Glucocorticoid Receptor-α and MKP-1 as Candidate Biomarkers for Treatment Response and Disease Activity in Vogt-Koyanagi-Harada Disease

CRISTHIAN A. URZUA, PING CHEN, BENJAMIN CHAIGNE-DELALANDE, BAOYING LIU, RODRIGO ANGUITA, JULIA GUERRERO, PABLO SABAT, VICTOR VELASQUEZ, H. NIDA SEN, RICHARD W.J. LEE, AND ANNELISE GOECKE

• PURPOSE: To investigate the potential of utilizing the expression of genes for glucocorticoid receptor (GR) and mitogen-activated protein kinase phosphatase-1 (MKP-1) as biomarkers of corticosteroid (CS) refractoriness and disease activity in patients with Vogt-Koyanagi-Harada (VKH) disease.

• DESIGN: Prospective cohort study.

• METHODS: Twenty VKH patients receiving their first cycle of CS treatment in the absence of additional systemic immunosuppressive therapy and a control group of fifteen healthy volunteers were recruited from the University of Chile (Santiago, Chile) and US National Institutes of Health (Bethesda, United States). Intraocular inflammation was clinically quantified at enrolment and all follow-up visits. CS refractoriness was defined as an ocular reactivation of VKH upon CS withdrawal at a daily oral prednisone dose of 10 mg or more. Quantitative Reverse transcription polymerase chain reaction (qRT-PCR) was performed to measure the mRNA levels of the alpha (α) and beta (β) isoforms of GR and MKP-1 in peripheral blood mononuclear cells (PBMC) after in vitro stimulation with either anti-CD3/anti-CD28 antibodies, lipopolysaccharide (LPS), or phytohemagglutinin (PHA), in the presence or absence of dexamethasone (Dex).

• RESULTS: After 6 hours of stimulation in the presence of Dex, PBMC from CS-refractory VKH patients had an impaired elevation in GRα expression ($P = .03$). Furthermore, inactive patients showed a significant Dex-induced upregulation of MKP-1 ($P = .005$).

• CONCLUSIONS: In this pilot study, the expression of GR isoforms and MKP-1 corresponded with patients' clinical response to systemic CS treatment and disease activity, respectively. Hence, these candidate biomarkers have potential clinical utility in the early identification of CS refractoriness and subclinical inflammation in patients with VKH disease. (Am J Ophthalmol 2019;207:319–325. © 2019 Elsevier Inc. All rights reserved.)

VOGT-KOYANAGI-HARADA (VKH) DISEASE IS A multisystem inflammatory disorder that includes the central nervous system and skin and ocular involvement, with a bilateral granulomatous panuveitis characterized by exudative retinal detachments.[1,2] The mainstay of treatment is systemic corticosteroid (CS) therapy initiated as early as possible and continued for at least 6 months.[3–5] However, despite this intensive approach, VKH patients are often refractory to CS therapy.[4,6,7]

Previously, the present group highlighted the importance of early immunomodulatory therapy (IMT) in CS-refractory VKH patients[6] and subsequently proposed a set of clinical predictive factors at diagnosis for CS refractoriness.[6] In addition, it was previously demonstrated in VKH disease that the change in levels of the glucocorticoid receptor (GR) isoforms alpha and beta in peripheral blood mononuclear cells (PBMC) after 2 weeks of treatment was a potential biomarker of CS refractoriness.[8] Furthermore, as true quiescence is also difficult to evaluate clinically by standard ocular examination[9] and as patients with active intraocular inflammation have a potential deficiency in the GC-inducible protein mitogen-activated protein kinase phosphatase-1 (MKP-1) in dendritic cells,[10,11] it was hypothesized that GC-induced MKP-1 could serve as a serum-derived biomarker of disease remission. MKP-1 is a known endogenous anti-inflammatory factor that limits p38 mitogen-activated protein kinase (MAPK) activity, and its impairment has been involved in the molecular

See Accompanying Editorial/Article on page xxx.
Accepted for publication Jun 23, 2019.
From the Department of Ophthalmology (C.A.U., R.A., J.G., P.S., V.V., A.G.) Faculty of Medicine. University of Chile, Santiago, Chile; Facultad de Medicina, Clinica Alemana-Universidad del Desarrollo (C.A.U.), Santiago, Chile; Laboratory of Immunology (C.A.U., P.C., B.C-D., B.L., H.N.S.), National Eye Institute, National Institutes of Health, Bethesda, Maryland, USA; Translational Health Sciences (R.W.J.L.), Faculty of Health Sciences. University of Bristol, Bristol, United Kingdom; and the Moorfields Eye Hospital National Health Service Foundation Trust (R.W.J.L.), London, United Kingdom.

