Predictive value of genetic testing for inherited retinal diseases in patients with suspected atypical autoimmune retinopathy

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**Abstract**

**Purpose:** The clinical features of autoimmune retinopathy (AIR) can resemble and be difficult to differentiate from inherited retinal degenerations (IRDs). Misdiagnosis of an IRD as AIR causes unnecessary treatment with immunosuppressive agents. The purpose of this study is to calculate the predictive value of genetic testing for AIR in patients with suspected AIR and provide clinical examples where genetic testing has been useful.

**Methods:** We identified patients seen at MEEI between April 2013 and January 2017 for whom the differentiation of AIR vs. IRDs was difficult based on clinical assessment alone. All patients had some atypical features for AIR, but tested positive for anti-retinal antibodies. Within this group, we identified six patients who had genetic testing for IRDs with the Genetic Eye Disease panel for retinal genes (GEDi-R). We calculated the positive predictive value (PPV) and negative predictive value (NPV) of genetic testing in a population with approximately equal numbers of IRD and AIR patients.

**Results:** Six patients had clinical features that made distinguishing between IRDs and AIR on a clinical basis difficult and were sent for genetic testing: four women and two men with a mean age of 59.5 years. In two of these six patients, genetic diagnoses were made based upon the identification of known pathogenic variants in the common IRD genes USH2A and RHO. Two patients had variants of unknown significance within genes associated with IRDs, and the other two had no relevant genetic findings. Given the 60% sensitivity and 3% false positive rate for GEDi-R testing and assuming a 50% pre-test probability of having an IRD, the PPV for GEDi-R for detecting IRD is 95.2% and the NPV is 70.8%.

**Conclusions and Importance:** In patients for whom the differential diagnosis of AIR and IRDs is unclear based on clinical information, genetic testing can be a valuable tool when it identifies an IRD, sparing the patient unnecessary immunosuppressive treatment. However, the test has a low NPV so a negative genetic testing result does not confidently exclude IRD as the true diagnosis.

1. Introduction

Autoimmune retinopathy (AIR) is a rare blinding retinal disorder characterized by the presence of antiretinal autoantibodies (ARAs), electroretinogram (ERG) abnormalities, and visual field defects.1 The spectrum of AIR includes nonparaneoplastic AIR (npAIR), cancer-associated retinopathy (CAR), melanoma-associated retinopathy (MAR), and autoimmune-related retinopathy and optic neuropathy (AARON). Though the exact pathogenesis of AIR is not known, AIR is thought to be the result of an immunologic attack on the retina by ARAs causing damage to ocular tissues resulting in vision loss.2–4

The presence of ARAs is an essential criterion for the diagnosis of AIR. However, ARAs can also be present in patients with other autoimmune disorders as well as in normal controls.5 Due to the low specificity of ARAs and lack of distinctive clinical features, the diagnosis of AIR is usually made after the exclusion of inherited retinal degenerations (IRDs) and other retinal degenerative disorders.1 However, the differentiation between IRDs and AIR is not always clear. IRDs are a phenotypically diverse set of diseases that affect the function of photoreceptors and the retinal pigment epithelium (RPE).6 Retinitis pigmentosa (RP) is the most common IRD, and its clinical characteristics are particularly similar to those of AIR.7 There are some clinical features which help differentiate these two diagnoses. A typical AIR patient will be older than the average IRD patient. AIR tends to present with sudden or subacute vision loss while more slowly progressive vision loss is usually seen in IRDs. AIR patients often have a relatively normal retinal examination, especially at disease onset. In contrast, patients with RP often have pigmentedary changes on the fundus
examination as well as abnormalities in retinal imaging. IRD patients sometimes, but not always, have a family history of retinal disease. Further complicating the issue, reports of patients with hereditary RP developing secondary ARAs have been published.8,9

As the treatments for AIR and IRDs vary greatly, the difficulty in clinically differentiating between these diagnoses can greatly impact patient outcomes. For example, the misdiagnosis of an IRD as AIR causes unnecessary treatment with immunosuppressive agents. Side effects of immunosuppressive agents include an increased risk of infections. In a study of 30 AIR patients, 10% of the patients had to stop at least one immunosuppressive medication due to adverse effects.10

Distinguishing IRD patients from potential AIR patients is essential to setting patient outcome expectations, providing risk assessment to family members, administering proper treatment, and preventing unnecessary side effects from treatment.

