# Evaluation of the Glucocorticoid Receptor as a Biomarker of Treatment Response in Vogt-Koyanagi-Harada Disease

Cristhian A. Urzua,[1–3] Julia Guerrero,[2] Hector Gatica,[4] Victor Velasquez,[1,3] and Annelise Goecke[2,4]

[1]Department of Ophthalmology, School of Medicine, Universidad de Chile, Santiago, Chile
[2]Physiology Program, School of Medicine, Universidad de Chile, Santiago, Chile
[3]Uveitis Department, Hospital del Salvador, Santiago, Chile
[4]Rheumatology Service, Department of Medicine, Hospital Clinico Universidad de Chile, Santiago, Chile

Correspondence: Annelise Goecke, Physiology Program, School of Medicine, Universidad de Chile, Independencia 1027, Santiago, Chile; agoecke@med.uchile.cl.

Submitted: September 19, 2016
Accepted: December 22, 2016

Citation: 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;58:974–980. DOI:10.1167/iovs.16-20783

**Purpose.** This study is aimed to investigate the role of glucocorticoid receptor (GR) isoforms in peripheral blood mononuclear cells (PBMC) as biomarkers of glucocorticoid (GC) resistance and to validate a set of clinical predictive factors in patients with Vogt-Koyanagi-Harada (VKH) disease.

**Methods.** This was a prospective cohort study that included a total of 21 patients with VKH. A complete ophthalmologic evaluation was carried out at baseline that recorded the presence of any clinical predictive factors (visual acuity $\leq$ 20/200, tinnitus, chronic disease, and fundus depigmentation). Real-time quantitative PCR was performed to measure the mRNA levels of GR alpha (GRα) and beta (GRβ) isoforms at baseline and at 2 weeks after prednisone therapy initiation.

**Results.** There were no differences between GRα and GRβ levels in GC-sensitive and GC-resistant patients at baseline before treatment initiation. After 2 weeks of prednisone treatment, GC-sensitive patients had a median 5.5-fold increase in levels of GRα, whereas GC-resistant patients had a median 0.7-fold decrease in levels of this isoform ($P = 0.003$). Similarly, GRβ increased in GC-sensitive patients, in comparison with GR-resistant patients (6.49-fold versus 1.01 fold, respectively, I = 0.04). The mRNA levels of GR isoforms were independent of disease activity. Fundus depigmentation and chronic disease at diagnosis were associated with GC resistance ($P = 0.03$, odds ratio = 21.0; and $P = 0.008$, odds ratio = 37.8, respectively). However, associations with visual acuity or tinnitus were not confirmed in this study.

**Conclusions.** The evaluation of clinical predictive factors and determination of the change in expression of GR isoforms as potential biomarkers can contribute to the early identification of GC-resistant patients with VKH.

Keywords: biomarker, glucocorticoid receptor, predictive factors, treatment response, Vogt-Koyanagi-Harada disease

Vogt-Koyanagi-Harada disease (VKH) is a multisystemic inflammatory disease that affects the eyes, central nervous system, and skin, with a bilateral granulomatous panuveitis characterized by posterior involvement with exudative retinal detachment.[1,2] The mainstay of treatment has been the implementation of systemic glucocorticoid (GC) therapy as early as possible that is intensive (1 mg/kg/day) for at least 6 months.[3–5] Despite the high dosage of GC treatment, up to one third of VKH patients have no response to therapy, retaining active inflammation and developing vision-threatening complications, thus requiring immunosuppressive therapy (IMT).[4,6,7]

In a previous report, we found that earlier IMT was associated with better functional outcomes in the subset of GC-resistant patients. Moreover, we proposed a set of clinical predictive factors at diagnosis for GC resistance: best corrected visual acuity (BCVA) < 20/200, tinnitus, chronic disease, and fundus depigmentation.[6]

The active mechanisms of GC are mediated by the glucocorticoid receptor (GR). Two classical transcriptional GR isoforms have been described: the alpha isoform (GRα) and the beta isoform (GRβ). GRα mediates most of the known GC actions. Conversely, GRβ does not bind to GC, and it has a dominant-negative effect on genes regulated by GRα.[8] In this regard, GR isoforms have been implicated in the mechanism of GC resistance in autoimmune and inflammatory diseases (e.g., ulcerative colitis, autoimmune hepatitis, and asthma).[8,9]

In this study, we evaluated GR isoforms in PBMC from VKH patients as a potential biomarker of GC resistance. We compared mRNA levels between GC-resistant and GC-sensitive patients and found significant differences in the profiles of GRα and GRβ expression levels after 2 weeks of prednisone treatment. In addition, we prospectively evaluated previously described clinical predictive factors.[6] We found that fundus depigmentation and chronic disease at diagnosis were

associated with a poor response to GC in this prospective cohort.

