more likely to be current smokers, have a higher white blood cell count and a higher mean serum cystatin C level, and to have mild or severe CKD, and were less likely to have early AMD or hypertension at baseline compared with participants included in the analyses.

Relationships of serum cystatin C level, serum BUN level, gross proteinuria, and CKD to sex and age are given in Table 2. With the exception of gross proteinuria, all increased with age. After controlling for age, men had higher levels of serum cystatin C and BUN levels compared with women (P < .001 for all associations). Also, men exhibited a higher prevalence of proteinuria (P < .001) and a lower prevalence of CKD (P = .001) compared with women.

CUMULATIVE INCIDENCE OF AMD

Of individuals at risk, 392 of 2825 developed incident early AMD, 63 of 3503 developed incident exudative AMD, 39 of 3489 developed geographic atrophy, and 400 of 3505 demonstrated progression of AMD. The 15-year cumulative incidence of early AMD was 14.3%; pure geographic atrophy, 1.3%; exudative AMD, 2.0%; and progression of AMD, 12.2%. The prevalence and 15-year cumulative incidence and progression of AMD were similar in men and women and increased with age (data not shown).40-42

BLOOD UREA NITROGEN LEVEL, SERUM CYSTATIN C LEVEL, GROSS PROTEINURIA, CKD, AND INCIDENCE OF AMD

While controlling for age and sex, smoking status, white blood cell count, body mass index, and history of vitamin use, BUN level, and the presence of gross proteinuria were not associated with the 15-year cumulative incidence of early AMD, exudative AMD, pure geographic atrophy, or progression of AMD (Table 3). Mild CKD
was associated with the 15-year cumulative incidence of early AMD but not the incidence of exudative AMD and pure geographic atrophy or progression of AMD (Table 3). Using time-dependent covariate analyses, we found a similar relation of mild CKD with the 5-year incidence of early AMD (OR [95% CI], 1.35 [1.01-1.81]; P=.04). For the first period (baseline to 5-year follow-up, n=272), in individuals with mild CKD at baseline and at risk of developing AMD, these values were 1.63 (95% CI [1.08-2.46]; P=.02), for the second period (5- to 10-year follow-up, n=177), 1.14 [0.66-1.98]; P=.65); and for the third period (10- to 15-year follow-up, n=120), 0.99 [0.50-1.96]; P=.98). Using time-dependent covariate analyses, we found no relation of mild CKD with the 5-year incidence of exudative AMD and geographic atrophy and the progression of AMD (data not shown).

### Table 2. Relationship Between Serum Cystatin C Level and Markers of Renal Disease, and CKD by Age and Sex at Baseline in the Beaver Dam Eye Study, 1988 to 1990

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Serum Cystatin C Level, mg/L</th>
<th>BUN Level, mg/dL</th>
<th>Gross Proteinuria, No. (%)</th>
<th>CKD, No. (% Mild/Severe)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. Mean (SD)</td>
<td>No. Mean (SD)</td>
<td>Value</td>
<td>No. Mean (SD)</td>
</tr>
<tr>
<td>All individuals</td>
<td>43-54</td>
<td>1183 0.79 (0.15)</td>
<td>1251 15.8 (4.6)</td>
<td>1249 (3.0)</td>
</tr>
<tr>
<td></td>
<td>55-64</td>
<td>983 0.86 (0.17)</td>
<td>1035 17.3 (4.8)</td>
<td>1037 (2.2)</td>
</tr>
<tr>
<td></td>
<td>65-74</td>
<td>865 0.94 (0.22)</td>
<td>919 18.2 (5.2)</td>
<td>916 (14.2/2.7)</td>
</tr>
<tr>
<td></td>
<td>75-86</td>
<td>320 1.11 (0.31)</td>
<td>343 20.5 (7.1)</td>
<td>342 (2.9)</td>
</tr>
<tr>
<td></td>
<td>All ages</td>
<td>3351 0.88 (0.21)</td>
<td>3548 17.3 (5.3)</td>
<td>3547 (3.2)</td>
</tr>
</tbody>
</table>

