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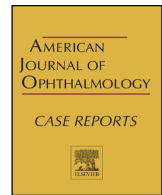
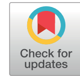


Predictive value of genetic testing for inherited retinal diseases in patients with suspected atypical autoimmune retinopathy



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A R T I C L E I N F O

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A B S T R A C T

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 IRDs 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 of 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 pigmentary changes on the fundus

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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). GVF 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.[14] It is performed by selective capture of exon, splice sites and specific intronic 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%.[14] 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.[18] 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 big blind spot syndrome versus acute zonal occult outer retinopathy. Over several years, pericentral scotomas developed subjectively as well as on perimetry and were accompanied by nyctalopia. Multi-focal and full-field ERGs were suggestive of mild macular and panretinal 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,[19] 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 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)

Table 1
The 267 genes analyzed using GEDi-R. All known isoform of the tabulated genes described below have been accounted for in this design. Whole mitochondrial genome sequencing is performed with this panel.

ABCA4	BBS5	CERKL	DHX38	GRM6	JAG1	MVK	PCDH15	PRCD	RLBP1	TEAD1	UNC119
ABCC6	BBS7	CHM	DRAM2	GRN	KCNJ13	MYO7A	PCYT1A	PRDM13	ROM1	TEK	USH1C
ABHD12	BBS9	CIB2	DTHD1	GUCA1A	KCNV2	NDP	PDE6A	PROM1	RP1	TIMM8A	USH1G
ACBD5	BEST1	CLN3	EFEMP1	GUCA1B	KCTD7	NEK2	PDE6B	PRPF3	RP1L1	TIMP3	USH2A
ADAM9	C1QTNF5	CLN5	ELOVL4	GUCY2D	KIAA1549	NEUROD1	PDE6C	PRPF31	RP2	TMEM126A	VCAN
ADAMTS18	C21orf2	CLN6	EMC1	HARS	KIF11	NMNAT1	PDE6G	PRPF4	RP9	TMEM231	VPS13B
AFG3L2	C2ORF71	CLN8	ERCC6	HMX1	KIZ	NPHP1	PDE6H	PRPF6	RPE65	TMEM237	WDPCP
AHI1	C5orf42	CLRN1	EYS	IDH3B	KLHL7	NPHP3	PDZD7	PRPF8	RPGR	TMEM67	WDR19
AIPL1	C8orf37	CNGA1	FAM161A	IFT122	LCA5	NPHP4	PEX1	PRPH2	RPGRIP1	TOPORS	WDR34
ALMS1	CA4	CNGA3	FLVCR1	IFT140	LRAT	NR2E3	PEX10	RAB28	RPGRIP1L	TPP1	WDR35
ARL13B	CABP4	CNGB1	FOXF2	IFT172	LRIT3	NRL	PEX14	RAX2	RS1	TREX1	WFS1
ARL2BP	CACNA1F	CNGB3	FSCN2	IFT27	LRP5	NYX	PEX16	RBP3	SAG	TRIM32	ZNF408
ARL6	CACNA2D4	CNNM4	FZD4	IFT43	LZTFL1	OAT	PEX19	RBP4	SDCCAG8	TRPM1	ZNF423
ASRGL1	CAPN5	COL11A1	GDF6	IFT80	MAK	OCA2	PEX2	RCBTB1	SEMA4A	TSPAN12	ZNF513
ATF6	CC2D2A	COL2A1	GNAT1	IFT88	MAPKAPK3	OFD1	PEX5	RD3	SLC24A1	TTC21B	
ATXN7	CDH23	COL9A1	GNAT2	IKBKG	MERTK	OPA1	PEX6	RDH12	SLC25A46	TTC8	
BBIP1	CDH3	CRB1	GNB1	IMPDH1	MFN2	OPA3	PEX7	RDH5	SLC45A2	TTLL5	
BBIP1	CDHR1	CRX	GNPTG	IMPG1	MFRP	OPN1LW	PHYH	REEP6	SLC4A5	TTPA	
BBS1	CEP164	CSPP1	GPR125	IMPG2	MFSD8	OPN1MW	PITPNM3	RGR	SLC7A14	TUB	
BBS10	CEP290	CSPP1	GPR143	INPP5E	MIR204	OPN1SW	PLA2G5	RGS9	SNRNP200	TUBGCP4	
BBS12	CEP41	CYP4V2	GPR179	INVS	MKKS	OTX2	PNPLA6	RGS9BP	SPATA7	TULP1	
BBS2	CEP78	DFNB31	GPR98	IQCB1	MKS1	PANK2	POC1B	RHO	SPP2	TYR	
BBS4	CEP83	DHDDS	GRK1	ITM2B	MTTP	PAX2	PPT1	RIMS1	SRD5A3	TYRP1	