## Methods

We conducted a prospective cohort study of VKH patients attending the Uveitis Department of the Hospital del Salvador (Santiago, Chile).

Two patient groups were included: those with active disease who had not received systemic treatment previously and those patients with inactive disease without current inflammatory activity and who had not received treatment for at least 1 month.

The protocol complied with the contents of the Declaration of Helsinki, and all participants gave written informed consent before being included. Institutional Review Board approval was obtained.

### Inclusion Criteria

We recruited adult subjects with a diagnosis of VKH according to the diagnostic criteria revised by the international nomenclature committee in uveitis.[2] Alternative diagnoses (i.e., syphilis and tuberculosis) were ruled out during our initial evaluation.

### Exclusion Criteria

Individuals were excluded if they had been diagnosed with other systemic autoimmune/inflammatory disorders, cancer, or pregnancy or if they had received systemic anti-inflammatory drugs within 1 month before recruitment or any intravitreal/periocular treatment.

### 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 BCVA, intraocular pressure, slit-lamp biomicroscopy, ophthalmoscopy under mydriasis, and ancillary testing, such as fundus fluorescein angiography and ocular coherence tomography.

For inactive patients, a retrospective review of medical records from the Uveitis Department database was performed. This database contains the complete clinical data of patients admitted to and followed in our department in a prospective and standardized manner. Additionally, the same evaluation as described above was performed at enrollment to confirm the absence of disease activity in these subjects with inactive disease.

Clinical predictive factors of GC resistance were evaluated in each patient at baseline, as described elsewhere.[6] In this regard, the eye with the worse vision was considered for the evaluation of BCVA as the predictor factor.

Follow-up included a workup for potential side effects: blood pressure, blood glucose levels, complete blood count, and liver function tests.[10]

### Definition of GC Resistance

We defined GC resistance as the absence of inflammatory improvement, persistence, or worsening of inflammation, namely, not achieving a two-step decrease in the level of inflammation or a decrease to grade 0+, as described by Standardization of Uveitis Nomenclature,[11] and/or the persistence of retinal detachment, despite 4 to 6 weeks of prednisone therapy, 1 mg/kg/day.[6]

### PBMC Preparation and GRα and GRβ Isoform Measurements

Peripheral venous blood samples (30 mL) were extracted from all subjects and placed in tubes with EDTA, 0.5 M at pH 8. PBMCs were isolated by using Ficoll density gradient centrifugation protocol (Histopaque; Sigma Diagnostic, St. Louis, MO, USA). RNA was isolated using TRIzol reagent (Gibco, Halethorpe, MD, USA) and 2 μg of RNA was reverse transcribed. The primers used for real time quantitative polymerase chain reaction (RT-qPCR) amplification of GRα were 5′-CCTAAGGACGGTCTGAAGAGC-3′ (upstream) and 5′-GCCAAGTCTTGGCCCTCTAT-3′ (downstream). The primers used for amplification of GRβ were 5′-CCTAAGGACGGTCT GAAGAGC-3′ (upstream) and 5′-CCACGTATCCTAAAAGGG-3′ (downstream). Measurements were normalized using human 18s rRNA as the housekeeping gene. All GR isoform measurements were performed by investigators blinded to patient clinical data.

Two samples were collected among active patients. The standard of care in our clinic is to review VKH patients 2 weeks after treatment initiation, which is too early to clinically categorize response to therapy. Therefore, we decided to obtain a baseline sample and a second sample after 2 weeks of prednisone treatment initiation, considering the potential clinical relevance of having an earlier categorization of steroid sensitivity based on GR levels within the regular protocol of visits.

### Treatment Scheme

Immediately after blood samples were obtained, all active patients received prednisone therapy, 1 mg/kg/day, for at least 4 weeks and were then carefully followed to evaluate the GC response. After inflammation was controlled, the prednisone dose was slowly tapered according to disease activity.[10]

If patients were considered GC-resistant at the 4- to 6-week follow-up examination as described above, azathioprine, 2 mg/kg/day, was added as a second line therapy.