A potential way to differentiate atypical AIR presentations from IRDs is through genetic testing; there are currently over 250 genes known to cause IRDs.11 Genetic testing for IRDs currently has a 50–60% sensitivity, with some testing achieving a sensitivity of more than 75% when using a clinically directed tiered testing strategy.12 A previous study has shown insight into the utility of genetic testing in distinguishing between IRDs,13 however no study has examined its utility in differentiation between IRDs and AIR.

In this retrospective case series, we examine the utility of genetic testing in distinguishing AIR from IRDs and providing a more accurate diagnosis that could spare patients unnecessary treatment with immunosuppressive therapy. We calculate the predictive value of genetic testing for IRDs in patients with suspected AIR and provide productive examples of this testing in clinical practice.

2. Methods

This retrospective case series was approved by the Massachusetts Eye and Ear Infirmary (MEEI) institutional review board. The study conformed to the tenets of the Declaration of Helsinki and HIPAA regulations.

We identified patients evaluated on both the Ocular Immunology and IRD Services at MEEI between April 2013 and January 2017 for whom the differentiation of AIR vs. IRD was questioned based on clinical assessment alone. We further identified the subset of these patients who had genetic testing for IRDs. All patients had some atypical features for AIR but tested positive for ARAs. The following data was collected for each patient: age at presentation, sex, clinical findings, visual acuity (VA), fundus photo interpretations, fluorescein angiography (FA) interpretations, full-field ERG results, optical coherence tomography (OCT) results, Goldmann Visual Field (GVF) results and ARAs, closest to the date of blood draw for genetic testing.

The best corrected VA, with correction by pinhole when applicable, was recorded. Full-field ERGs were performed with Burian-Allen electrodes (Hansen Labs, Coralville, Iowa, USA) at MEEI. Dim scotopic, bright scotopic, 30 Hz flicker amplitudes, and 30 Hz flicker implicit times were obtained. OCT imaging was performed with a spectral domain OCT instrument (Spectralis, Heidelberg, Germany). Fovea-centered images were acquired (25 lines within a 20-degree horizontal scan and 25 lines within a 20-degree vertical scan). GFV testing with I2e, I4e, and V4e test lights was performed. ARA testing was done by Western blot either at the Ocular Immunology Laboratory, Casey Eye Institute, Oregon Health Sciences University (Patients 1, 4, 5 and 6) or at the University of Michigan Medical School (Patients 2 and 3).

Genetic testing performed at The Ocular Genomic Institute at MEEI with Genetic Eye Disease panel for Retinal genes (GEDi-R) using next-generation sequencing. It is performed by selective capture of exon, splice sites and specific intrinsic variants for 267 genes associated with IRDs. A list of genes analyzed by GEDi-R can be found in Table 1. Variants were annotated using a custom human base-pair codon resource. Variant interpretation was performed according to American College of Medical Genetics practice guidelines.15–17

GEDi-R testing has a 60% clinical sensitivity in patients with IRDs and a false positive rate of approximately 3%.15 We calculated the positive predictive value (PPV) and negative predictive value (NPV) of genetic testing assuming a 33%, 50%, and 66% prevalence of IRDs in the population using standard formulas. The determination of the range of IRD prevalences was based on the clinical experience of the physician authors since there is a lack of data in the literature regarding this topic.

