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Retina

RANTES (CCL5) in Patients With Geographic Atrophy Age-Related Macular Degeneration

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Purpose: A previous study from our research group showed significantly lower levels of RANTES (Regulated upon Activation, Normal T Cell Expressed and Secreted) in patients with intermediate age-related macular degeneration (AMD) compared to control patients with no AMD. The primary aim of this study was to assess levels of RANTES in a cohort of patients with a more advanced form of the disease, geographic atrophy (GA), in comparison with controls.

Methods: The study was conducted on a cohort of patients with GA recruited into a Colorado AMD registry. Cases and controls were defined with multimodal imaging. Plasma levels of the chemokine RANTES were measured using a multiplex assay. A nonparametric (rank-based) regression model was fit to RANTES with a sex by AMD category interaction.

Results: The plasma levels of RANTES were significantly higher in the control group in comparison to the GA AMD group (median [interquartile range]): 10,204 [5799–19,554] pg/mL vs. 5435 [3420–9177] pg/mL, respectively, $P < 0.01$). When moderated by sex, there was no statistical difference between the male and female GA AMD or the male and female controls.

Conclusions: We found lower level of RANTES in patients with GA AMD compared with controls. This finding is consistent with the findings from our previous intermediate AMD study. However, in contrast to the results of our previous research, when moderated by sex there was no statistical difference between male and female GA patients.

Translational Relevance: The biomarker RANTES is significantly lower in GA AMD patients compared to controls.

Introduction

One of the advanced forms of age-related macular degeneration (AMD) is geographic atrophy (GA), affecting five to six million people globally.[1,2] GA is characterized by progressive loss of photoreceptors, retinal pigment epithelium (RPE), and the underlying loss of the choriocapillaris. These pathological events result in a sharply demarcated atrophic lesions of the outer retina, that eventually lead to irreversible vision loss.[1,2] The pathophysiology of RPE cell atrophy with accompanying atrophy of the outer neurosensory retinal layers and choriocapillaris remains unclear. There are currently no approved treatments to prevent, reduce, or reverse the rate of GA progression.[2] Several trials are, however, ongoing that involve testing interventions such as stem cell therapies, complement pathway inhibitors, and interventions targeting oxidative stress and inflammation.[1,3–8] Identifying systemic and retinal biomarkers associated with GA onset and progression could lead to earlier detection and more

focused treatment options for this devastating vision-threatening disease.[1]

Several studies have shown that both local[9] and systemic inflammation[10–13] have a role in AMD. In recent times, we have studied the role of the chemokine RANTES (regulated on activation, normal T cell expressed and secreted) in the plasma of patients with the intermediate phenotype of AMD (iAMD).[14,15] RANTES has been associated with acute and chronic phases of inflammation, has been implicated in moderating immune responses, is a chemotactic for eosinophils, basophils, and T-cells, and can activate natural killer cells.[9,16,17] We found that levels of RANTES were significantly lower in patients with iAMD in comparison to controls with no AMD.[14] A second finding of our prior research was that females had a significantly higher levels of RANTES in comparison to males in patients with iAMD.[15]

The goal of this study was to extend our findings in iAMD patients to a cohort of patients with GA to determine (1) whether there are differences in levels of RANTES with GA compared with controls with no AMD and (2) whether there are sex differences in RANTES levels in patients with GA. To our knowledge, sex differences have not been studied before in this subtype of AMD.

