Elevated Retinol Binding Protein 3 Concentrations Are Associated With Decreased Vitreous Inflammatory Cytokines, VEGF, and Progression of Diabetic Retinopathy

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OBJECTIVE

To correlate inflammatory cytokines and vascular endothelial growth factor (VEGF) in vitreous and plasma with vitreous retinol binding protein 3 (RBP3), diabetic retinopathy (DR) severity, and DR worsening in a population with type 1 and type 2 diabetes.

RESEARCH DESIGN AND METHODS

RBP3, VEGF, and inflammatory cytokines were measured in plasma and vitreous samples (n = 205) from subjects of the Joslin Medalist Study and Beetham Eye Institute.

RESULTS

Higher vitreous RBP3 concentrations were associated with less severe DR ($P < 0.0001$) and a reduced risk of developing proliferative DR (PDR) ($P < 0.0001$). Higher RBP3 correlated with increased photoreceptor segment thickness and lower vitreous interleukin-12 (IL-12), tumor necrosis factor-α (TNF-α), and TNF-β ($P < 0.05$). PDR was associated with lower vitreous interferon-γ and IL-10 and higher VEGF, IL-6, and IL-15 ($P < 0.05$), but was not associated with their plasma concentrations.

CONCLUSIONS

Higher vitreous RBP3 concentrations are associated with less severe DR and slower rates of progression to PDR, supporting its potential as a biomarker and therapeutic agent for preventing DR worsening, possibly by lowering retinal VEGF and inflammatory cytokines.

Findings from the Joslin Medalist Study cohort, composed of individuals who have had insulin-dependent diabetes for ≥50 years, provided strong support that protective factors exist that can reduce the development of advanced diabetic retinopathy (DR) (1–3). Proteomic analysis of cadaveric eyes from Medalists showed that levels of retinol binding protein 3 (RBP3) were significantly higher in both retina and vitreous, with no to mild DR compared with those with advanced DR, and may inhibit glucose uptake and inflammation in the retina of rodent models of DR (1). RBP3 is produced in the photoreceptors with the critical function of binding and transporting *cis*/*trans*-retinols, chemicals involved in capturing light, between

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photoreceptors and retinal pigment epithelium. This study correlated inflammatory cytokines and vascular epidermal growth factor (VEGF) in the vitreous and plasma from the same individual with vitreous RBP3, DR severity and worsening, and retinal layer thickness in a large cohort with diabetes.

RESEARCH DESIGN AND METHODS

We performed an observational study of all available postmortem vitreous samples from U.S. residents with at least 50 years of insulin-dependent diabetes who participated in the Medalist Study and vitreous samples collected from individuals with type 1 and type 2 diabetes who underwent vitreoretinal surgery between May 2018 and May 2021 at the Beetham Eye Institute (BEI) of the Joslin Diabetes Center. The Joslin institutional review board approved the study, and informed consent was obtained from all participants. Postmortem and surgical vitreous samples were analyzed together. Plasma samples from the same individuals were collected. Longitudinal data on retinopathy progression was obtained through chart review and eye examinations from the BEI. Proliferative diabetic retinopathy (PDR) was defined as Early Treatment Diabetic Retinopathy Study severity level >53. Neural retinal layer thicknesses in the foveal area were obtained from optical coherence tomography (SPECTRALIS; Heidelberg Engineering, Heidelberg, Germany) images using automated layer segmentation software as previously described (4). Inflammatory markers were measured in plasma and vitreous samples using the Q-Plex Human Cytokine HS Screen kit (Quidel, San Diego, CA). Vitreous RBP3 concentration was measured by ELISA as previously described (1). Plasma and vitreous VEGF concentrations also were assessed by ELISA (R&D Systems).

RESULTS

Clinical Characteristics

Patient characteristics (N = 165) for 205 vitreous samples are shown in Supplementary Table 1. DR severity distribution in eyes with type 1 diabetes was 29.8% no to mild DR, 7.8% moderate or severe DR, and 62.4% PDR. In eyes from individuals with type 2 diabetes, DR severity distribution was 22.6% no to mild DR, 9.7% moderate or severe DR, and 67.7% PDR.

RBP3 Association With DR Severity

Increased vitreous RBP3 concentrations were associated with less severe DR ($P < 0.0001$) (Fig. 1*A*). RBP3 concentrations in eyes with no to mild DR were increased compared with those with moderate to severe DR ($P < 0.01$), active PDR, and quiescent PDR ($P < 0.0001$). A higher vitreous RBP3/VEGF ratio, a possible index of protective capacity (1), was associated with less severe DR ($P < 0.01$) (Supplementary Fig. 1).