3. Results

Six patients with clinical features making differentiation between IRDs and AIR difficult underwent genetic testing: four women and two men with a mean age of 59.5 years. These patients presented with clinical features typical for both AIR and IRD, making the diagnosis difficult. For example, there may have been fundus findings atypical for but potentially consistent with an IRD in a middle-aged patient without a family history of retinal disease. Clinical characteristics of these six patients can be found in Table 2. In two of these six patients, genetic diagnoses were made based upon the identification of known pathogenic variants in the common IRD genes: USH2A [c.2299delG (p.Glu767Serfs) and c.2276G > T (p.Cys759Phe)] and RHO [c.745G > T (p.Glu249Ter)]. Genetic diagnoses were not identified for the other four patients. Table 3 shows the GEDi-R results, along with the ARA results and final diagnosis of each patient.

Pathogenic variants in IRD-causing genes were identified in patients GT-01 and GT-02 (see Table 3). Color fundus photos, fundus autofluorescence (FAF) photos, and OCT images of the central macula for patient GT-01 can be found in Fig. 1. The differential diagnosis for GT-01 included AIR vs. RP. This patient first presented with photopsias and initial visual fields suggesting acute idiopathic blind spot syndrome versus acute zonal occult outer retinopathy. Over several years, pericentral scotomata developed subjectively as well as on perimetry and were accompanied by nyctalopia. Multi-focal and full-field ERGs were suggestive of mild macular and periretinal photoreceptor degeneration. The full-field ERG pattern was atypically mild for RP: 50% decrease in scotopic amplitudes and normal 30 Hz amplitudes but prolonged implicit times. The FAF and OCT imaging demonstrated symmetric findings that, in combination with her ERGs, could be pericentral RP; however, the possibility of AIR was also raised. Taking into account the lack of family history of retinal disease and positive ARAs which included enolase, a common antibody found in patients with AIR,18 the diagnosis of GT-01 was ambiguous. GEDI-R testing revealed compound heterozygous recessive pathogenic mutations in the USH2A gene with parental segregation. Mutations in USH2A are known to cause autosomal recessive Usher syndrome as well as non-syndromic RP.

Color fundus photos, FAF, and OCT images of the central macula for patient GT-02 can be found in Fig. 2. GT-02 initially presented with photopsias. Further testing revealed slight superior GVF constriction as well as decreased cone and rod responses with a slight delay in implicit times on full-field ERG testing. GT-02 had a history of cutaneous squamous cell carcinoma and pulmonary nodules making CAR a possibility, particularly given his age at presentation. He tested positive for several ARAs and had no family history of IRDs, however, his symptoms were very slowly progressive for AIR and warranted further investigation. Genetic testing identified a heterozygous pathogenic mutation in RHO. In the absence of familial clinical data or genetic samples, it remained unclear whether this was a de novo mutation or if it was associated with a mild and undetected phenotype in other family members.

The genetic testing for the remaining four patients was inconclusive. GT-03 was a 59-year old Asian female who was thought to have AIR based on clinical features including inner retinal thinning. Of note, family history was significant for two siblings with congenital hearing loss but no reported vision loss. Genetic testing did not identify a clear genetic diagnosis. Testing did identify variants of unknown significance (VUSs)
in two genes known to cause autosomal recessive Usher syndrome (USH1C and USH2A; Table 3). Two additional VUSs were found genes associated with IRDs (EYS and SLC24A1; Table 3).

GT-04 presented with photopsia of the left eye. Upon examination she had visual field loss, pigmented features typical of RP, and an extinguished full-field ERG responses. It was thought his clinical findings help “rule in” a genetic disease, but a negative result does not “rule out” a genetic disease. In fact, Table 4 shows that this finding holds true over a range of clinical scenarios where a patient is more or less likely to have a genetic diagnosis before testing. This is a natural consequence of the imperfect sensitivity of the current genetic testing for IRDs (50–60 diagnostic rate). Another collection of patients may have different pre-genetic testing probabilities of having an IRD, based on the criteria used to assemble the cohort. For that reason, we provided PPV and NPV calculations for a variety of pre-test probabilities. With progress in diagnostics, more disease-causing variants in both known and undiscovered genes will be found, and the sensitivity and therefore the predictive value of genetic testing will increase. Similarly, the diagnostic testing for AIR (e.g. ARA testing) is imperfect in positively identifying AIR patients, and improvements in this testing would be very helpful as well. 20, 21