Inquiries to Cristhian A. Urzua, Department of Ophthalmology, Faculty of Medicine, Universidad de Chile, Independencia 1027, Santiago, Chile; e-mail: cristhian.urzua@uchile.cl

0002-9394/$36.00
https://doi.org/10.1016/j.ajo.2019.06.032

mechanisms of inflammatory disorders, such as asthma, psoriasis, and rheumatoid arthritis.[12–14]

Given the widely recognized clinical challenge of minimizing CS use in uveitis patients with sight-threatening disease[15] and the ambition to augment clinical measurements of intraocular inflammation with biological readouts of immune quiescence to guide the use of IMT, the goal of this study was to evaluate candidate biomarkers of VKH CS refractoriness and disease activity using expression of GR isoforms and GC-induced MKP-1, respectively. Accordingly, this study obtained pilot data regarding the sensitivity and specificity of these measurements for the evaluation of ocular manifestations of VKH disease and confirmed that expression of GRα correlated with response to systemic CS therapy and that GC-induced MKP-1 levels discriminated between active and inactive patients.

SUBJECTS AND METHODS

A PROSPECTIVE STUDY OF PATIENTS PRESENTING WITH VKH disease was conducted. The protocol complied with tenets of the Declaration of Helsinki, and all participants gave written informed consent before being included. Ethical approval was obtained from Institutional Review Board (IRB) in involved institutions (Comite Etica Cientifica Hospital Clinico Universidad de Chile IRB, Comite de Etica Cientifico Servicio de Salud Metropolitano Oriente IRB, Chile; and Office of Human Subjects Research, United States).

VKH subjects and healthy controls were recruited from the Department of Ophthalmology, University of Chile (Santiago, Chile) and the Blood Bank of the US National Institutes of Health (Bethesda, Maryland), respectively.

• **INCLUSION CRITERIA:** Adult subjects with a diagnosis of VKH according to the diagnostic criteria revised by the international nomenclature committee in uveitis were recruited. Participants had not received any IMT treatment for at least 1 month.[2] Alternative infectious diagnoses, particularly syphilis and tuberculosis, were ruled out during the initial evaluation. Two patient groups were established: patients with active disease and a recent diagnosis of VKH disease and patients with inactive VKH disease without any evidence of inflammatory activity, either clinically or on ancillary ocular imaging results.

• **EXCLUSION CRITERIA:** Subjects with other systemic autoimmune/inflammatory disorders, cancer, and pregnancy were excluded.

• **CLINICAL EVALUATION:** A comprehensive evaluation that included demographic data, medical and family history, and a complete ophthalmologic examination of the study subjects was performed.

The ophthalmologic evaluation included best-corrected visual acuity, intraocular pressure, slit-lamp biomicroscopy examination, ophthalmoscopy under mydriasis, and ancillary testing, such as fundus fluorescein angiography and ocular coherence tomography (OCT).

For inactive patients, a retrospective review of the medical records from the Uveitis Department database was performed. This included the complete clinical data of patients admitted and followed in the department in a prospective and standardized manner. Additionally, the same clinical evaluation as described above plus indocyanine green angiography (ICG) was performed at enrollment to confirm the absence of disease activity in inactive subjects.

Follow-up visits included a systemic workup for potential CS treatment side effects, including blood pressure, blood glucose levels, complete blood count, and liver function tests.[15]

• **DEFINITION OF CS REFRACTORINESS:** CS refractoriness was defined as a patient with a reactivation with an equivalent dose of prednisolone of 10 mg or more during the first cycle of CS treatment.[16] Reactivation was considered if patients had anterior chamber cells and/or vitreous haze ≥1+ as described by Standardization of Uveitis Nomenclature,[17] or presence of subretinal fluid, serous retinal detachment, or signs compatible with active inflammation on ancillary testing (fundus fluorescein angiography, OCT, ICG).

• **PBMC PREPARATION AND MEASUREMENTS:** Peripheral venous blood samples (30 ml) were extracted from all subjects (VKH patients and healthy controls) in 0.5 M EDTA tubes at pH 8. PBMC were isolated by a Ficoll density gradient centrifugation protocol (Histopaque, Sigma Diagnostic, St Louis, Missouri).

PBMC were cultured in complete RPMI medium (Invitrogen, Carlsbad, California) supplemented with 10% fetal bovine serum and penicillin/streptomycin for 3 days, stimulated with 1 μM dexamethasone (Sigma) in the last 6 and 24 hours of culture. Thereafter, cells were transferred to 500 μL RLT buffer (Qiagen, Hilden, Germany), and RNA was isolated using RNeasy microkit (Qiagen) and RNase-free DNase set for genomic DNA digestion. A total of 0.5 μg of the obtained RNA was reverse transcribed. The primers used for the real time quantitative polymerase chain reaction (qRT-PCR) amplification of GRα were as follows: 5′-CCTAAGGACGGTCTGAAGAGC-3′ (upstream) and 5′-GCCAAGTCTTGGCCCTCTAT-3′ (downstream). Measurements were normalized using human *GAPDH* as a housekeeping gene. The primers used for the amplification of GRβ and MKP-1 were Hs00354508_m1 and Hs00610256_g1 (TaqMan, Applied Biosystems, Waltham, Massachusetts), respectively. Measurements were normalized using *GAPDH* and human 18S rRNA as

housekeeping genes. All experiments were performed by investigators blinded to patient clinical data.