### Statistics

Descriptive statistics were calculated for the whole cohort and subgroups, including frequency distribution and means or medians as appropriate. Univariate analyses were performed using Student's *t*-tests to compare GRα and GRβ levels between groups. We used the nonparametric Fisher exact test to evaluate the clinical predictive factors, as described elsewhere.[6] Receiver operating characteristic (ROC) curves were used to evaluate the accuracy of the GR isoform measurements as classifiers of GC response (GC-sensitive patients vs. GC-resistant patients) and also for clinical activity (active patients vs. inactive patients). The measurements with the largest area under the curve (AUC) were chosen as the significant classifier and, therefore, were eligible for calculation of sensitivity, specificity, positive predictive value and negative predictive value. Cutoff values for both of the GR isoforms were selected from the ROC curves, considering the point with the highest Youden's index in order to maximize the sensitivity and specificity. A sample size of 12 VKH patients was calculated by estimating a difference of 73% in the primary outcome (baseline levels of GR) between GC-sensitive versus GC-resistant samples.[9] Sample size was calculated using StudySize version 1.0.9 software (CreoStat HB Co., Gothenburg, Sweden). $P$ values of $<0.05$ were considered statistically significant. Statistical analyses were performed using Prism software version 6 (GraphPad Software Inc., La Jolla, CA, USA).

Investigative Ophthalmology & Visual Science

**Table 1.** Demographics and Clinical Characteristics of Subjects ($n$ = 21)

| Demographic and Clinical Characteristic | Value(s) |
|---|---|
| Mean (±SD),y | 36.4 ± 11.8 |
| Sex (%) | |
| Males | 3 (14.2) |
| Females | 18 (85.7) |
| VKH diagnosis (%) | |
| Probable | 13 (61.9) |
| Incomplete | 4 (19) |
| Complete | 4 (19) |
| Clinical inflammation (%) | |
| Active | 16 (76.2) |
| Acute | 12 |
| Chronic | 4 |
| Inactive | 5 (23.8) |
| Median duration of symptoms at diagnosis (range), days | 14 (10–180) |

SD, standard deviation.

## Results

Twenty-one VKH patients were recruited. Their demographic and clinical features are shown in Table 1. The subjects were mostly females (85.7%) of mean 36.4 ± 11.8 years of age. The study sample included 16 patients with active disease who presented with a broad range of duration of symptoms at diagnosis (10–180 days), as well as 5 patients with inactive disease.

### Treatment Outcomes

Using the standard evaluation for categorization of GC response described above, we identified 13 GC-sensitive patients and 8 GC-resistant patients.

All GC-resistant patients received azathioprine as a second-line therapy and achieved an improvement in intraocular inflammation after IMT initiation. None of them require switching or adding another IMT drug.

No severe side effects were reported secondary to the use of GC or azathioprine therapy.

### Glucocorticoid Receptor as a Potential Biomarker of Glucocorticoid Resistance

Glucocorticoid actions are mediated by GR. Therefore, we evaluated the mRNA levels of GRα and GRβ before treatment initiation (pretreatment levels) in PBMCs from active patients, later classified as GC-sensitive or GC-resistant. As shown in Figure 1, there were no significant differences in mRNA pretreatment levels of GRα or GRβ between both groups of patients.

After 2 weeks of prednisone treatment, the mRNA levels of GR in PBMC were also analyzed (post-treatment levels). We did not observe any significant differences in post-treatment levels of GR isoforms between both groups of patients (data not shown).

However, changes in mRNA post-treatment levels of GRα and GRβ isoforms compared to pretreatment levels (change ratio) were significantly different between GC-sensitive and GC-resistant patients. Glucocorticoid-sensitive patients had a median 5.5-fold increase in the levels of GRα, whereas GC-resistant patients had a median 0.7-fold decrease in the levels of this isoform ($P = 0.003$). Similarly, GRβ expression increased in both groups, with a significantly increase in GC-sensitive patients in comaprison with GC-resistant patients (6.49-fold versus 1.01-fold, respectively, $P = 0.04$) (Fig. 2).