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, pigmentary features typical of RP, and an extinguished full-field ERG in the left eye. The right eye was normal. She further developed night blindness and floaters. Initially, she was thought to have unilateral late onset RP, although unilateral presentation of RP is very rare. About two years later, she began to exhibit visual field constriction and ERG revealed marked attenuation of right eye retinal function. At this point, rapid progression of her disease was thought to be unusually fast for RP and AIR was considered in the differential diagnosis. ARAs were detected in her serum, and GEDi-R testing was negative in this patient.

GT-05 presented with symptoms indicative of AIR including glare at night, decreased color vision, and large central scotomas. She had a normal rod responses and low normal cone responses on ERG. Medical history was significant for Crohn's disease and breast cancer. FAF results showed a speckled bull's eye pattern of pigment loss which is atypical in AIR patients. ARAs were detected. Genetic testing, however, did not identify any pathogenic variants but did identify two VUSs in two genes known to cause IRDs (*CEP290* and *MERTK*; Table 3).

GT-06 presented with an inability to see in bright lights, trouble looking at objects against a white background, and "black and white throbbing spots." Upon examination he was found to have bilateral central scotomas, sub-normal vision, abnormal multi-focal ERG, and reduced and delayed full-field ERG responses. It was thought his clinical symptoms were consistent with a rod-cone dystrophy such as RP, but the clinical picture was atypical given preferential central versus peripheral retinal involvement. The progression of disease was also more rapid than normally seen in RP patients and as a result he was sent for GEDi-R testing which was negative. ARAs were detected in his serum.

For the purposes of calculating PPV and NPV, we roughly estimated that in the cohort of patients used in this study, the prevalence of IRD was 50%, before genetic testing. Given the 60% sensitivity and 3% false positive rate of GEDi-R testing, we calculated the PPV of genetic testing to be 95.2% and the NPV to be 70.8%. Different clinicians might produce a cohort leaning more toward one diagnosis or the other. Table 4 demonstrates how the predictive value changes in higher- or lower-probability cohorts/patients. These calculations rely only on the equations defining sensitivity and specificity and the known characteristics of the genetic test, rather than using any data explicitly from our cohort. If a similar population with an estimated 33% IRD incidence were tested in the future, the PPV and NPV would be 90.8% and 83.1%, respectively. In a population with 66% estimated prevalence of IRD, the PPV and NPV would be 97.5% and 55.5%, respectively.

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).[14] 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 immunosuppressing patients in this situation.[8,10] The cases with genetic diagnoses are reminders of the spectrum of severity with which IRDs can manifest.

Table 2
Patient clinical characteristics.

Patient	Age/Sex/Race	Presentation Symptoms	VA	ERG results	OCT results	Fundus Results	GVF
GT-01	41/F/CA	pericentral scotomas, photopsias, nyctalopia	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 in temporal mid-periphery. OS: mild PPA, arteriolar attenuation, mild atrophic appearance in temporal macula; suggestion of outer retinal atrophy in temporal macula superonasal to nerve and superior to arcade	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.	I-4e and V-4e subtle superior field constriction
GT-03	59/F/AS	photopsias, visual field constriction	OD: 20/63 -2 +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.	Pale, cupped nerves and attenuated vessels	V-4e light revealed general constriction. I-2e severely constricted.
GT-04	64/F/CA	nyctalopia, visual field constriction, and mid-peripheral scotomas	OU: 20/20	No detectable rod responses and decreased cone responses. Decreased and delayed 30 Hz cone signal.	Thinning of photoreceptor layer peripherally.	Early attenuation of retinal blood vessels, mild pigment granularity in macula	OD: I-4e constricted to 18; V-4e constricted to 140°; midperipheral scotomas. OS: I-4e constricted to 10°; V-4e constricted to 3°; midperipheral scotomas
GT-05	80/F/CA	nyctalopia, visual field constriction, decreased color vision, and central scotomas	OD: 20/40 -2 OS: 20/50 -1	The rod isolated amplitudes are just below normal. The maximal responses show amplitudes in the low normal range. The cone isolated and 30 Hz cone flicker responses show subnormal amplitudes, however the implicit times are normal.	OD: loss of IS/OS junction; overall thinning; subretinal hyper-reflective foci; choroid is thin OS: loss of IS/OS junction; subretinal hyper-reflective foci; choroid is thin	PVD, RPE mottling and atrophy throughout macula, mild peripheral pigment changes	NA
GT-06	59/M/CA	Photophobia, central scotomas	OU: 20/600 -3	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 subtly attenuated close to the center; ONL thin in peripheral macula without recognizable ELM/EZ	OD: Blurred nerve margins, mild arteriolar attenuation, subtle areas of increased paravenous pigmentation, nasal bone spicules OS: Large vitreous opacity, blurred nerve margins, mild arteriolar attenuation, subtle areas of increased paravenous pigmentation	OD:I-4e light revealed general constriction; V-4e light revealed mid-peripheral scotoma; unable to see I-2e OS: I-4e light revealed temporal island; V-4e light revealed central scotoma; unable to see I-2e