The results in Table 4 demonstrate that when IRD genetic testing is performed in a patient population with a moderate chance of having a genetic disease, such as our patient population, a positive genetic finding helps “rule in” a genetic disease, but a negative result does not “rule out” a genetic disease. In fact, Table 4 shows that this finding holds true over a range of clinical scenarios where a patient is more or less likely to have a genetic diagnosis before testing. This is a natural consequence of the imperfect sensitivity of the current genetic testing for IRDs (50–60 diagnostic rate). Another collection of patients may have different pre-genetic testing probabilities of having an IRD, based on the criteria used to assemble the cohort. For that reason, we provided PPV and NPV calculations for a variety of pre-test probabilities. With progress in diagnostics, more disease-causing variants in both known and undiscovered genes will be found, and the sensitivity and therefore the predictive value of genetic testing will increase. Similarly, the diagnostic testing for AIR (e.g. ARA testing) is imperfect in positively identifying AIR patients, and improvements in this testing would be very helpful as well. 20, 21

4. Discussion

The results in Table 4 demonstrate that when IRD genetic testing is performed in a patient population with a moderate chance of having a genetic disease, such as our patient population, a positive genetic finding helps “rule in” a genetic disease, but a negative result does not “rule out” a genetic disease. In fact, Table 4 shows that this finding holds true over a range of clinical scenarios where a patient is more or less likely to have a genetic diagnosis before testing. This is a natural consequence of the imperfect sensitivity of the current genetic testing for IRDs (50–60 diagnostic rate). Another collection of patients may have different pre-genetic testing probabilities of having an IRD, based on the criteria used to assemble the cohort. For that reason, we provided PPV and NPV calculations for a variety of pre-test probabilities. With progress in diagnostics, more disease-causing variants in both known and undiscovered genes will be found, and the sensitivity and therefore the predictive value of genetic testing will increase. Similarly, the diagnostic testing for AIR (e.g. ARA testing) is imperfect in positively identifying AIR patients, and improvements in this testing would be very helpful as well. 20, 21