• **IN VITRO STIMULATION OF PBMC AND MKP-1 LEVELS:** In order to evaluate the role of inflammation in MKP-1 levels, PBMC of healthy controls were isolated and stimulated with anti-CD3/anti-CD28 (5 μg/ml), lipopolysaccharide (LPS) (10 ng/ml) and phytohemagglutinin (PHA) (5 μg/ml) for 24 hours. In addition, 1 μM dexamethasone (Sigma) was added the last 6 and 24 hours of culture. Thereafter, MKP-1 levels were evaluated by qRT-PCR as described above.

• **TREATMENT SCHEME:** Immediately after blood samples were obtained, all active patients received prednisone, 1 mg/kg/day, for at least 4 weeks and were then carefully followed to evaluate their CS response. After inflammation was controlled, the prednisone dose was slowly tapered according to disease activity.[15] Azathioprine, 2 mg/kg/day, was added as a second-line therapy if patients were considered CS refractory as described above or significant CS side effects were observed.

• **STATISTICS:** Descriptive statistics were used for the whole cohort and subgroups, including frequency distribution and means or medians as appropriate. Univariate analyses were performed using Student *t*-tests to compare GRα, GRβ, and MKP-1 levels between groups. Receiver operating characteristic (ROC) curves were used to evaluate the accuracy of the biomarkers as classifiers of CS response (CS-sensitive patients vs. CS-refractory patients) and also for clinical activity (active patients vs. inactive patients). *P* values <.05 were considered statistically significant. Statistical analyses were performed using Prism 6 software (GraphPad Inc., La Jolla, California).

RESULTS

TWENTY FEMALE VKH SUBJECTS WITH A MEAN AGE OF 35.8 ± 10.7 years old were included. Patient clinical and demographic characteristic are summarized in Table 1. Most of them had incomplete VKH disease, with ocular involvement associated with neurological findings (headache, nausea, vomiting, and tinnitus). Seven subjects were newly diagnosed VKH patients, and 13 had previously been treated with currently inactive disease. The median time with no CS therapy or IMT in the inactive group was 25.5 months (range, 4.4-213.2 months) and 25.9 months (range, 5.2-70.1 months), respectively. No evidence of inflammatory activity was found on ICG test images of these clinically inactive patients.

Using the standard definition for categorization of CS response described above, 10 CS-sensitive patients and 9 CS-refractory patients were identified (Table 1).

During the course of the disease and on retrospective review of clinical data from inactive subjects, 11 patients had recurrences of inflammation with a mean dose of 22.7 ± 17.2 mg per day of prednisone. The primary site of inflammation during flare up was anterior segment (anterior chamber cells, keratic precipitates, and/or iris nodules) and posterior segment (new subretinal fluid, serous retinal detachment, and/or papillitis) in 7 and 4 subjects, respectively.

During follow-up, a total of 10 patients received azathioprine, 2 mg/kg/day, and 2 patients were switched to cyclophosphamide due to uncontrolled inflammation. The indication for IMT was CS refractoriness in 9 subjects,

TABLE 1. Demographics and Clinical Characteristics of VKH Patients (N = 20)

Mean age (y)	35.8 ± 10.7
VKH diagnosis	
Probable	6 (30.0)
Incomplete	10 (50.0)
Complete	4 (20.0)
Visual acuity	
20/40 or better	25 (62.5)
20/50 to 20/100	8 (20.0)
20/200 or worse	7 (17.5)
Clinical inflammation	
Active	7 (35.0)
Inactive	13 (65.0)
Inflammatory findings[a]	
Median AC cells (range)	1.25 (0.5-3)
Median flare (range)	0.25 (0-2)
Posterior synechiae	4 (28.5)
Iris nodules	8 (57.1)
Median vitreous haze (range)	0.75 (0-2)
Disc swollen or hyperemia	10 (71.4)
RD or subretinal fluid	14 (100)
Complications	
Cataract	2 (5.0)
Ocular hypertension	2 (5.0)
Subretinal fibrosis	3 (7.5)
Treatment response	
CS sensitive	10 (50.0)
CS refractory	9 (45.0)
Unknown[b]	1 (5.0)

Values are n (%), median (range), or mean ± SD.