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; ONL = outer nuclear layer; PVD = posterior viterous detachment; RPE = retinal pigment epithelium; PPA = peripapillary atrophy.

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[a]	Gene	Type	Genetic Variant	ExAC Frequency (total frequency)	GERP[b]	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	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 and NA	Pathogenic
				Chr1:216420460	*USH2A*	het	c.2276G > T (p.Cys759Phe)	95/121178	5.79	Deleterious Probably Damaging	Pathogenic
				Chr7:92118724	*PEX1*	het	c.3650T > G, p.(Met1217Arg)	18/276762	5.320	Tolerated and Benign	VUS
GT-02	IRD	16-kDa, 20-kDa, 26-kDa, and 36-kDa	NA	Chr3:129251424	*RHO*	het	c.745G > T (p.Glu249Ter)	6/246242	5.510	NA/NA	Pathogenic
Patients with negative GEDI-R results (N = 4)											
GT-03	AIR/optic neuropathy	aldolase, enolase, tubulin, PKM2, GADPH, Rab6	moderate staining of the outer and inner nuclear layers and ganglion cell layer in human retina	Chr11:17539012	*USH1C*	het	c.1220G > A (p.Gly407Glu)	24/41422	4.41	Tolerated and Benign	VUS
				Chr15:65917980	*SLC24A1*	het	c.1562G > A (p.Ser407Asn)	3/120776	5.39	Tolerated and Possibly Damaging	VUS
				Chr6:66112486	*EYS*	het	c.1069A > T, p.(Ile357Phe)	Absent	2.18	Tolerated and Possibly Damaging	VUS
				Chr1:215847770	*USH2A*	het	c.13483C > T (p.Arg4495Cys)	15/121332	4.22	NA and Benign	VUS
GT-04	npAIR	40-kDa (aldolase), 42-Kda, and 46-kDa (enolase)	strong staining of the outer limiting membrane and mild staining in the bipolar cell layer	–	–	–	–	–	–	–	–
GT-05	AIR/CAR-maculopathy	70-72-kDA	moderate staining of the bipolar cell layer	Chr12:88462318	*CEP290*	het	c.6116A > G (p.Asp2039Gly)	31/96416	3.32	NA and Benign	VUS
				Chr2:112722783	*MERTK*	het	c.773C > A (p.Ala258Glu)	20/121384	5.6	Deleterious and Probably Damaging	VUS
GT-06	AIR (possibly on top of RP)	30 –kDa (carbonic anhydrase II) and 46-kDa (enolase)	moderate staining of the outer segments in photoreceptor cells	–	–	–	–	–		–	–

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; PKM2 = Pyruvate kinase muscle isozyme 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.

[I]multiethnic frequency includes African, European, East Asian, South Asian, and Latino populations.

[a] Genomic location based on GRCh37 assembly.

[b] GERP range: 12.3 to 6.17 where variants with higher scores being more conserved.