The diagnosis of a genetic disorder not only avoids use of potentially ineffective anti-inflammatory or immunomodulatory therapies but also can provide risk information for other family members as well as potentially providing eligibility for gene-based therapies. As a result of our findings, we were able to definitely diagnose two patients with an IRD thus sparing them immunosuppressive therapy. All the patients in this series whose genetic results were equivocal or negative were treated for AIR with immunosuppression and counseled that there was still a small chance they have a genetic cause of their disease that we could not identify and that their exposure to immunosuppressive therapy would not be beneficial and could be potentially harmful. This point is controversial; there are published reports of AIR coexisting with IRD, and some clinicians have advocated immunosuppressive patients in this situation. The cases with genetic diagnoses are reminders of the spectrum of severity with which IRDs can manifest.
| Patient | Age/ Sex/ Race | Presentation Symptoms | VA | ERG results | OCT results | Fundus Results |
|---------|----------------|-----------------------|----|-------------|-------------|----------------|
| GT-01   | 41/ F/ CA      | pericentral scotomas, photopsias | OU: 20/20 | Easily measurable but depressed rod responses, 30 Hz cone flicker signals at the lower end of normal but with significantly prolonged implicit times. OS shows a depressed maximal combined response. Pericentral loss of photoreceptor bands (IS/OS line, external limiting membrane, and outer nuclear layer). | OD: mild PPA, arteriolar attenuation, outer retinal atrophy. OS: mild PPA, arteriolar attenuation, mild atrophic appearance in temporal macula, suggestion of outer retinal atrophy in superior peripheral retina. | 1-4e light revealed normal central vision. I-4e light revealed paracentral scotoma. |
| GT-02   | 54/M/ CA       | photopsias OD: 20/20 -2 OS: 20/25 -2 | | Mildly reduced cone and rod responses with minimally delayed implicit time in both eyes. No significant findings. OD: Pink optic disc, mildly attenuated vessels at the inferior quadrant, scattered peripheral pigment clumps and dropout worse at the inferior. OS: Pink optic disc, scattered peripheral pigment clumps and dropout worse at the inferior. | OD: irregular foveal contour; general inner retinal thinning. OS: general inner retinal thinning with loss of foveal contour. Pale, cupped nerves and attenuated vessels. V-4e light revealed general constriction. I-2e severely constricted. | OD: I-4e constricted to 18; V-4e constricted to 140\(^\circ\); midperipheral scotomas. OS: I-4e constricted to 10; V-4e constricted to 3; midperipheral scotomas. |
| GT-03   | 59/F/ AS       | photopsias, visual field constriction | OD: 20/63 -2 OS: 20/40 | Scotopic rod responses are barely recordable. Maximum combined responses are reduced. The 30 Hz cone signal is decreased and delayed. OD: irregular foveal contour; general inner retinal thinning. OS: general inner retinal thinning with loss of foveal contour. | OD: loss of IS/OS junction; overall thinning; subretinal hyperreflective foci; choroid is thin. OS: loss of IS/OS junction; subretinal hyperreflective foci; choroid is thin. | OD: I-4e light revealed general constriction. V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. OS: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. |
| GT-04   | 64/F/ CA       | nyctalopia, visual field constriction, and midperipheral scotomas OU: 20/20 | | No detectable rod responses and decreased cone responses. Decreased and delayed 30 Hz cone signal. Early attenuation of retinal blood vessels, mild pigment granularity in macula. OD: I-4e constricted to 18; V-4e constricted to 140\(^\circ\); midperipheral scotomas. OS: I-4e constricted to 10; V-4e constricted to 3; midperipheral scotomas. | OD: Blurred nerve margins, mild arteriolar attenuation, submacular hyperpigmentation, nasal bone picaules, and atrophy in macula. OS: Blurred nerve margins, mild arteriolar attenuation, submacular hyperpigmentation, nasal bone picaules, and atrophy in macula. | OD: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. OS: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. |
| GT-05   | 80/F/ CA       | nyctalopia, visual field constriction, decreased color vision, and central scotomas | | The rod isolated amplitudes are just below normal. The maximal responses show decreased rod responses and decreased rod responses show delay and delay. 30 Hz cone signal. OD: Blurred and poorly defined ELM/EZ complex that is mildly atrophied close to the center; ONL thin in peripheral retina. OS: Blurred and poorly defined ELM/EZ complex that is mildly atrophied close to the center; ONL thin in peripheral retina. | OD: loss of IS/OS junction; outer retinal thinning with loss of outer nuclear layer. OS: loss of IS/OS junction; outer retinal thinning with loss of outer nuclear layer. | OD: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. OS: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. |
| GT-06   | 59/M/ CA       | photophobia, central scotomas | OD: 20/600 -3 OS: 20/500 -1 | Reduced and delayed cone ERGs. Rod responses are again non-detectable. Decreased 30 Hz signal amplitude. Blurred and poorly defined ELM/EZ complex that is mildly atrophied close to the center; ONL thin in peripheral retina. OS: Blurred and poorly defined ELM/EZ complex that is mildly atrophied close to the center; ONL thin in peripheral retina. | OD: Blurred nerve margins, mild arteriolar attenuation, submacular hyperpigmentation, nasal bone picaules, and atrophy in macula. OS: Blurred nerve margins, mild arteriolar attenuation, submacular hyperpigmentation, nasal bone picaules, and atrophy in macula. | OD: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. OS: I-4e light revealed general constriction; V-4e light revealed midperipheral scotomas. Both eyes with marginal atrophy, mild peripheral pigment changes. |

**CA** = Caucasian; **AS** = Asian; **VA** = Best-corrected visual acuity; **ERG** = Electroretinogram; **OCT** = Optical coherence tomography; **GVF** = Goldmann visual fields; **OD** = right eye; **OS** = left eye; **OU** = both eyes; **IS/OS** = inner and outer segment; **ELM** = external limiting membrane; **EZ** = ellipsoid zone; **PVD** = posterior vitreous detachment; **BRG** = Bruch's membrane.
Table 3
Results of GEDi-R genetic testing for IRDs, ARA testing, and final diagnosis of all patients in the study.