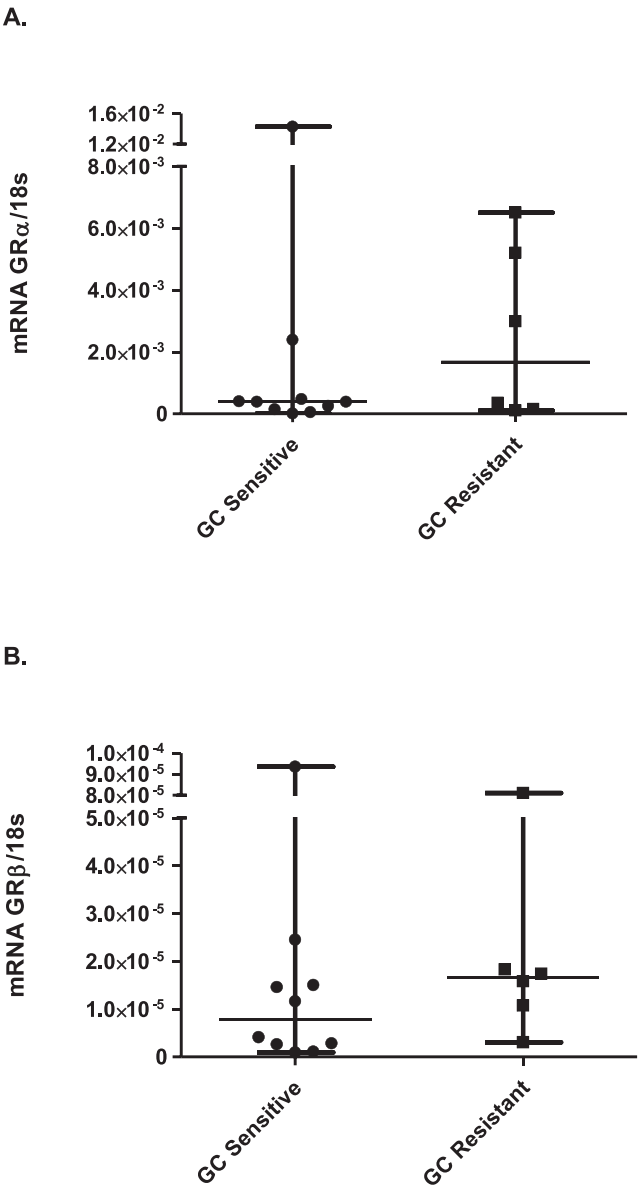


**Figure 1.** Scatter-plots showing the mRNA levels of glucocorticoid receptor isoforms in PBMCs from Vogt-Koyanagi-Harada patients with active disease at diagnosis (before treatment initiation). Patients were divided into two groups based on their clinical response to glucocorticoids (GC), GC-sensitive and GC-resistant. (**A**) Levels of glucocorticoid receptor isoform α (GRα). No significant differences were observed between GRα levels in GC-sensitive and those in GC-resistant patients (Mann-Whitney $U$ test, $P = 0.94$). (**B**) Levels of glucocorticoid receptor isoform β (GRβ). No significant differences were observed between GC-sensitive and GC-resistant patients in the levels of GRβ (Mann-Whitney $U$ test, $P = 0.66$). Results were normalized by mRNA levels of human 18s rRNA. *Horizontal lines* represent medians and ranges.

To evaluate the accuracy of these strategies to predict GC resistance, ROC curve analysis was performed, and AUC was calculated. As shown in Table 2, change ratios of GRα and GRβ after 2 weeks of prednisone treatment, baseline levels were useful as a classifier for discrimination of GC-resistant patients from GC-sensitive patients. Using a cutoff for the GRα ratio and