Correlating RBP3 With DR Progression and Photoreceptor Segment Thickness

Analysis of DR progression in Medalists with longitudinal follow-up showed that higher RBP3 concentrations were associated with a reduced risk of PDR development over time ($P < 0.0001$) (Fig. 1*B*). The majority of eyes (82%) with high RBP3 concentration (>20 nmol/L) remained free of PDR, while only two eyes with high RBP3 concentrations progressed to PDR, which occurred after 70 years of diabetes duration. However, >90% of eyes with low RBP3 concentration (<10 nmol/L), developed PDR in the first 30–40 years of diabetes duration. In a cohort of 15 Medalist eyes, higher vitreous RBP3 concentration was associated with increased photoreceptor segment layer thickness (P = 0.04) (Fig. 1*C*).

Associations With Vascular Complications and Systemic Factors

Higher vitreous RBP3 concentrations correlated with younger age at diagnosis (P = 0.02). They also were associated with lower albumin-to-creatinine ratio (P = 0.01), higher diabetes duration ($P < 0.0001$), older age (P = 0.01), and the absence of panretinal photocoagulation (P = 0.0002) (Supplementary Table 2).

Inflammatory Markers and DR

Inflammatory markers were assessed in 198 paired vitreous and plasma samples from the same individual (Supplementary Table 3). Presence of PDR was associated with lower interferon-γ (IFN-γ) (P = 0.001) and interleukin-10 (IL-10) (P = 0.01) and higher IL-6 (P = 0.02) and IL-15 in the vitreous (P = 0.01) but was not associated with these markers' plasma concentrations (Supplementary Fig. 2). No relationships were observed between vitreous and plasma inflammatory markers from the same individual. VEGF concentration in circulation was not associated with vitreous VEGF or RBP3 concentration or the presence of PDR (Supplementary Fig. 3). A higher concentration of RBP3 was associated with lower IL-12 (P = 0.03), tumor necrosis factor-α (TNF-α) (P = 0.02), and TNF-β (P = 0.04) in the vitreous (Fig. 1*D*).

CONCLUSIONS

In this study, the findings showed that high concentrations of vitreous RBP3 in people with type 1 and 2 diabetes are associated with less severe DR and slower rates of progression to PDR, supporting the potential of RBP3 as a biomarker and therapeutic agent for the prevention of worsening DR. The association of decreased RBP3 concentrations with thinning of the photoreceptor layer is consistent with the fact that RBP3 is primarily expressed in the rods and cones, which are reported to decrease with increasing DR severity (1,5,6). It is possible that panretinal photocoagulation decreased RBP3 content in some eyes because of the loss of photoreceptors in the peripheral retina. It is unlikely that the reduction of RBP3 in eyes with PDR is wholly related to photocoagulation since reduced RBP3 concentrations were also present in eyes with moderate to severe and active PDR without panretinal photocoagulation. Furthermore, RBP3 concentrations were decreased in the retina of diabetic rodents that had not undergone laser treatment (1).

This study demonstrates that higher RBP3 concentration was associated with less risk and delayed onset of PDR. These findings and previous reports suggest that preservation of RBP3 expression by photoreceptors might protect the neuroretina and vascular retina from diabetes-induced retinal pathology (7–11). Our findings suggest that persistent high concentrations of RBP3 are associated with protection from severe DR. This is consistent with our previous report that individuals who do not develop advanced DR after 20 years of diabetes are not likely to progress even after ≥50 years of follow-up (2).

The mechanism of retinal protective actions could be related to anti-inflammatory actions of RBP3 since it correlated inversely with levels of TNF-α and TNF-β. Previously, we reported that RBP3 reduced glucose uptake in Müller cells and decrease the expressions of