Methods

This research study included patients with GA whose samples and records were part of the University of Colorado AMD research repository and registry developed by the Division of Ophthalmic Epidemiology, Department of Ophthalmology at the University of Colorado (details described elsewhere).[18–20] Controls for patients with AMD were individuals after cataract surgery with no AMD. Cases and controls all received eye care at the UCHealth Sue Anschutz-Rogers Eye center. This registry was approved by the Colorado Multiple Institutional Review Board and conforms with the Declaration of Helsinki. Recruitment, informed consent, and inclusion and exclusion criteria are described elsewhere.[18,19] To summarize, each patient in the registry is consented for collection of plasma and serum for biomarker studies, a review of the medical and ocular history, and AMD classification[21] following an assessment of multimodal imaging (autofluorescence, color fundus photography and ophthalmic coherence tomography).[18,19] Inclusion criteria for cases was age between 55 and 99 years, AMD in one or both eyes, and the capacity to provide consent. Exclusion criteria included active inflammatory ocular disease, proliferative and nonproliferative diabetic retinopathy, diabetic macula edema, cystoid macula edema, macula off retinal detachment, central serous retinopathy, full-thickness macular hole, ocular melanoma, pattern or occult macular dystrophy, macular telangiectasia, corneal transplant, drusen not caused by AMD, prior treatment with antivascular endothelial growth factor, panretinal photocoagulation, branch and central retinal vein occlusion, terminal illness, current systemic treatment for cancer, or any serious mental health or advanced dementia issues. Control patients were cataract surgery patients registered one month after cataract surgery, with no evidence of AMD by multimodal imaging review.[18–20,22] The patient's epidemiological and image data were entered into REDCap, a secure web-based Institutional Review Board–approved database.

Image Review

Two vitreoretinal specialists reviewed the images on cases and controls, focusing on the examination of the anatomic macula. Any discrepancies were resolved by a third vitreoretinal specialist. Using the classification described by the Beckman Initiative for Macular Research Classification Committee,[21] images were classified into early, iAMD, or advanced (neovascular or GA) AMD. GA was further described using the complete RPE and outer retinal atrophy per the Classification of Atrophy Meeting classification.[23,24] Complete RPE and outer retinal atrophy are defined by the following criteria: (i) a region of choroidal hyper transmission of 250 mm or more in diameter; (ii) a zone of attenuation or disruption of the RPE of 250 mm or more in diameter; and (iii) evidence of overlying photoreceptor degeneration, all occurring in the absence of signs of an RPE tear.[23,24] An example of the images used for classification are shown in Figure 1.

Collection and Processing Blood Samples

We used plasma samples for this study. We spun the plasma ethylenediaminetetraacetic acid tube at 3000 rpm for 10 minutes in a chilled (4°C) centrifuge. After pipetting into aliquots, the plasma was immediately stored in a −80°C freezer. The plasma aliquots were then transferred to the laboratory for measurement of RANTES.[15]

Measurement of Plasma RANTES

The Clinical Translational Core laboratory, located at the Children's Hospital Colorado measured the