| Patient   | Final Diagnosis | ARAs                                      | Immunohistochemistry                                      | Genomic location<sup>a</sup> | Gene     | Type          | Genetic Variant | ExAC Frequency (total frequency) | GERP<sup>b</sup> SIFT and PolyPhen-2 predictions | Pathogenesis           |
|-----------|-----------------|-------------------------------------------|-----------------------------------------------------------|-------------------------------|----------|---------------|------------------|---------------------------------|-----------------------------------------------|------------------------|
| Patients with positive GEDI-R results (N = 2) |                  |                                           |                                                            |                               |          |               |                  |                                 |                                |                        |
| GT-01     | IRD 36-kDa (GADPH), 46-kDa (enolase), and 112-kDa (tubulin, GAPDH, Rab6) | moderate staining of outer segments in photoreceptor cells, inner plexiform and nerve fiber layers | Chr1:216420437 USH2A het | c.2299delG (p.Glu767Serfs) | 96/121284 | NA            | NA               | Pathogenic                      |                                |                        |
| GT-02     | IRD 16-kDa, 20 kDa, 26 kDa, and 36 kDa (GADPH, GAPDH, Rab6) | NA                                         | Chr2:112722783 MERTK het | c.773C > A (p.Ala258Glu)    | 20/121384 | NA            | NA               | Pathogenic                      |                                |                        |

Patients with negative GEDI-R results (N = 4)

| Patient | Final Diagnosis | ARAs                                      | Immunohistochemistry                                      | Genomic location<sup>a</sup> | Gene     | Type          | Genetic Variant | ExAC Frequency (total frequency) | GERP<sup>b</sup> SIFT and PolyPhen-2 predictions | Pathogenesis           |
|---------|-----------------|-------------------------------------------|-----------------------------------------------------------|-------------------------------|----------|---------------|------------------|---------------------------------|-----------------------------------------------|------------------------|
| GT-03   | AIR/optic neuropathy | aldolase, enolase, GAPDH, Rabα | moderate staining of the outer and inner nuclear layers and ganglion cell layer in human retina | Chr1:215847770 USH2A het | c.13483C > T (p.Arg449Cys) | 15/121332 | 4.22          | NA/Benign       | VUS                             |                                |                        |
| GT-04   | npAIR 40-kDa (aldolase), 42-kDa (GADPH, Rabα) | strong staining of the outer limiting membrane and mild staining in the bipolar cell layer | – – – – – – – – | – – – – – – | – – – – – | – – – – | – – – – | – – – – | – – – – | – – – – |
| GT-05   | AIR/CAR-maculopathy 70-72-kDa (carbonic anhydrase II) and 46-kDa (enolase) | moderate staining of the bipolar cell layer | Chr1:212722783 MERTK het | c.773G > A (p.Ala258Glu) | 20/121384 | 5.6           | Deleterious and Possibly DAMAGING | VUS                             |                                |                        |

GEDi-R = Genetic Eye Disease Panel for Retinal Genes; AIR = autoimmune retinopathy; npAIR = non-paraneoplastic retinopathy; CAR = cancer associated retinopathy; IRD = inherited retinal disease; RP = retinitis pigmentosa; ARAs = antiretinal antibodies; chr = chromosome; GADPH = Glyceraldehyde 3-phosphate dehydrogenase; GAPDH = glyceraldehyde 3-phosphate dehydrogenase; PKM2 = pyruvate kinase muscle isoform M2; Rab6 = Ras-related protein Rab-6A; het = heterozygous; SIFT = Sorting Intolerant from Tolerant; PolyPhen-2 = Polymorphism Phenotyping v2; GERP = genomic evolutionary rate profiling; ExAC = Exome Aggregation Consortium; VUS = variant of uncertain significance.