SD = standard deviation; VKH = Vogt-Koyanagi-Harada disease; CS = corticosteroids; RD = retinal detachment; VKH = Vogt-Koyanagi-Harada disease.

[a]Patients with active VKH.

[b]Patients who had immunomodulatory therapy as a first-line treatment.

and only 1 patient was treated with IMT as a CS-sparing agent. No severe side effects requiring cessation of therapy were reported secondary to the use of CS or IMT.

Valid duplicated values for GR isoforms were not feasible in 5 patients. There were no significant differences among different groups of patients (active vs. inactive or refractory versus sensitive) in terms of RNA quality and feasibility for assessing mRNA levels.

Correlations among mRNA levels of GRα, GRβ, and MKP-1 were evaluated, and no significant associations among these variables were found.

• **EVALUATION OF POTENTIAL BIOMARKERS OF CS REFRACTORINESS:** In the present study, mRNA levels of GRα, GRβ, and MKP-1 after 6 and 24 hours of in vitro stimulation with Dex were measured.

As shown in Figure 1, PBMC from CS-sensitive patients showed a significant upregulation of GRα expression after 6 hours of in vitro stimulation with CS, in comparison with PBMC from CS-refractory subjects. However, no differences in the levels of either GRβ or MKP-1, between CS-sensitive and CS- refractory subjects, were observed in this cohort of VKH patients.

To evaluate the accuracy of this strategy to predict CS refractoriness and, thus, its clinical applicability, ROC curve analysis was performed, and area under the curve (AUC) was calculated. Expression of GRα after 6 hours of Dex treatment was found useful as a classifier for discrimination of CS-refractory patients from CS-sensitive patients (AUC of 0.83; $P = .03$). Furthermore, using a cut-off of 0.92, the sensitivity, specificity, positive predictive value, and negative predictive values were estimated. We found that GRα showed a good performance as a test to evaluate response to therapy (Table 2).

In addition, the GRα:GRβ ratio was calculated for each subject, and no differences between refractory and sensitive patients were found. Finally, experiments conducted with an interval of Dex stimulation of 24 hours showed no significant differences between CS-sensitive and CS-refractory subjects.

• **EVALUATION OF POTENTIAL BIOMARKERS OF DISEASE ACTIVITY:** MKP-1 is a known mediator of GR effects, and this phosphatase displayed a significantly distinct profile in PBMC from active patients in comparison with PBMC from subjects with no disease activity. As shown in Figure 2, after 6 hours of in vitro Dex stimulation, inactive patients had a mean of 5.12 increase in the levels of MKP-1, whereas active patients had a mean of 1.05 increase in these levels ($P = .005$). Conversely, no differences were observed in either GRα, GRβ, or the GRα:GRβ ratio among active and inactive patients.

In addition, MKP-1 showed a good performance for diagnosis of inflammatory activity with an AUC of 0.89 ($P = .004$) and sensitivity, specificity, and predictive values parameters between 75% and 91.6% (Table 2).