### Table 3. Multivariate Analyses of Markers of Renal Disease and CKD at Baseline and 15-Year Cumulative Incidence and Progression of AMD in the Beaver Dam Eye Study, 1988 to 2003

<table>
<thead>
<tr>
<th>Variable</th>
<th>Incidence of Early AMD</th>
<th>Incidence of Exudative AMD</th>
<th>Incidence of Pure Geographic Atrophy</th>
<th>Progression of AMD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. at Risk</td>
<td>OR (95% CI)</td>
<td>P</td>
<td>No. at Risk</td>
</tr>
<tr>
<td>Log serum cystatin C per SD</td>
<td>2660 374 1.16 (0.50-2.21)</td>
<td>.04</td>
<td>3294 56 1.42 (0.86-2.34)</td>
<td>.03</td>
</tr>
<tr>
<td>Log BUN per SD</td>
<td>2812 392 0.99 (0.88-1.12)</td>
<td>.92</td>
<td>3486 62 0.90 (0.67-1.20)</td>
<td>.47</td>
</tr>
<tr>
<td>Gross proteinuria</td>
<td>2809 391 0.97 (0.62-1.90)</td>
<td>.92</td>
<td>3483 62 None</td>
<td>.</td>
</tr>
<tr>
<td>MDRD, ml/min/1.73 m²</td>
<td>2799 390</td>
<td></td>
<td>3470 60 None</td>
<td>.</td>
</tr>
</tbody>
</table>

Abbreviations: AMD, age-related macular degeneration; BUN, blood urea nitrogen; CI, confidence interval; CKD, chronic kidney disease; MDRD, Modification of Diet in Renal Disease [Study] equation; OR, odds ratio; SD, standard deviation; ellipsis, not applicable.

**a** Multivariate analysis adjusted for age group, sex, smoking history, white blood cell count, body mass index, and vitamin use.

**b** Multivariate analysis adjusted for age group, sex, smoking history, white blood cell count, body mass index, history of physical activity, and vitamin use.

**c** Multivariate analysis adjusted for age group, sex, smoking history, white blood cell count, body mass index, vitamin use, and baseline AMD severity.

**d** No persons with gross proteinuria at baseline developed late AMD.
Higher levels of serum cystatin C were associated with the 15-year cumulative incidence of early AMD and exudative AMD but not with the incidence of geographic atrophy or progression of AMD (Table 3). While controlling for other risk factors, higher levels of serum cystatin C were associated with the incidence of exudative AMD in individuals without CKD (OR [95% CI], 1.77 [1.09-2.88]; P=.02) but not in those with CKD (1.33 [0.73-2.42]; P=.36; P-interaction=.40), whereas the associations of higher levels of serum cystatin C with early AMD in individuals with CKD (0.93 [0.69-1.24]; P=.62) and those without CKD (1.17 [0.96-1.43]; P=.11; P-interaction=.79) did not reach statistical significance.

COMMENT

In the Beaver Dam Eye Study, when controlling for age, sex, smoking status, and other risk factors, a higher level of serum cystatin C was associated with increased risk of incident early AMD and exudative AMD. The effect was stronger for incident exudative AMD in individuals without CKD compared with those with CKD, which suggests that this relationship might not be due to kidney-related processes. To our knowledge, no other published articles from population-based studies have examined the relationship of the level of serum cystatin C with the incidence of AMD. The underlying reasons for our finding are not known.