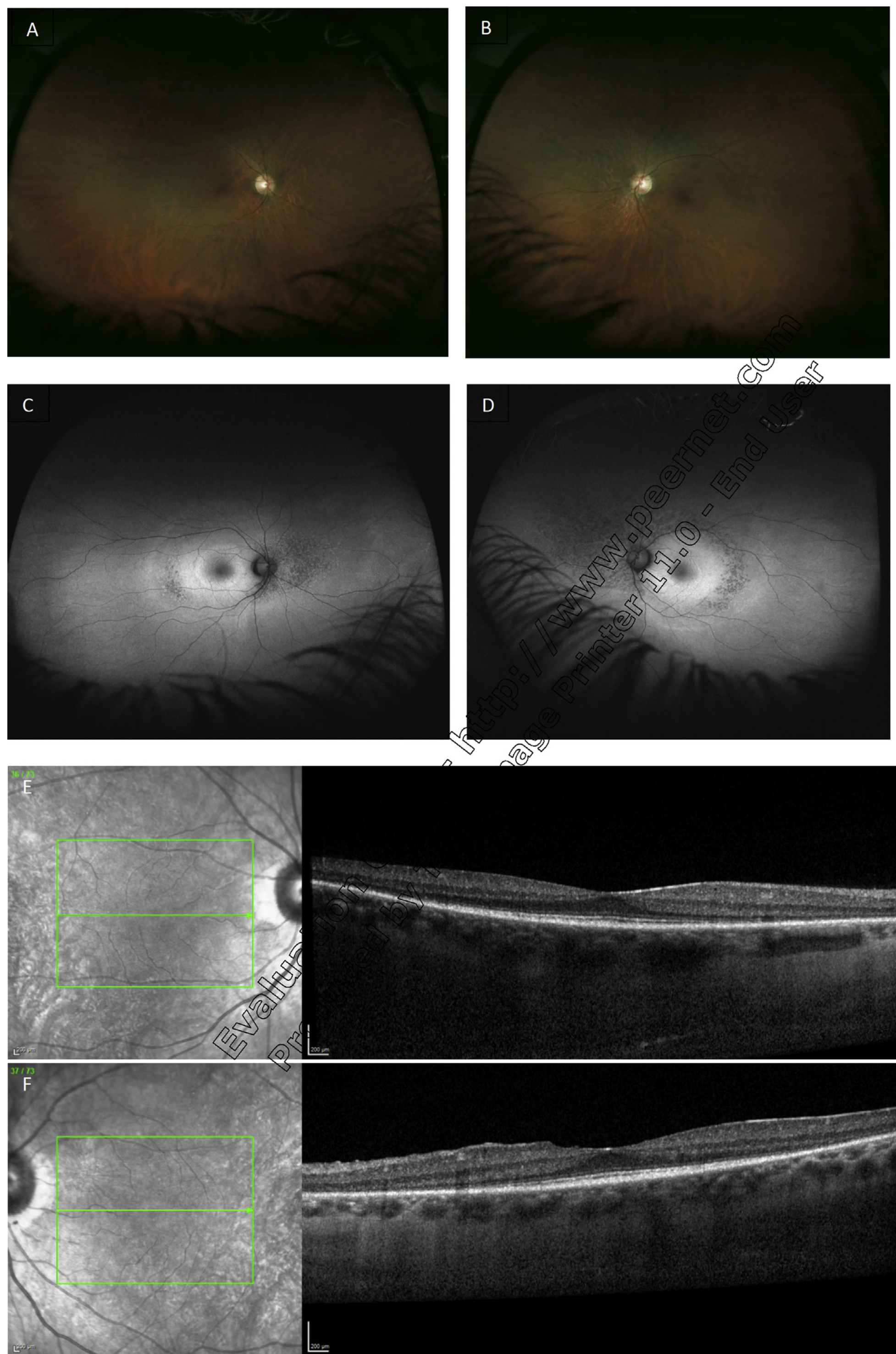

Fig. 1. Imaging for patient GT-01 where GEDi-R testing returned positive for known pathogenic mutations in *USH2A*. Wide field fundus photographs of the right (A) and left (B) eyes show arteriolar attenuation and areas outer retinal atrophy. Wide-field fundus autofluorescence of the right (C) and left (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.