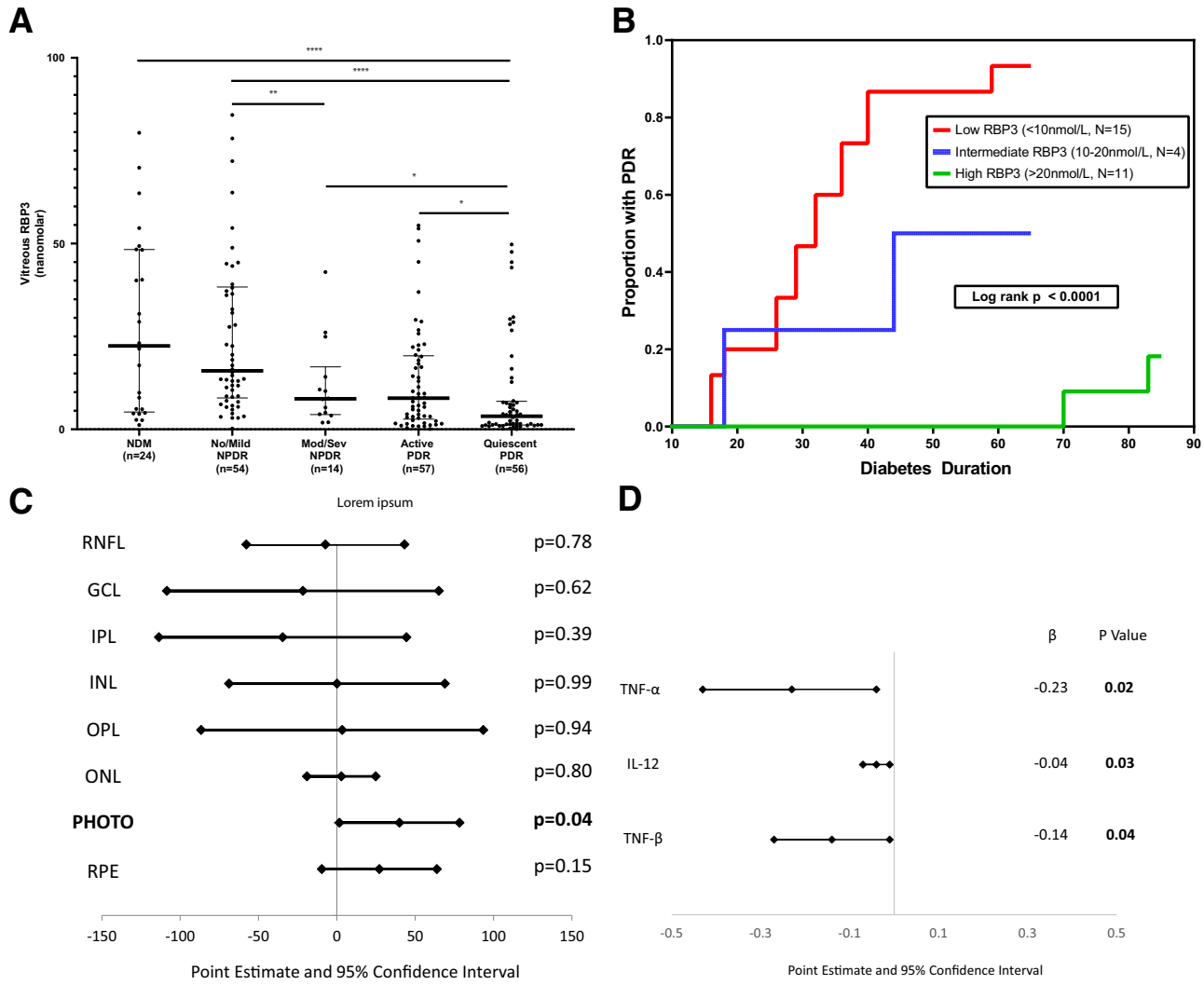

Figure 1—Vitreous RBP3 is associated with DR severity (*A*), decreased and slower progression to PDR (*B*), photoreceptor segment thickness (*C*), and vitreous TNF-α, TNF-β, and IL-12 (*D*) in type 1 and type 2 diabetes. *A*: Analysis by ELISA of vitreous RBP3 concentration in individuals without diabetes and with type 1 and type 2 diabetes and no to mild nonproliferative DR (NPDR), moderate to severe (Mod/Sev) NPDR, active PDR, and quiescent PDR. Each dot represents one eye. Data are median ± interquartile range for each DR severity group. ANOVA was used for DR severity group comparison. *B*: A proportion of the study population with PDR is plotted against diabetes duration. Vitreous RBP3 concentration groups are indicated in the key. Log-rank test was used to compare differences in survival distributions between groups. *C*: Forest plot showing point estimates and 95% CIs for the associations between vitreous RBP3 concentration and individual retinal layer thicknesses (n = 15). Associations were examined using generalized estimating equations with unstructured correlation matrix. *D*: Forest plot showing point estimates and 95% CIs demonstrating associations between vitreous RBP3 and vitreous TNF-α, and TNF-β, and IL-12. Associations were examined using generalized estimating equations with unstructured correlation matrix. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; NDM, nondiabetes mellitus; ONL, outer nuclear layer; OPL, outer plexiform layer; PHOTO, photoreceptor segment layer; RNFL, retinal nerve fiber layer. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