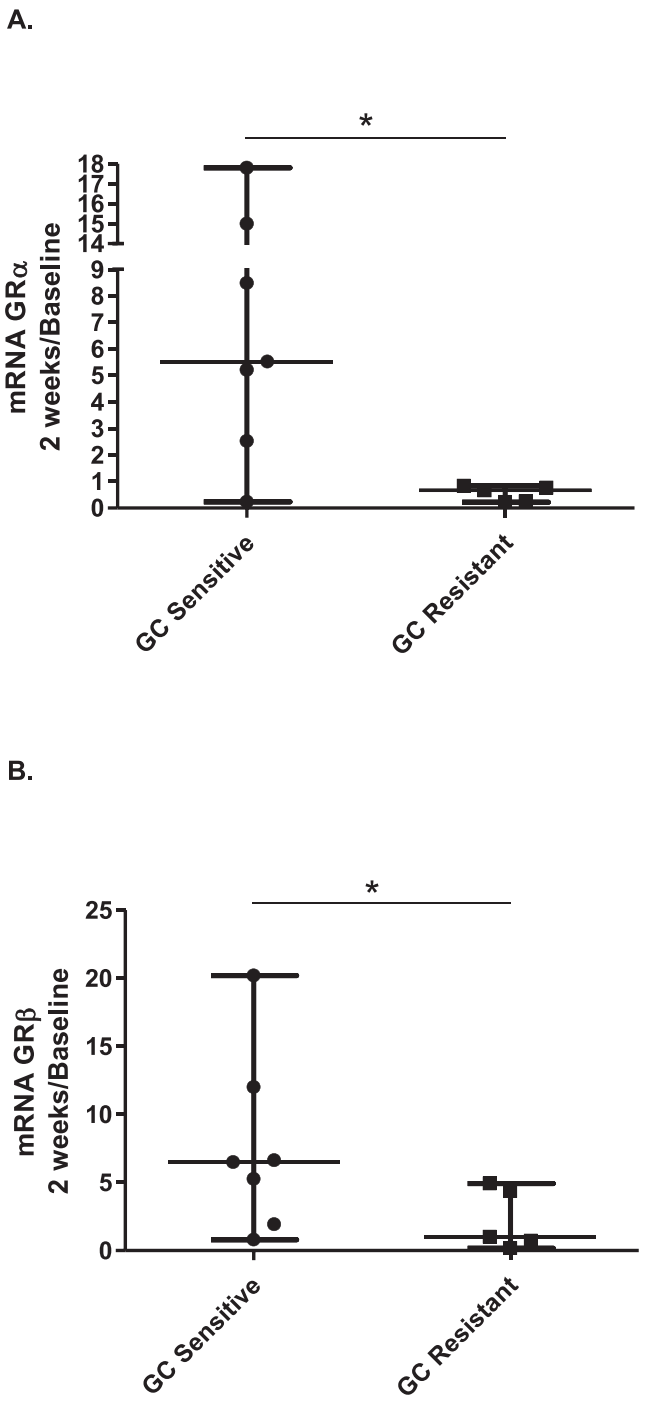


**Figure 2.** Scatter plots show the changes in expression of glucocorticoid receptor isoforms after 2 weeks of prednisone treatment in PBMCs from Vogt-Koyanagi-Harada patients with active disease. Patients were divided into two groups based on their clinical response to glucocorticoids (GC), GC-sensitive and GC-resistant. (**A**) Change in the ratio of mRNA levels of glucocorticoid receptor isoform α (GRα) after 2 weeks of GC initiation: baseline levels. A significant increase in the change in the ratio of GRα measured from GC-sensitive patients in comparison to GC-resistant patients was obtained. (Mann-Whitney *U* test, $P = 0.003$). (**B**) Change in the ratio of the mRNA levels of glucocorticoid receptor isoform β (GRβ) after 2 weeks of GC initiation: baseline levels. There was a significant increase in the ratio of GRβ in GC-sensitive patients, in comparison with GC-resistant patients (Mann-Whitney *U* test, $P = 0.04$). *Horizontal lines* represent medians and ranges. *$P < 0.05$.

**Table 2.** AUC of GRα and GRβ Measurements for Diagnosis of GR and Clinical Activity in VKH Patients

| | AUC | |
|---|---|---|
| **Parameter** | **Glucocorticoid Resistance (95% CI)** | **Clinical Activity (95% CI)** |
| GRα | 0.56 (0.19–0.93) | 0.58 (0.31–0.85) |
| GRβ | 0.58 (0.25–0.91) | 0.58 (0.3–0.85) |
| GRα ratio* | 0.88† (0.66–1.1) | NA |
| GRβ ratio* | 0.85† (0.63–1.07) | NA |

AUC, area under receiver operating characteristic curve; CI, confidence interval; GR, glucocorticoid receptor; NA, not applicable.
* Two weeks' prednisone treatment:baseline.
† $P < 0.05$.

GRβ ratio of 1.68 and 5.09, respectively, the sensitivity, specificity, positive predictive value, and negative predictive value parameters were estimated (Table 3). In summary, we found that the GRα ratio presented the best accuracy for GC-response classification.

It is possible to postulate that the different GR ratios between GC-sensitive and GC-resistant patients could be secondary to a significantly decreased inflammatory state in the GC-sensitive patients after 2 weeks of treatment, in contrast to the GC-resistant patients who would not have experienced a significant reduction in inflammation despite treatment. To evaluate this hypothesis, we measured the mRNA levels of GRα and GRβ in PBMCs from active and inactive patients. Despite there were 5 subjects and 2 subjects in the active group with higher levels of GRα and GRβ, respectively, our results showed that the levels of GR expression were independent of disease activity, with no statistically significant differences between active patients and inactive patients (Fig. 3).

## Clinical Predictive Factors for GC Resistance

Clinical predictive factors for GC resistance described elsewhere (tinnitus, chronic disease, fundus depigmentation, and BCVA ≤ 20/200) were also evaluated in this prospective cohort study. In this regard, most of the included subjects with active disease had tinnitus (11 of 16). Four of them had chronic disease, and a BCVA equal to or worse than 20/200 was measured in 8 patients; 3 patients presented with fundus depigmentation. There were no significant differences in the number of such factors between GC-sensitive and GC-resistant patients. However, after the removal of tinnitus from the analysis, a significantly greater number of factors were found in the GC-resistant group compared with the GC-sensitive group (Fig. 4).

Additionally, a univariate analysis of these clinical characteristics was performed. Fundus depigmentation and chronic disease at diagnosis were associated with GC resistance ($P =$

**Table 3.** Performance of GRα and GRβ Ratios as Diagnostic Tests for Glucocorticoid Resistance in VKH Patients

| **Parameter** | **GRα Ratio* (95% CI)** | **GRβ Ratio† (95% CI)** |
|---|---|---|
| Sensitivity | 100% (47.8–100) | 100% (47.8–100) |
| Specificity | 85.7% (42.1–99.6) | 71.4% (29–96.3) |
| Positive predictive value | 83.3% (35.8–99.5) | 71.4% (29–96.3) |
| Negative predictive value | 100% (54–100) | 100% (47.8–100) |

CI, confidence interval; GR, glucocorticoid receptors.
* Cutoff: 1.68.
† Cutoff: 5.09.