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*

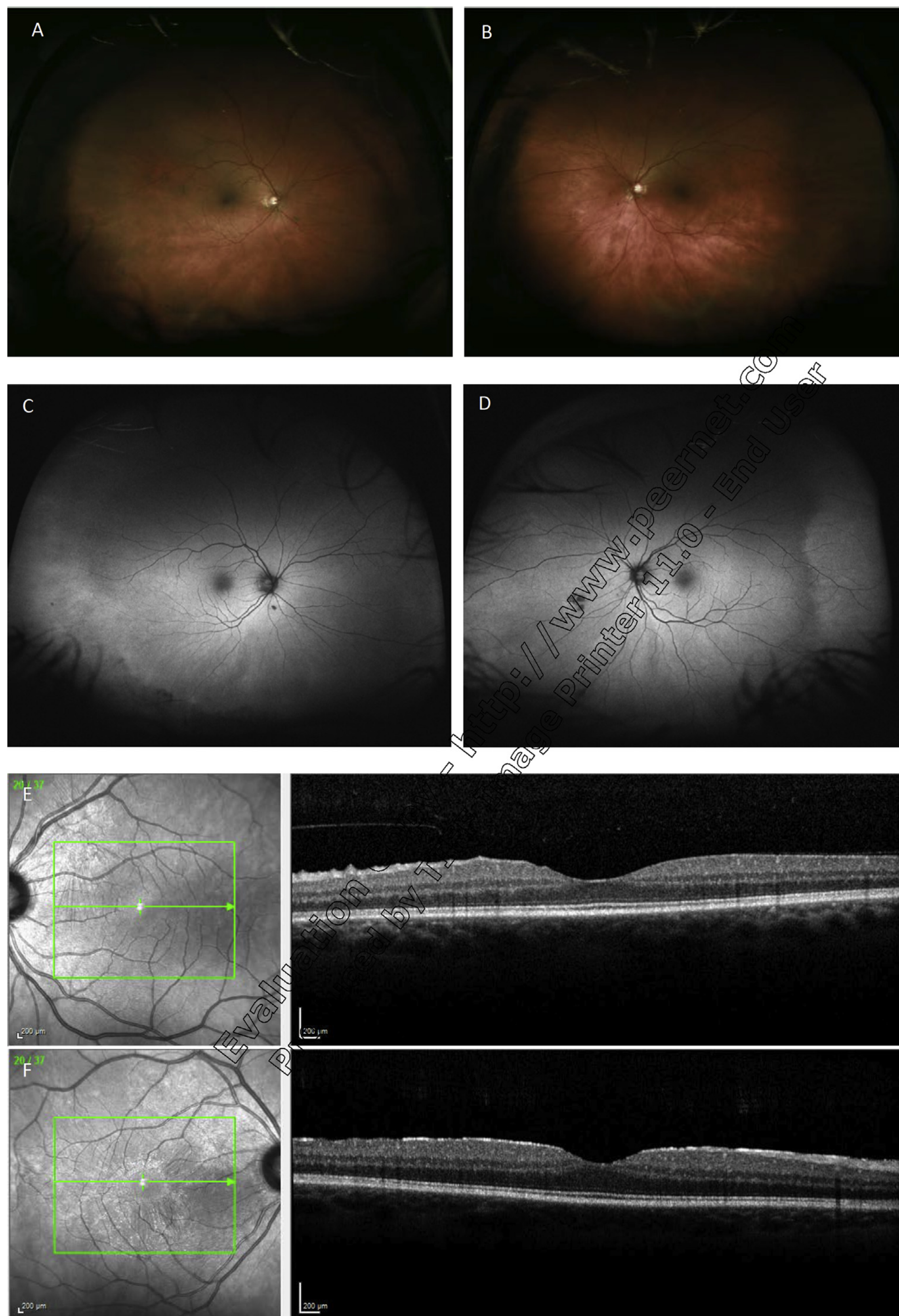

Fig. 2. Imaging for patient GT-02 where GEDi-R testing returned positive for a known pathogenic mutation in *RHO*. Wide field fundus photographs of the right (A) and left (B) eyes scattered areas of peripheral pigment loss/drops out. Wide-field fundus autofluorescence of the right (C) and left (D) eyes show some areas of hypoautofluorescence corresponding to areas of RPE loss in the nasal mid-periphery of the left eye. E and F: Optical coherence tomography of the macula of the right (E) and left (F) eyes shows no abnormalities.

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.[22] 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.[23]

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.[24] 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.[25] Rosenfeld et al. reported that while heterozygous carriers of this variant had a normal ophthalmologic exams, ERG testing demonstrated decrease rod signals.[25] This may explain the mild nature

Table 4
Sensitivity and specificity calculations for Genetic Eye Disease Panel for Retinal Genes (GEDi-R) genetic testing for populations with varying risks of inherited retinal diseases.

	GEDi-R		
Prevalence	66%	50%	33%
Sensitivity	60%	60%	60%
Specificity	97%	97%	97%
PPV	97.5%	95.2%	90.8%
NPV	55.5%	70.8%	83.1%

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.[17] 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.[26]

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.