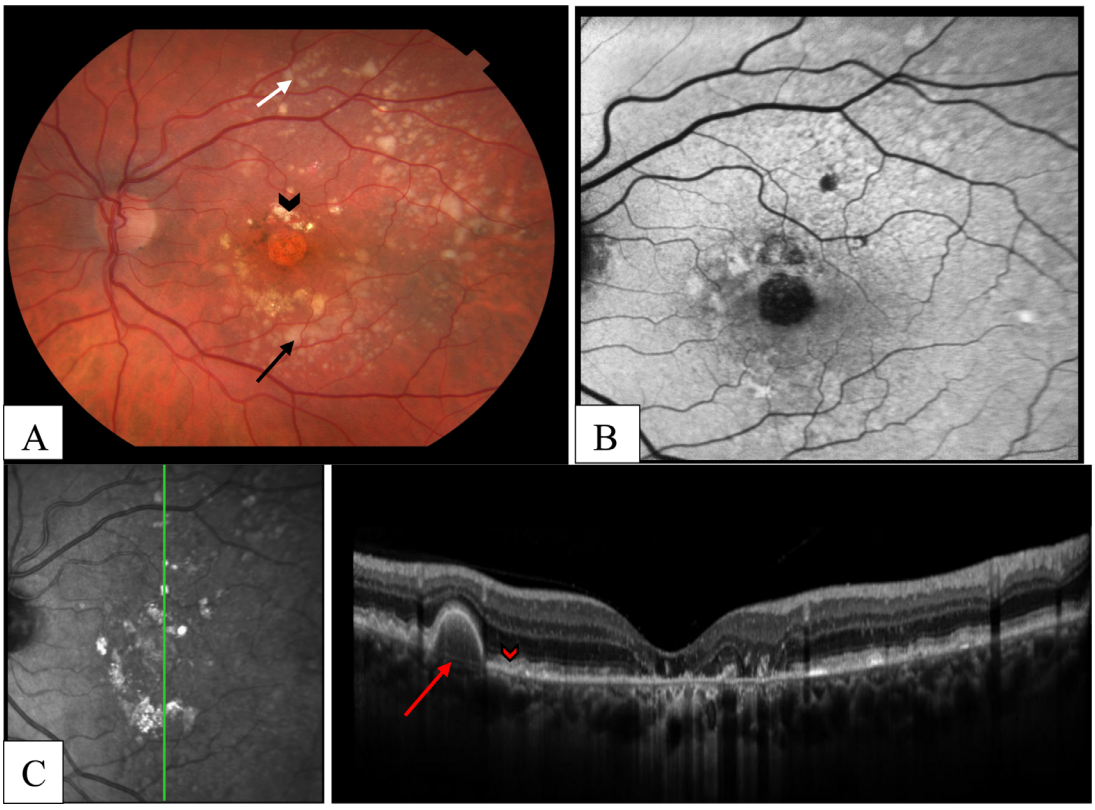

Figure 1. Images of a patient consistent with GA AMD. (A) Color fundus showing hard drusen (*white arrow*), soft drusen (*black arrow*), calcified drusen (*arrowhead*), and pigmentary changes. (B) Fundus autofluorescence showing drusen and geographic atrophy. (C) Optical coherence tomography showing drusen, pigment migration (*arrowhead*), and drusenoid pigment epithelial detachment (*red arrow*).

RANTES levels. Using multiplex kits produced by the R&D Systems that use color-coded microparticles coated with analyte-specific antibodies that are analyzed on dual-laser suspension array platforms, multiplex assays were completed. Using a magnetic bead–based multiplex method and read on a Luminex FlexMap platform, we examined 150 µL of plasma. All samples were performed in duplicate and had an acceptance threshold coefficient of variance of less than 15%.[15]

Statistical Analysis

When comparing patient characteristics between groups, a two-sample *t*-test was used for continuous variables and a χ^2 test or Fisher's exact test for categorical variables when indicated. RANTES with a sex by AMD category interaction was fitted using a regression model using a rank transformation for the RANTES outcome. Least square means were used to analyze pairwise comparisons, including GA cases versus control, male versus female cases, male versus female controls, male cases versus female cases, and female controls versus female cases. Furthermore, a sensitivity analysis was performed, adjusting for age and batch effect. SAS version 9.4 (The SAS Institute, Cary, NC, USA) was used to perform all analysis.

Results

The Table shows differences in the demographic characteristics and select comorbidities between controls (n = 128) and the GA AMD group (n = 104). This cohort included 83 females in the control group and 56 in the GA AMD group. As expected, GA AMD patients had significantly higher mean age and higher rates of family history of AMD in comparison to controls, $P < 0.01$ for both. Furthermore, the GA AMD group had higher rates of treated hypertension and cardiac disease in comparison to controls. This is in line with existing literature, describing late-stage AMD associated with hypertension and cardiovascular disease.[25]