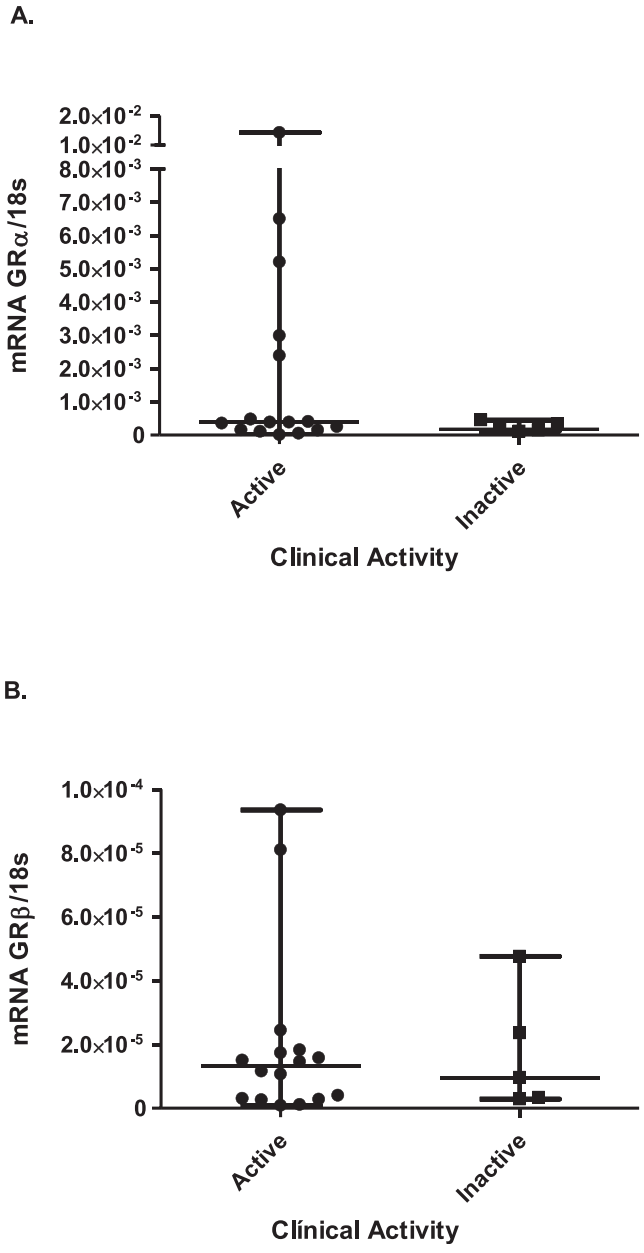


FIGURE 3. Scatter plots show the association between clinical activity and mRNA levels of glucocorticoid receptor isoforms in PBMCs from Vogt-Koyanagi-Harada patients. Patients were divided into two groups based on clinical inflammatory activity, active and inactive. (**A**) Levels of glucocorticoid receptor isoform α (GRα). No significant differences were observed between active patients and inactive patients (Mann-Whitney *U* test, $P = 0.61$). (**B**) Levels of glucocorticoid receptor isoform β (GRβ). No significant differences were obtained between active patients and inactive patients (Mann-Whitney *U* test, $P = 0.57$). Results were normalized by the mRNA levels of human 18s rRNA. *Horizontal lines* represent medians and ranges.

0.03, odds ratio = 21.0; and $P = 0.008$, odds ratio = 37.8, respectively). However, associations with BCVA or tinnitus were not confirmed in this study.

### Clinical and Molecular Markers are Independent Predictive Factors for GC Resistance

It is important to understand if the above-described molecular predictive factors for GC resistance improve the capacity of clinical factors to predict GC resistance or if these data are redundant. To answer this question, we compared the mRNA levels of GRα with those of GRβ at baseline and changes in the GR isoform ratios after 2 weeks of prednisone treatment between the subgroup of patients with each clinical predictive factor for GC resistance (tinnitus, chronic disease, fundus depigmentation and BCVA $\leq$ 20/200) and the subgroup of patients without each of these factors.

There were no significant differences in either the levels of GR isoforms at baseline or the changes in the ratios between the analyzed subgroups.