References

1. Fox AR, Gordon LK, Heckenlively JR, et al. Consensus on the diagnosis and management of nonparaneoplastic autoimmune retinopathy using a modified delphi approach. *Am J Ophthalmol.* 2016;168:183–190.
2. Adamus G. Autoantibody targets and their cancer relationship in the pathogenicity of paraneoplastic retinopathy. *Autoimmun Rev.* 2009;8(5):410–414.
3. Adamus G, Machnicki M, Elerding H, et al. Antibodies to recoverin induce apoptosis of photoreceptor and bipolar cells in vivo. *J Autoimmun.* 1998;11(5):523–533.
4. Thirkill CE, FitzGerald P, Sergott RC, et al. Cancer-associated retinopathy (CAR syndrome) with antibodies reacting with retinal, optic-nerve, and cancer cells. *N Engl J Med.* 1989;321(23):1589–1594.
5. Shimazaki K, Jirawuthiworavong GV, Heckenlively JR, Gordon LK. Frequency of anti-retinal antibodies in normal human serum. *J Neuro Ophthalmol.* 2008;28(1):5–11.
6. Sahel JA, Marazova K, Audo I. Clinical characteristics and current therapies for inherited retinal degenerations. *Cold Spring Harb Perspect Med.* 2014;5(2):a017111.
7. Grange L, Dalal M, Nussenblatt RB, Sen HN. Autoimmune retinopathy. *Am J Ophthalmol.* 2014;157(2):266–272 e1.
8. Heckenlively JR, Jordan BL, Aptsiauri N. Association of antiretinal antibodies and cystoid macular edema in patients with retinitis pigmentosa. *Am J Ophthalmol.* 1999;127(5):565–573.
9. Heckenlively JR, Solish AM, Chant SM, Meyers-Elliott RH. Autoimmunity in hereditary retinal degenerations. II. Clinical studies: antiretinal antibodies and fluorescein angiogram findings. *Br J Ophthalmol.* 1985;69(10):758–764.
10. Ferreyra HA, Jayasundera T, Khan NW, et al. Management of autoimmune retinopathies with immunosuppression. *Arch Ophthalmol.* 2009;127(4):390–397.
11. Hafler BP. Clinical progress in inherited retinal degenerations: gene therapy clinical trials and advances in genetic sequencing. *Retina.* 2017;37(3):417–423.
12. Stone EM, Andorf JL, Whitmore SS, et al. Clinically focused molecular investigation of 1000 consecutive families with inherited retinal disease. *Ophthalmology.* 2017;124(9):1314–1331.
13. Men CJ, Bujakowska KM, Comander J, et al. The importance of genetic testing as demonstrated by two cases of CACNA1F-associated retinal generation misdiagnosed as LCA. *Mol Vis.* 2017;23:695–706.
14. Consugar MB, Navarro-Gomez D, Place EM, et al. Panel-based genetic diagnostic testing for inherited eye diseases is highly accurate and reproducible, and more sensitive for variant detection, than exome sequencing. *Genet Med.* 2015;17(4):253–261.
15. Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res.* 2002;30(17):3894–3900.
16. Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003;31(13):3812–3814.
17. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med.* 2015;17(5):405–424.
18. Simundic AM. Measures of diagnostic accuracy: basic definitions. *EJIFCC.* 2009;19(4):203–211.
19. Weleber RG, Watzke RC, Shults WT, et al. Clinical and electrophysiologic characterization of paraneoplastic and autoimmune retinopathies associated with antienolase antibodies. *Am J Ophthalmol.* 2005;139(5):780–794.
20. Sobrin L. Progress toward precisely diagnosing autoimmune retinopathy. *Am J Ophthalmol.* 2018;188:xiv–xv.
21. Heckenlively JR, Lundy SK. Autoimmune retinopathy: an immunologic cellular-driven disorder. *Adv Exp Med Biol.* 2018;1074:193–201.
22. Bhattacharya G, Miller C, Kimberling WJ, Jablonski MM, Cosgrove D. Localization and expression of usherin: a novel basement membrane protein defective in people with Usher's syndrome type IIa. *Hear Res.* 2002;163(1-2):1–11.
23. Sandberg MA, Rosner B, Weigel-DiFranco C, et al. Disease course in patients with autosomal recessive retinitis pigmentosa due to the USH2A gene. *Invest Ophthalmol Vis Sci.* 2008;49(12):5532–5539.
24. Dryja TP, McGee TL, Hahn LB, et al. Mutations within the rhodopsin gene in patients with autosomal dominant retinitis pigmentosa. *N Engl J Med.* 1990;323(19):1302–1307.
25. Rosenfeld PJ, Cowley GS, McGee TL, et al. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. *Nat Genet.* 1992;1(3):209–213.
26. Shashi V, McConkie-Rosell A, Schoch K, et al. Practical considerations in the clinical application of whole-exome sequencing. *Clin Genet.* 2016;89(2):173–181.