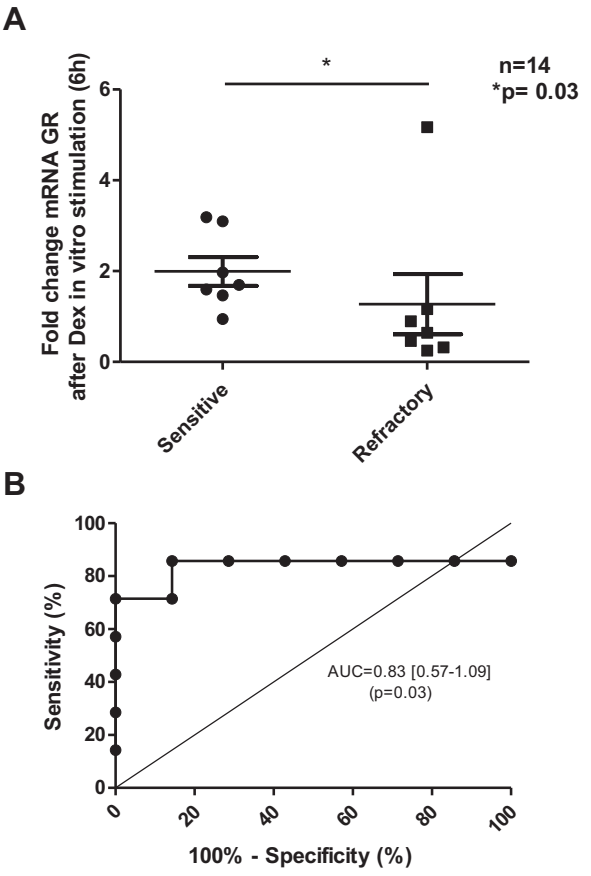

FIGURE 1. The value of glucocorticoid receptor-α as a predictive biomarker of corticosteroid treatment response in a prospective cohort of patients with Vogt-Koyanagi-Harada disease. (A) Scatter dot plot shows the change in the expression of glucocorticoid receptor-α (GRα) after six hours of in vitro stimulation with dexamethasone (Dex), 1 μM in peripheral blood mononuclear cells of patients with Vogt-Koyanagi-Harada disease. The cohort was divided into refractory and sensitive patients, considering the clinical response to corticosteroids. Fourteen valid duplicated GRα measurements were analyzed. Data are mean ± SEM (Mann-Whitney *U* test, *$P = .03$). (B) Receiver operating characteristic curve show evaluation of GRα to predict corticosteroid response. AUC = area under the curve (95% confidence interval).

Experiments conducted with an interval of Dex stimulation of 24 hours showed no significant differences between active and inactive subjects.

• **INFLAMMATION DRIVES DISTINCTIVE CHANGES IN LEVELS OF MKP-1 AFTER IN VITRO STIMULATION WITH DEXAMETHASONE:** As described above, in order to evaluate the role of inflammation in MKP-1 levels, PBMC from healthy controls were exposed to different inflammatory stimuli (anti-CD3/anti-CD28, LPS, PHA). In this regard, Dex failed to upregulate MKP-1 in PBMC stimulated with LPS and PHA. PBMC from healthy controls with no previous inflammatory stimulation had a mean of

TABLE 2. Performance of the Evaluation of GRα and MKP-1 Changes After Dexamethasone In Vitro Stimulation for the Diagnosis of Corticosteroid Refractoriness and Disease Activity in Vogt-Koyanagi-Harada Patients

	GRα[a]	MKP-1[b]
Sensitivity	71.4% (29-96.3)[c]	85.7% (42.1-99.6)
Specificity	100% (59-100)[c]	84.6% (54.5-98)[c]
Positive predictive value	100% (47.8-100)[c]	75% (34.9-96.8)[c]
Negative predictive value	77.7% (39.9-97.1)[c]	91.6% (61.5-99.7)[c]

GRα = glucocorticoid receptor-α; MKP-1 = Mitogen-activated protein kinase phosphatase-1.
[a]Cutoff of 0.92 for diagnosis of glucocorticoid refractoriness.
[b]Cutoff of 1.85 for diagnosis of disease activity.
[c]95% confidence interval.

9.81-fold increase in the levels of MKP-1. Conversely, PBMC prestimulated with anti-CD3/anti-CD28, LPS, anti-CD3/anti-CD28 plus LPS, or PHA had increments of 4.34, 1.83, 2.92, and 3.69, respectively ($P = .20$ for anti-CD3/anti-CD28 vs. control, and $P = .02$ for LPS or anti-CD3/anti-CD28 plus LPS or PHA vs. control) (Figure 3).

No differences in the levels of GRα and GRβ were found in PBMC exposed to inflammatory stimuli and cells with no stimulation.

DISCUSSION

IN OUR PREVIOUS REPORT, EARLIER IMT INITIATION SHOWED better functional outcomes in CS-refractory patients with VKH.[6] This finding highlights the importance of an early diagnosis of CS refractoriness in this subset of patients and, more promising, the possibility of predicting the response to therapy in order to anticipate the clinical scenario and to make decisions in a safer manner, avoiding potential complications in patients with no suboptimal responses and thus overt clinical or subclinical inflammation.

The evaluation of response to therapy is a challenge in VKH disease, especially considering the lack of a standard criteria for the definition of treatment refractoriness in this specific disorder and the difficulties in the clinical evaluation of these subjects. In particular, it is well-known that there can be persistent subclinical activity (evaluated with ancillary testing) in VKH patients with no clinically observed signs of inflammation, such as anterior chamber cells, vitreous haze, papillitis, or retinal detachments. Kawaguchi and associates[8] showed that VKH patients with subclinical inflammation had a higher probability of developing late stage signs, such as sunset glow fundus.[8] In addition, it is widely recognized that there is significant disagreement among experts in assessing clinical inflammatory activity and a lack of consensus regarding the definition of treatment response or refractoriness, with heterogeneity of clinical trial endpoints as a consequence.[18–21] In this regard, previously proposed definitions for CS-refractory disease in uveitis include persistence of inflammatory activity despite CS treatment,[8] dependence on a specified dose of systemic corticosteroids to control inflammation,[16,22] or the need to add IMT.[23] Given this heterogeneity, for the purpose of this proof-of-principle study, the inability to taper CS treatment below 10 mg of prednisone daily was selected as the threshold for defining CS-refractory uveitis, in accordance with previous reports by the present group.[16]