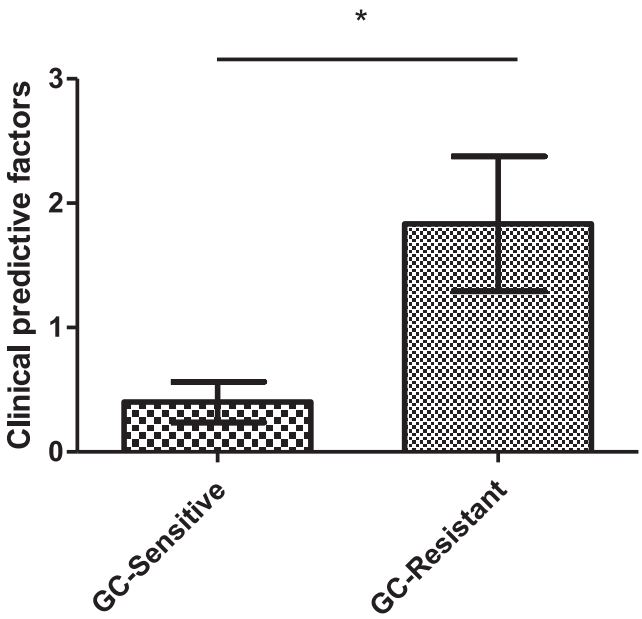


FIGURE 4. Bar graph shows clinical predictive factors for glucocorticoid (GC) resistance in Vogt-Koyanagi-Harada disease at diagnosis. Patients were divided into two groups based on their clinical response to GC, GC-sensitive and GC-resistant. Three factors were considered in the analysis: best-corrected visual acuity $\leq$ 20/200, chronic disease, and fundus depigmentation. GC-resistant patients had more predictive factors than GC-sensitive patients (Mann-Whitney *U* test, $P = 0.03$). Data are mean $\pm$ standard error of the mean. $^*P < 0.05$.

## DISCUSSION

High-dose and prolonged GC treatment has been considered the first-line therapy for VKH patients, with second-line IMT indicated for specific cases, such as patients presenting with severe side effects or GC-resistant patients.[12,13] We have shown that, in these nonresponding patients, early initiation of IMT correlates with better functional outcomes, underscoring the importance of the timing of IMT in GC-resistant VKH patients.[6]

Therefore, an early categorization of GC response has become an important issue in VKH patients. In a large retrospective study, we identified some clinical predictive factors for GC resistance: tinnitus, fundus depigmentation, chronic disease, and BCVA $\leq$ 20/200 at diagnosis.[6] In the present prospective cohort study, we confirmed that fundus depigmentation and chronic disease at diagnosis are associated with GC resistance. These clinical features have been related to a poor prognosis in other studies.[14,15]

Lee et al.[16] showed an in vitro classification of GC resistance by describing a proliferation profile of CD4-positive cells from a heterogeneous group of uveitis patients in terms of causes,

treatment, and disease activity. Here, we described a new biomarker strategy for early GC-response categorization based on the main active pathway of steroids by exploring the predictive role of measuring the mRNA levels of GR before and soon after treatment initiation in a cohort of patients with VKH.

An association has been described in the GC response with the levels of GRβ in PBMCs of patients with asthma and autoimmune diseases, such as ulcerative colitis and autoimmune hepatitis.[17–19] Additionally, changes in expression of GR isoforms after starting therapy have been reported elsewhere, in different diseases and cell types. Lauten et al.[20] evaluated the expression kinetics of GRα in pediatric patients with acute lymphoblastic leukemia. The authors found that sensitive patients presented with higher levels of GRα at different time points after prednisone initiation compared with resistant patients.

In the present study, changes in expression of GRα and GRβ were evaluated in PBMCs from active VKH patients by measuring the levels of the isoforms at baseline and at 2 weeks after GC initiation and then calculating the change ratio. A distinctive profile was observed in both GRα and GRβ measurements in GC-sensitive patients in comparison with GC-resistant patients (Fig. 2). In this regard, GRα appears to be a more feasible and attractive alternative, considering the technical challenges in the quantification of GRβ described previously due to its low relative expression.[8] Thus, the GRα ratio was evaluated as a potential diagnostic biomarker of GC resistance. It showed good efficacy for the distinction between sensitive versus resistant patients (sensitivity and specificity of 100% and 85.7%, respectively) (Tables 2, 3).

To our knowledge, this study is the first to report a potential biomarker of GC response in VKH disease. However, the data from this study may have some biases related to the design and outcome definitions. For instance, due to the referral nature of our center, it is possible that the included patients have special features (i.e., severity, duration of symptoms, follow-up, and others). Moreover, there is no consensus about a standard definition of GC resistance in patients with uveitis. Here, a disease-specific definition was used that considered the improvement of inflammation and retinal detachment.

Based on our study results and the review studies commented on above, we propose a comprehensive exploration of patients with VKH at diagnosis to make treatment decisions by including the evaluation of the clinical predictive factors and the GRα ratio as a new potential biomarker.