Table. Differences in Clinical Characteristics Across Ferris Groups

	Clean Controls (n = 128)	GA AMD (n = 104)	*P* Value
Sex, female	83 (65%)	56 (54%)	0.09
Family history of AMD			<0.01*
None	99 (77%)	55 (53%)	
Yes	23 (18%)	35 (34%)	
Uncertain	6 (5%)	14 (13%)	
Age, mean (SD)	74.3 (4.7)	81.9 (7.1)	<0.01
Body mass index, mean (SD)	27.6 (5.8) n = 142	26.6 (4.9) n = 90	0.21
Smoking			0.24*
Never	66 (52%)	43 (41%)	
Current	1 (1%)	2 (2%)	
Former	61 (48%)	59 (57%)	
History of			
Treated hypertension	70 (55%)	77 (74%)	<0.01
Kidney disease	13 (10%)	20 (19%)	0.05
Peripheral vascular disease	23 (18%)	11 (11%)	0.09
Cardiac disease	37 (29%)	45 (43%)	0.02
Autoimmune disorders	17 (13%)	10 (10%)	0.39
History of Infectious Disease	7 (5%)	4 (4%)	0.76*
Patient report current medication use			
Aspirin	60 (47%)	51 (49%)	0.74
NSAIDS	23 (18%)	16 (15%)	0.60

P values obtained from χ^2 for categorical variables and *t*-test for continuous variables unless noted otherwise.
**P* value calculated from Fisher's exact test.

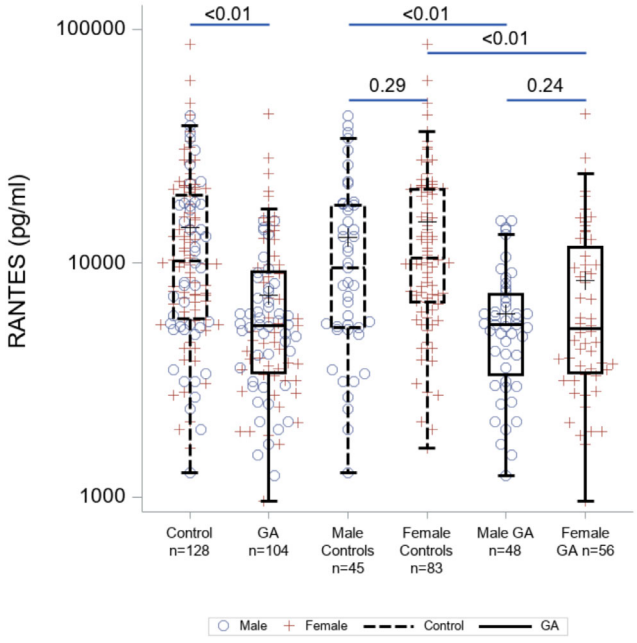

Figure 2. Boxplot showing the RANTES levels for GA AMD patients and controls stratified by sex. The *box* extends to the 25th and 75th percentiles, the *line* represents the median. Individual colored *circles/plus points* illustrate the observed values. *Y-axis tick marks* are displayed on a log (10) scale. Male patients are represented as *blue circles* and female patients are represented as *red plus signs*.

In Figure 2, we illustrate RANTES levels for controls and GA AMD cases. Significantly higher level of RANTES were found in controls compared with cases of GA (median [interquartile range]: 10,204 (5799–19,554] pg/mL vs. 5435 [3420–9177] pg/mL, respectively; $P < 0.01$). When moderated by sex, although the lower levels in GA compared to controls remained after stratifying by sex, there was no difference in the median levels between male and female GA patients. The results remain unchanged after adjusting for age and potential laboratory batch effects.

Discussion

We describe plasma levels of RANTES in the study among GA AMD patients in comparison to controls with no AMD, stratified by sex in a cohort of patients who were part of the University of Colorado AMD registry. In this cohort, the levels of RANTES were significantly lower in patients with GA compared with controls with no AMD. There were no significant differences between the male and female GA patients.