The evaluation of GRα and MKP-1 promises to be a helpful biomarker of CS refractoriness and disease quiescence, respectively, in VKH patients, enabling clinicians to decide which patients would benefit from early IMT in order to achieve better disease outcomes and to minimize CS side effects.[6]

Advances in the use of OCT as an objective method to quantify the inflammation at the level of anterior chamber and vitreous cavity have been reported.[24,25] However, relevant challenges must be addressed in order to translate this evidence into clinical practice, such as the need for manual measurement, the time it takes to acquire images, and the difficulty of using this approach to distinguish between low grades of inflammation. Thus, there remains a failure to include quantitative methods for quantifying ocular inflammation in the clinical practice.[26]

Hence, a laboratory-based approach could provide an objective tool to evaluate disease activity in the follow-up of patients with VKH disease. In the present study, measurements of MKP-1 expression showed an adequate performance as a classifier of activity. As described above, MKP-1 is a known mediator of GR effects, and our results showed that Dex failed to upregulate MKP-1 in active VKH patients. In that sense, other studies have shown similar findings in PBMC from obese asthmatic patients, with a lower ability to upregulate MKP-1 after Dex

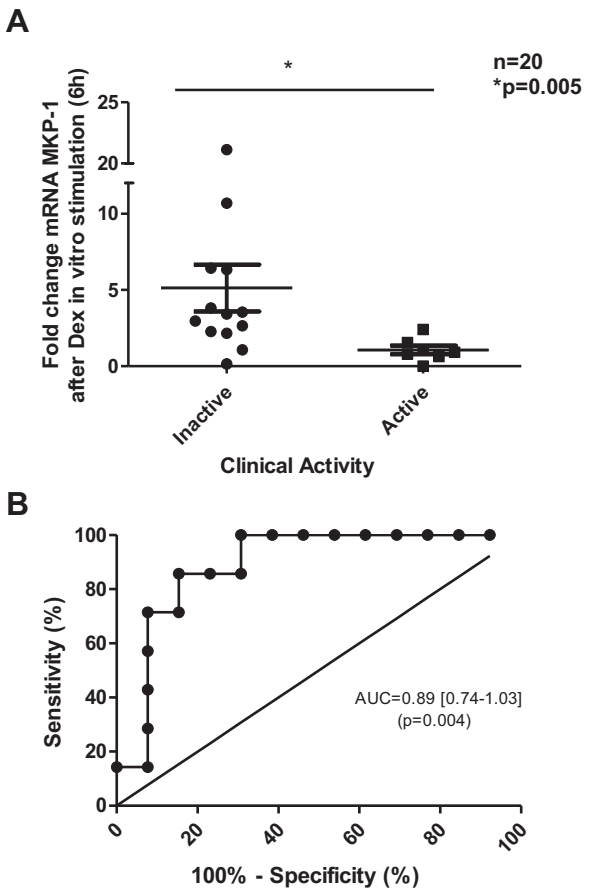

FIGURE 2. Value of MKP-1 as a predictive biomarker of disease activity in a prospective cohort of patients with Vogt-Koyanagi-Harada disease. (A) Scatter dot plot shows the change in expression level of MKP-1 after 6 hours of in vitro stimulation with dexamethasone, 1 μM, in peripheral blood mononuclear cells of patients with Vogt-Koyanagi-Harada disease. The cohort was divided into inactive and active patients. Data are mean ± SEM (Mann-Whitney *U* test, **P* = .005). (B) Receiver operating characteristic curve evaluation of MKP-1 in determining disease activity. AUC = area under the curve (95% confidence interval).