<sup>a</sup> Genomic location based on GRCh37 assembly.
<sup>b</sup> GERP range: 12.3 to 6.17 where variants with higher scores being more conserved.
4.1. Application to our cohort

In six patients who had a differential diagnosis of AIR vs. IRD, two had genetically confirmed diagnosis of an IRD and four had no genetic diagnoses. While the emphasis of this report is on the use of genetic testing to distinguish IRD from AIR, it is also important to apply the diagnostic criteria\(^1\) for these disorders clinically before considering ARA and/or genetic testing. Proper application of the criteria can point towards the correct diagnoses, thus avoiding unnecessary blood testing. For example, FAF findings consistent with an IRD could lead the clinician away from an AIR diagnosis and preclude need for ARA testing.

The known IRD mutations found in our patients were found in the genes RHO and USH2A. GT-01 had compound heterozygous mutations in USH2A which were confirmed to be bi-parentally inherited. USH2A

Fig. 1. Imaging for patient GT-01 where GEDI-R testing returned positive for known pathogenic mutations in USH2A. Wide field fundus photographs of the right (A) and left (B) eyes show arteriolar attenuation and areas outer retinal atrophy. Wide-field fundus autofluorescence of the right (C) and left (D) eyes show hypoautofluorescent areas corresponding to the areas of outer retinal atrophy in the fundus photographs. E and F: Optical coherence tomography of the macula of the right (E) and left (F) eyes shows pericentral loss of photoreceptor bands.
encodes Usherin, a protein found in the basement membrane and thought to be important in the development and homeostasis of the inner ear and retina. The two USH2A mutations identified in GT-01 are among the most common mutations in USH2A-related retinal disease. While USH2A-associated vision loss usually progresses to a greater degree by adulthood than what was observed in this patient, mutations in the USH2A gene can display a wide phenotypic spectrum as exemplified by this patient and potentially lead to the overlap with an AIR-like presentation.

The second patient with positive genetic testing, GT-02, had a single heterozygous mutation in the gene RHO. RHO encodes rhodopsin, a photosensitive protein found exclusively in rod cells. While mutations in RHO are associated with autosomal dominant RP, mutations in this gene can also cause autosomal recessive disease. The c.745G > T mutation identified in our patient has been reported to cause autosomal recessive RP. Rosenfeld et al. reported that while heterozygous carriers of this variant had a normal ophthalmologic exams, ERG testing demonstrated decrease rod signals. This may explain the mild nature...
Table 4

|                      | GEDi-R |
|----------------------|--------|
| Prevalence           | 66%    |
| Sensitivity          | 60%    |
| Specificity          | 97%    |
| PPV                  | 97.5%  |
| NPV                  | 55.5%  |

PPV = positive predictive value; NPV = negative predictive value.

of symptoms in patient GT-02.

In addition to the pathogenic mutations identified in our cohort, seven heterozygous VUSs were identified across seven genes. None of these seven variants, which all occurred in genes associated with autosomal recessive inheritance, were accompanied by a second variant in the same gene. It is not uncommon for patients with no ocular disease to have a number of VUSs.

In conclusion, genetic testing can be a valuable tool when it identifies an IRD in a patient for whom the differential diagnosis of AIR versus IRD is unclear based only on clinical information, thus sparing the patient unnecessary treatment with immunosuppressive agents. However, the test has a low NPV, meaning that a negative genetic testing result does not confidently exclude IRD as the true diagnosis. We presented cases demonstrating how IRD genetic testing can be successfully utilized in a patient population with moderate risk of IRD.

Patient consent

This study was approved by the Massachusetts Eye and Ear Infirmary institutional review board. The study conformed to the tenets of the Declaration of Helsinki and HIPAA regulations.

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Conflicts of interest

The following authors have no financial disclosures: LKS, EMP, JC, RMH, LS.

The Massachusetts Eye and Ear Infirmary (which employs EMP, JC, RMG, and LS) offers the diagnostic genetic test for inherited retinal degenerations (GEDi-R) described in this manuscript.

Authorship

All authors attest that they meet the current ICJME criteria for Authorship.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ajoc.2019.100461.

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