However, some challenges related to the biomarker described herein must be considered. Because the change in expression occurs in the context of in vivo prednisone treatment, patient compliance is a crucial issue. Additionally, two-sample collections are needed to calculate the change in the expression between two time points. In this regard, a less invasive and non–treatment-dependent strategy may be developed. For instance, an assay that includes in vitro GC treatment of PBMCs from VKH patients which would require collection of just one sample and be independent of the effect of patient compliance.

In conclusion, determination of the changes in expression of GR isoforms as a potential biomarker and the evaluation of clinical predictive factors can contribute to the early identification of GC-resistant patients. These results may facilitate clinical decision making in the management of patients with VKH.

## Acknowledgments

Supported by National Fund for Scientific and Technological Development (FONDECYT), Santiago, Chile, Grant1080529. The funding organization had no role in the design or conduct of this research.

Disclosure: **C.A. Urzua**, P; **J. Guerrero**, None; **H. Gatica**, None; **V. Velasquez**, None; **A. Goecke**, P

## References

1. Rajendram R, Evans M, Rao NA. Vogt-Koyanagi-Harada disease. *Int Ophthalmol Clin*. 2005;45:115–134.
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;131:647–652.
3. Moorthy RS, Hinomata H, Rao NA. Vogt-Koyanagi-Harada Syndrome. *Surv Ophthalmol*. 1995;39:265–292.
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; 142:119–124.
5. Nazari H, Rao NA. Resolution of subretinal fluid with systemic corticosteroid treatment in acute Vogt-Koyanagi-Harada disease. *Br J Ophthalmol*. 2012;96:1410–1414.
6. Urzua CA, Velasquez V, Sabat P, et al. Earlier immunomodulatory treatment is associated with better functional outcomes in a subset of patients with Vogt-Koyanagi-Harada. *Acta Ophthalmol*. 2015;93:e475–e480.
7. Kim SJ, Yu HG. The use of low-dose azathioprine in patients with Vogt-Koyanagi-Harada disease. *Ocul Immunol Inflamm*. 2007;15:381–387.
8. Goecke A, Guerrero J. Glucocorticoid receptor in acute and chronic inflammatory conditions: clinical implications. *Immunobiology*. 2006;211:85–96.
9. Honda O, Ayabe T. Expression of glucocorticoid receptor β in lymphocytes of patients with glucocorticoid-resistant ulcerative colitis. *Gastroenterology*. 2000;118:859–866.
10. 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;130:492–513.
11. Jabs DA, Nussenblatt RB, Rosenbaum JT; for the Standardization of Uveitis Nomenclature (SUN) Working Group. Standardization of uveitis nomenclature for reporting clinical data. Results of the First International Workshop. *Am J Ophthalmol*. 2005;140:509–516.
12. Errera MH, Fardeau C, Cohen D, et al. Effect of the duration of immunomodulatory therapy on the clinical features of recurrent episodes in Vogt-Koyanagi-Harada disease. *Acta Ophthalmol*. 2011;89:e357–366.
13. Lai TY, Chan RP, Chan CK, Lam DS. Effects of the duration of initial oral corticosteroid treatment on the recurrence of inflammation in Vogt-Koyanagi-Harada disease. *Eye* (*Lond*). 2009;23:543–548.
14. Abu El-Asrar AM, Al Tamimi M, Hemachandran S, Al-Mezaine HS, Al-Muammar A, Kangave D. Prognostic factors for clinical outcomes in patients with Vogt-Koyanagi-Harada disease treated with high-dose corticosteroids. *Acta Ophthalmol*. 2013;91:e486–e493.
15. Yang P, Ren Y, Li B, Fang W, Menq Q, Kijlstra A. Clinical characteristics of Vogt-Koyanagi-Harada syndrome in Chinese patients. *Ophthalmology*. 2007;114:606–614.
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*. 2007;50: 4273–4278.

17. Leung D, Hamid Q, Vottero A, et al. Association of glucocorticoid insensitivity with increased expression of glucocorticoid receptor beta. *J Exp Med*. 1997;186:1567–1574.
18. Hamid QA, Wenzel SE, Hauk PJ, et al. Increased glucocorticoid receptor in airway cells of glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med*. 1999;159:1600–1604.
19. Rai T, Ohira H, Tojo J, et al. Expression of human glucocorticoid receptor in lymphocytes of patients with autoimmune hepatitis. *Hepatol Res*. 2004;29:148–152.
20. Lauten M, Fernandez-Munoz I, Gerdes K, et al. Kinetics of the in vivo expression of glucocorticoid receptor splice variants during prednisone treatment in childhood acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. 2009;52:459–463.

Investigative Ophthalmology & Visual Science