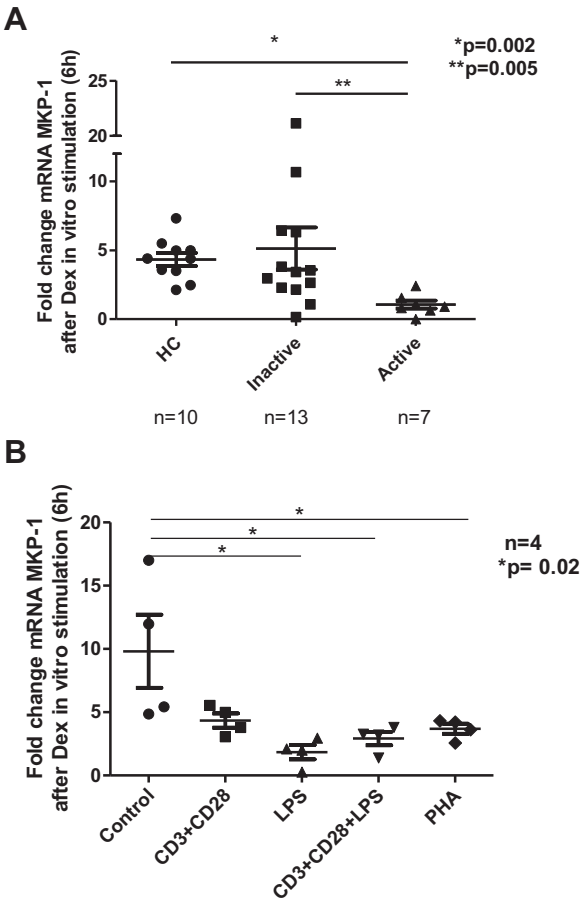

FIGURE 3. Role of inflammation in the expression of MKP-1 in healthy controls. (A) Scatter dot plot shows the change in the expression of MKP-1 after 6 hours of in vitro stimulation with Dex, 1 μM, in PBMC of healthy controls. Data from active patients with Vogt-Koyanagi-Harada disease are compared with those from inactive patients with Vogt-Koyanagi-Harada disease. The cohort of Vogt-Koyanagi-Harada patients was divided into inactive and active patients, considering the disease activity. Data are mean ± SEM (Mann-Whitney *U* test, **P* = .002 and ***P* = .005). (B) Scatter dot plot shows the change in the expression of MKP-1 after 6 hours of Dex, 1 μM, in PBMC of healthy controls, prestimulated with CD3+CD28, LPS, LPS plus CD3+CD28, and PHA. Data are mean ± SEM (Mann-Whitney *U* test, **P* = .02). Dex = dexamethasone; HC = healthy controls; LPS = lipopolysaccharide; PBMC = peripheral blood mononuclear cells; PHA = phytohemagglutinin.

in vitro stimulation in this subset of subjects, which may be related to the elevated systemic inflammatory state of patients with obesity.[27]

To the best of the present authors' knowledge, this study is the first to describe an in vitro assay for the evaluation of disease activity in VKH patients. In addition, a refined and simplified GR isoform biomarker of CS treatment response is proposed. Hence, these preliminary results support the rationale for further studies, with larger cohorts including different subsets of VKH patients to be conducted alongside refined laboratory protocols with a view to formal prospective evaluation of these biomarkers in the clinic.

In conclusion, the evaluation of changes in the levels of GRα and MKP-1 in PBMC may contribute to a potential objective classification of treatment response and disease activity in patients with VKH, and these findings may contribute to the development of a future biomarker in the field of uveitis.

ALL AUTHORS HAVE COMPLETED AND SUBMITTED THE ICMJE FORM FOR DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST and none were reported. Financial Disclosures: Drs. Urzua and Goecke hold patent rights (University of Chile). Funding/Support: This study was supported by the Intramural Research Program of the US National Institutes of Health/National Eye Institute and by Vicerrectoria de Investigacion y Desarrollo

(University of Chile) grant 560872-PR. The funding organizations had no role in the design or conduct of this research. Dr. Lee has received support from National Institute for Health Research (NIHR) Biomedical Research Centre, Moorfields Eye Hospital National Health Service Foundation Trust and University College London Institute of Ophthalmology. The views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR or the Department of Health.

The authors thank Dr. Robert B. Nussenblatt, Chief of Laboratory of Immunology of the National Eye Institute, for advice, critical review of experiments and protocols, and comments in support of this study.