Serum cystatin C is ubiquitous in the body, including human RPE.50 Serum cystatin C is an inhibitor of cysteine proteinases (eg, the cathepsins B, L, and S) found in the eye.51-53 It has been hypothesized that cathepsins are involved in the pathogenesis of AMD.54 Data from cell culture and animal studies suggest that cathepsins may maintain homeostasis of the retinal photoreceptors and the extracellular environment of the Bruch membrane, including the release of antiangiogenic endostatins from Bruch membrane collagen XVIII.55-57 Im et al58 hypothesized that high levels of serum cystatin C may have both deleterious and protective effects on the angiogenic response in the eye through its regulation of cathepsins. The deleterious effect of a high serum cystatin C concentration is postulated to be due to its reduction of the protective effect of cathepsins in the release of antineoangiogenic endostatins from Bruch membrane collagen, resulting in higher risk of exudative AMD. This is consistent with our findings. A protective effect of higher cellular levels of serum cystatin C is hypothesized to be due to its suppression of the vascuogenic effect of cathepsins at the level of the endothelium. This is consistent with the fact that B precursor cystatin C, CST3 genotype variant on chromosome 20, thought to result in lower levels of serum cystatin C in the RPE, is related to a higher risk of earlier onset of exudative AMD in human beings.59 However, to our knowledge, no data show that higher levels of serum cystatin C are associated with higher RPE cellular levels of cystatin C and that the latter result in changes in level and function of cathepsins in the aged human retina.

We hypothesized that CKD and its markers, including cystatin C, would be associated with increased risk of incident AMD. This was based on an earlier finding by us that complement factor H Y402H (1277T→C) genotype status is related to both progression of AMD and incidence of CKD.16 We speculated that CKD would be associated through systemic inflammation and oxidative stress with an increase in the risk of incident AMD. In the Beaver Dam Eye Study, we found a statistically significant relationship of mild CKD with the incidence of early AMD but not with exudative AMD, geographic atrophy, or progression of AMD. This is consistent with data from 2 earlier studies that examined these relationships.20,27 The Blue Mountains Eye Study showed that after adjusting for age, sex, and other risk factors, individuals with moderate CKD are 3 times as likely to develop early AMD compared with individuals with no or mild CKD (OR [95% CI], 3.2 [1.8-5.7]; P<.001).27 When using the same definition of CKD (based on the Cockcroft-Gault equation60) as used in the Blue Mountains Eye Study, while controlling for other factors in a multivariable model, we found a relationship of CKD to incident early AMD (OR [95% CI], 1.53 [1.10-2.11]; P=.01) but not to incident exudative AMD (1.38 [0.68-2.66]; P=.37).

The strengths of our study include its high participation rate, long follow-up, and use of standardized protocols to measure CKD risk factors and AMD end points. However, any conclusions or explanations about associations or lack thereof described herein must be made with caution for several reasons. First, the concomitant low frequency of some risk factors (eg, CKD) and of the incidence of some lesions (eg, pure geographic atrophy) limit our ability to detect (or reject) meaningful relations. Second, some findings that may be of potential biological significance may be entirely owing to chance, given the number of associations examined. Third, it is possible that we did not find significant relationships between some risk factors and AMD because individuals with these factors (eg, CKD) who developed AMD did not live to participate in the follow-up examination. However, nonparticipation because of death is probably unlikely to have biased our findings because AMD was not associated with mortality in the Beaver Dam Eye Study cohort.61 In addition, time-dependent covariate modeling for CKD showed similar strengths of association for CKD as those reported for the 15-year cumulative incidence of these end points. Fourth, there may have been misclassification of CKD. This might be expected to bias the results to the null. Fifth, there may have been uncontrolled confounding.

In conclusion, data from the Beaver Dam Eye Study show an association of the level of serum cystatin C and CKD with the incidence of AMD independent of smoking and other risk factors. Confirmation of these findings in other studies and a better understanding of the biological processes underlying our findings are needed.

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Author Contributions: Dr R. Klein had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES


In 1795, Dr Isaac Thompson concocted an eye water of zinc sulfate, saffron, camphor, and rose water. It was sold as late as 1939. This is 1 of a series of 32 medical trade cards advertising the product from 1875 through 1895.

Courtesy of: Daniel M. Albert, MD, MS.