VEGF and TNF-α (1). Interestingly, anti-inflammatory cytokine IL-10 correlated with both PDR (P = 0.01) and RBP3 (P = 0.09), suggesting that RBP3 may also regulate the expressions of anti-inflammatory cytokines, although no significant correlation between concentrations of VEGF and inflammatory markers in the vitreous or between systemic and vitreous concentrations of VEGF, TNF-α and TNF-β, and other inflammatory markers were found. Systemic VEGF and inflammatory cytokine concentrations were not associated with severity of DR. Thus, circulating inflammatory markers, including TNF and VEGF, may not be reflective of retinal pathology in diabetes because of the much greater contribution of these factors from nonretinal tissues relative to the retina (12–15).

Limitations to this study include that RBP3 measurements in the Medalists were not taken longitudinally and often after DR worsening had already occurred. Furthermore, changes in vitreous RBP3 concentrations with diabetic macular edema, the integrity of the ellipsoid zone, and retinal procedures such as anti-VEGF therapy, laser photocoagulation, and vitrectomy need to be determined.

Preservation of the retinal-specific neuroprotein RBP3 is associated with less severe DR and reduced progression to PDR in type 1 and 2 diabetes. RBP3 may protect against DR progression by decreasing inflammatory cytokines and inhibiting the actions of VEGF in the retina. These findings support RBP3 as a potential diagnostic marker and a

therapeutic approach for preventing or delaying progression of DR.

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References

1. Yokomizo H, Maeda Y, Park K, et al. Retinol binding protein 3 is increased in the retina of patients with diabetes resistant to diabetic retinopathy. Sci Transl Med 2019;11:499
2. Sun JK, Keenan HA, Cavallerano JD, et al. Protection from retinopathy and other complications in patients with type 1 diabetes of extreme duration: the Joslin 50-Year Medalist Study. Diabetes Care 2011;34:968–974
3. Keenan HA, Costacou T, Sun JK, et al. Clinical factors associated with resistance to microvascular complications in diabetic patients of extreme disease duration: the 50-Year Medalist Study. Diabetes Care 2007;30:1995–1997
4. Fickweiler W, Wolfson EA, Paniagua SM, et al. Association of cognitive function and retinal neural and vascular structure in type 1 diabetes. J Clin Endocrinol Metab 2021;106:1139–1149
5. Garcia-Ramírez M, Hernández C, Villarroel M, et al. Interphotoreceptor retinoid-binding protein (IRBP) is downregulated at early stages of diabetic retinopathy. Diabetologia 2009;52:2633–2641
6. Lammer J, Prager SG, Cheney MC, et al. Cone photoreceptor irregularity on adaptive optics scanning laser ophthalmoscopy correlates with severity of diabetic retinopathy and macular edema. Invest Ophthalmol Vis Sci 2016;57:6624–6632
7. Antonetti DA, Klein R, Gardner TW. Diabetic retinopathy. N Engl J Med 2012;366:1227–1239
8. Fortmann SD, Grant MB. Molecular mechanisms of retinal ischemia. Curr Opin Physiol 2019;7:41–48
9. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–1625
10. Simó R, Stitt AW, Gardner TW. Neurodegeneration in diabetic retinopathy: does it really matter? Diabetologia 2018;61:1902–1912
11. Duh EJ, Sun JK, Stitt AW. Diabetic retinopathy: current understanding, mechanisms, and treatment strategies. JCI Insight 2017;2:e93751
12. Fickweiler W, Aiello LP, Sun JK, King GL. Retinol binding protein 3 as biomarker for diabetic retinopathy. Ann Transl Med 2019;7:706
13. Tang J, Kern TS. Inflammation in diabetic retinopathy. Prog Retin Eye Res 2011;30:343–358
14. Vujosevic S, Simó R. Local and systemic inflammatory biomarkers of diabetic retinopathy: an integrative approach. Invest Ophthalmol Vis Sci 2017;58:BIO68–BIO75
15. Klein BE, Knudtson MD, Tsai MY, Klein R. The relation of markers of inflammation and endothelial dysfunction to the prevalence and progression of diabetic retinopathy: Wisconsin Epidemiologic Study of Diabetic Retinopathy. Arch Ophthalmol 2009;127:1175–1182