REFERENCES

1. Rajendram R, Evans M, Rao NA. Vogt-Koyanagi-Harada disease. *Int Ophthalmol Clin* 2005;45(2):115–134.
2. Read RW, Holland GN, Rao NA, et al. Revised diagnostic criteria for Vogt-Koyanagi-Harada disease: report of an international committee on nomenclature. *Am J Ophthalmol* 2001; 131(5):647–652.
3. Moorthy RS, Inomata H, Rao NA. Vogt-Koyanagi-Harada syndrome. *Surv Ophthalmol* 1995;39(4):265–292.
4. Read RW, Yu F, Accorinti M, et al. Evaluation of the effect on outcomes of the route of administration of corticosteroids in acute Vogt-Koyanagi-Harada disease. *Am J Ophthalmol* 2006;142(1):119–124.
5. Nazari H, Rao NA. Resolution of subretinal fluid with systemic corticosteroid treatment in acute Vogt-Koyanagi-Harada disease. *Br J Ophthalmol* 2012;96(11):1410–1414.
6. Urzua CA, Velasquez V, Sabat P, et al. Earlier immunomodulatory treatment is associated with better visual outcomes in a subset of patients with Vogt-Koyanagi-Harada. *Acta Ophthalmol* 2015;93(6):e475–480.
7. Kim SJ, Yu HG. The use of low-dose azathioprine in patients with Vogt-Koyanagi-Harada disease. *Ocul Immunol Inflamm* 2007;15(5):381–387.
8. Urzua CA, Guerrero J, Gatica H, Velasquez V, Goecke A. Evaluation of the glucocorticoid receptor as a biomarker of treatment response in Vogt-Koyanagi-Harada disease. *Invest Ophthalmol Vis Sci* 2017;58(2):974–980.
9. Kawaguchi T, Horie S, Bouchenaki N, Ohno-Matsui K, Mochizuki M, Herbort CP. Suboptimal therapy controls clinically apparent disease but not subclinical progression of Vogt-Koyanagi-Harada disease. *Int Ophthalmol* 2010;30(1): 41–50.
10. Chen P, Urzua CA, Knickelbein JE, et al. Elevated CD1c^{+} myeloid dendritic cell proportions associated with clinical activity and predict disease reactivation in noninfectious uveitis. *Invest Ophthalmol Vis Sci* 2016;57(4):1765–1772.
11. Huang G, Wang Y, Shi LZ, Kanneganti TD, Chi H. Signaling by the phosphatase MKP-1 in dendritic cells imprints distinct effector and regulatory T cell fates. *Immunity* 2011;35(1):45–58.
12. Bhavsar P, Hew M, Khorasani N, et al. Relative corticosteroid insensitivity of alveolar macrophages in severe asthma compared with non-severe asthma. *Thorax* 2008;63(9): 784–790.
13. Kjellerup RB, Johansen C, Kragballe K, Iversen L. The expression of dual specificity phosphatase 1 mRNA is downregulated in lesional psoriatic skin. *Br J Dermatol* 2013; 168(2):339–345.
14. Toh ML, Yang Y, Leech M, Santos L, Morand EF. Expression of mitogen-activated protein kinase phosphatase-1, a negative regulator of the mitogen-activated protein kinases, in rheumatoid arthritis: up-regulation by interleukin-1beta and glucocorticoids. *Arthritis Rheum* 2004;50(10):3118–3128.
15. Jabs DA, Rosenbaum JT, Foster CS, et al. Guidelines for the use of immunosuppressive drugs in patients with ocular inflammatory disorders: recommendations of an expert panel. *Am J Ophthalmol* 2000;130(4):492–513.
16. Lee RW, Schewitz LP, Nicholson LB, Dayan CM, Dick AD. Steroid refractory CD4+ T cells in patients with sight-threatening uveitis. *Invest Ophthalmol Vis Sci* 2009;50(9): 4273–4278.
17. Jabs DA, Nussenblatt RB, Rosenbaum JT, et al. Standardization of uveitis nomenclature for reporting clinical data. Results of the first international workshop. *Am J Ophthalmol* 2005;140(3):509–516.
18. Denniston AK, Holland GN, Kidess A, et al. Heterogeneity of primary outcome measures used in clinical trials of treatments for intermediate, posterior, and panuveitis. *Orphanet J Rare Dis* 2015;10:97.
19. Agrawal R, Keane P, Singh J, Saihan Z, Kontos A, Pavesio CE. Comparative analysis of anterior chamber flare grading between clinicians with different levels of experience and semi-automated laser flare photometry. *Ocul Immunol Inflamm* 2016;24(2):184–193.
20. Kempen JH, Ganesh SK, Sangwan VS, Rathinam SR. Interobserver agreement in grading activity and site of inflammation in eyes of patients with uveitis. *Am J Ophthalmol* 2008; 146(6):813–818.
21. Yeo TH, Ilangovan S, Keane PA, Pavesio C, Agrawal R. Discrepancies in assessing anterior chamber activity among uveitis specialists. *Jpn J Ophthalmol* 2016;60(3):206–211.
22. Pacheco PA, Taylor SR, Cuchacovic MT, Diaz GV. azathioprine in the management of autoimmune uveitis. *Ocul Immunol Inflamm* 2008;16(4):161–165.
23. Simonini G, Paudyal P, Jones GT, Cimaz R, Macfarlane GJ. Current evidence of methotrexate efficacy in childhood chronic uveitis: a systematic review and meta-analysis approach. *Rheumatology (Oxford)* 2013; 52(5):825–831.
24. Invernizzi A, Marchi S, Aldigeri R, et al. Objective quantification of anterior chamber inflammation: measuring cells and flare by anterior optical coherence tomography. *Ophthalmology* 2017;124(11):1670–1677.
25. Keane PA, Karampelas M, Sim DA, et al. Objective measurement of vitreous inflammation using optical coherence tomography. *Ophthalmology* 2014;121(9):1706–1714.
26. Herbort CP, Tugai-Tutkun I, Neri P, et al. Failure to integrate quantitative measurement methods of ocular inflammation hampers clinical practice and trials on new therapies for posterior uveitis. *J Ocul Pharmacol Ther* 2017;33(4):263–277.
27. Fantuzzi G. Adipose tissue, adipokines, and inflammation. *J Allergy Clin Immunol* 2005;115(5):911–919.