Levels of RANTES have been studied in several diseases, including age-related neurodegenerative

diseases such as Parkinson's,[26] cardiovascular,[27–32] and autoimmune diseases.[33,34] In a previous study from our group, we demonstrated higher levels of RANTES in controls compared to iAMD, even when stratified by sex.[15] This study followed our prior study that found that RANTES levels differed between iAMD cases and controls to determine whether differences were also present in advanced AMD cases. Aligned with the results of our previous study, levels of plasma RANTES were significantly higher in controls compared to cases with GA; however, in this study we found no differences in RANTES levels among GA cases by sex. The median and interquartile range of RANTES was 10,204 (5799–19,554) pg/mL in the control group versus 5435 (3420–9177) pg/mL in the GA group and 5479 (3140-11,067) in the iAMD group. Thus, in our cohort, RANTES is significantly lower in patients with iAMD, as well as patients with GA in comparison to controls.

Our results are in contrast to findings from study by Nielsen et al.,[9] which reports a higher level of plasma RANTES in the GA phenotype of AMD compared to controls.[9] The reasons for this conflicting results are unclear. One possibility is differences in the patient demographics. In the Nielson study, there was no significant difference in age between controls and cases.[9] In contrast, patients with GA were older in our study, although this difference was still present after adjusting for age. In our study, patients with GA had significantly higher rates of treated hypertension, kidney disease, and heart disease in comparison to controls. There was no difference in patient characteristics in the study by Nielsen et al.[9] This suggests that GA patients in our cohort might have poor health, which might affect the levels of plasma RANTES. Further study between the interaction of this chemokine and its receptor is needed understand these mechanisms.[14]

In nonocular pathologies other authors have reported lower levels of RANTES. Cavusoglu et al.[35] reported that a lower baseline RANTES was independently associated with increased risk of cardiac death at two-year follow up in male patients who were referred for coronary angiography. Rothenbacher et al.[36] also showed decreased levels of serum RANTES in patients with coronary artery disease in comparison to age- and sex matched controls. Although the role of RANTES is not fully understood, there are several proposed mechanisms to explain the lower levels of RANTES in some pathologies. One proposed mechanism is that low RANTES levels may cause the upregulation of CCR5 receptor.[35,37] Another suggested mechanism is that RANTES is deposited on the vascular endothelium in cardiovascular disease, leading to increased CCR5 stimulation.[35,38] More studies are needed to elucidate the role of RANTES in age-related pathologies.

There are well-established sex differences in the presentation and prevalence of several systemic and ocular pathologies.[39–43] Thus incorporating sex (being male or female) as a biological variable is crucial in study design, analysis, and reporting of study results. Failure to do so may obscure important sex-related outcomes.[44] Fonteh et al.[15] showed novel sex differences in the plasma levels of RANTES in patients with iAMD, with female iAMD patients having significantly higher levels of RANTES compared to male iAMD patients. In this follow-up study with GA patients, there were sex difference within the GA cases. As comprehensively reviewed by Hagg and Jylhava,[45] there are clear sex differences in aging between men and women, with women having a better maintenance of molecular and cellular mechanisms of aging. However, after menopause, the cellular and molecular mechanisms in women seem to catch up and reach similar levels of aging in men.[45] This could explain why significant sex differences in RANTES were found in our previously research iAMD cohort,[15] but not in the current older GA cohort. As AMD progresses from iAMD to GA, the patients are typically older, and it is possible that the sex differences in aging mechanisms and RANTES in the female AMD patients dissipate with increasing age.

Single-point measurement of the biomarker is one of the limitations in this study. A relatively small sample size is the second limitation. A third limitation is that sex-related risk factors such as hormonal status of the patients, which has been reported by several authors to have a role in aging and the immune system,[46] were not measured in this study. There is ongoing recruitment into our registry, and, with future studies, we intend to include other systemic biomarkers/hormone levels and additional risk factors.

To conclude, we demonstrated that GA patients had significantly lower levels of plasma RANTES in comparison to controls, and there were no significant differences when stratified by sex. In future studies, we intend to examine levels of RANTES collected over time and moderated by sex in a larger cohort. Our understanding of the role of inflammatory makers as AMD progresses in both male and female patients can direct future therapies in managing this visually threatening eye disease.

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