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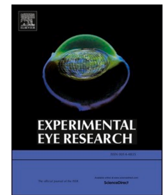

Research article

Anti-retinal IgG antibodies in patients with early and advanced type 2 macular telangiectasia

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ABSTRACT

Type 2 idiopathic macular telangiectasia (MacTel-2) is a progressive adult-onset macular disease associated with bilateral perifoveal vascular changes, Muller cell degeneration and increased blood-retinal barrier permeability. The pathophysiological mechanisms of MacTel-2 remain unclear, however it was previously reported that anti-retinal antibodies in MacTel-2 patients are a significant feature of the disease. In this study, we aimed to compare the prevalence of anti-retinal antibodies in patients MacTel-2, healthy controls and patients with other retinal diseases. MacTel-2 patients diagnosed with multimodal imaging were enrolled and their disease severities were graded using spectral-domain optical coherence tomography. For comparison, patients with age-related macular degeneration (AMD), inherited retinal diseases (IRDs) or no retinal disease (healthy controls) were recruited as controls. Blood serum samples were screened for immunoglobulin G anti-retinal antibodies by western blotting, followed by densitometry analysis. Odds ratios (OR) with 95% confidence intervals (CI) were calculated and $p < 0.05$ considered statistically significant. Overall, anti-retinal antibody-positive cases were older (64 ± 15 *vs* 53 ± 17 years, $p < 0.001$) and females were more likely to develop anti-retinal antibodies (OR: 2.41, CI: 1.12–5.18). The frequency of anti-retinal antibody detection in MacTel-2 patients ($n = 42$, 36%) was not significantly different from healthy controls ($n = 52$, 25%) or IRD patients ($n = 18$, 25%) and the majority of MacTel-2 patients had no anti-retinal antibodies. In contrast, the frequency of anti-retinal antibody detection was significantly higher in patients with AMD ($n = 15$, 73%, $p < 0.001$). The lack of a greater anti-retinal antibody frequency or specificity in the MacTel-2 cohort suggests that antibody mediated immunological mechanisms may play a less significant role in MacTel-2 disease pathogenesis.

Abbreviations: AMD, age-related macular degeneration; ARA, anti-retinal antibody; BRB, blood-retinal barrier; BCVA, best-corrected visual acuity; CI, confidence interval; IRDs, inherited retinal diseases; MacTel-2, Type 2 idiopathic macular telangiectasia; OR, Odds ratio; SD, standard deviation; SD-OCT, spectral-domain optical coherence tomography.

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1. Introduction

Type 2 idiopathic macular telangiectasia (MacTel-2) is a progressive adult-onset macular disease associated with bilateral perifoveal vascular changes, Muller cell degeneration and increased blood-retinal barrier (BRB) permeability (Charbel Issa et al., 2013; Powner et al., 2010). The breakdown of the BRB is also a feature of other, more common retinal diseases including age-related macular degeneration (AMD) and inherited retinal diseases (IRDs) (Adamus, 2015; Ahn et al., 2006; Fishman et al., 1986). Impaired function of the BRB may release retinal antigens to the systemic circulation, triggering non-specific autoimmune antibody-antigen responses that may contribute to retinal disease pathogenesis and progression (Vinores et al., 2001; Zhu et al., 2013).

An association between anti-retinal antibodies (ARAs) and MacTel-2 was previously reported by Zhu et al. (2013) in a cohort of 45 patients (69%) compared to 58 healthy controls (16%). The three most prevalent ARAs in MacTel-2 patients were found to bind the 40 kilo-Dalton (kDa) creatine kinase type B (CK-B, 20%), the 120 kDa retinol-binding protein 3 (RBP3, 24%) and the 150 kDa glycogen debranching enzyme (AGL, 9%) (Zhu et al., 2013). Although each of these specific ARA were present in less than a quarter of MacTel-2 patients tested, the detection of at least one ARA in 69% of MacTel-2 patients raised the question of whether the development of autoimmune responses directed against the retina could play a role in the etiology of MacTel-2. Subsequent genetic and metabolomic studies have implicated altered amino acid and lipid metabolism as the more likely putative basis of photoreceptor damage in patients with MacTel-2, and any definite role of ARAs in the pathogenesis of this disease process has yet to be elucidated (Gantner et al., 2019).

This study aims to further clarify the clinical relevance of ARAs associated with MacTel-2, by providing broader comparisons of the IgG ARA profile with other non-MacTel-2 retinal disease entities that also exhibit impaired BRB, as well as healthy controls. Furthermore, the relationships between ARA profile and MacTel-2 disease severity are explored.

2. Methods

2.1. Human participants

The study was conducted under the research protocol approved by the Human Ethics Office of Research Enterprise, The University of Western Australia (RA/4/1/7916) and the North Shore-Long Island Jewish Health System (IRB#14–655), New York, USA. Collection and use of human retinal tissue obtained from the Lions Eye Bank of Lions Eye Institute was approved by the University of Western Australia Human Research Ethics committee (RA/4/1/9327 and RA/4/20/5717). Written informed consent was obtained from all participants who provided serum samples. This research adhered to the tenets of the Declaration of Helsinki and was performed in accordance with the Health Insurance Portability and Accountability Act regulations.

2.2. Clinical phenotyping

Participants were recruited from the Vitreous, Retina Macula Consultants of New Year, New York, United States of America and the Lions Eye Institute, Perth, Western Australia, Australia. All eligible subjects underwent a complete ophthalmic assessment including best-corrected visual acuity (BCVA), dilated slit lamp and fundus examination. Detailed multi-modal imaging was performed, incorporating color fundus photography (California, Optos plc., Dunfermline, UK), spectral-domain optical coherence tomography (SD-OCT, Spectralis, Heidelberg Engineering, Heidelberg, Germany) and short-wave fundus autofluorescence (HRA2 or HRA+OCT, Heidelberg Engineering, Heidelberg, Germany). Fluorescein angiography or OCT angiography (RTVue XR Avanti system, Optovue, Inc, CA, USA) were performed to confirm the vascular changes in MacTel-2.

2.3. Study subjects

Patients with MacTel-2, age-related macular degeneration (AMD) or inherited retinal diseases (IRDs) and subjects with no retinal disease were recruited. The criteria for the MacTel-2 enrolment included a clinical diagnosis of MacTel-2 supported by multimodal imaging and with no signs of diabetic retinopathy or AMD. Severity of MacTel-2 was graded by two retinal specialists independently (CB, FKC) on the basis of colour fundus photographs, SD-OCT images, fundus autofluorescence and fluorescein angiography (where available) and/or OCT angiography, using the staging system described by Chew et al. (2019) (Supplementary Table S1**)**. The cohort was divided into early (grades 0-3) and advanced stages (grades 4–7) for further analysis (Supplementary Table S2). A control cohort consisted of patients with AMD and IRDs, We used the Beckman classification for AMD cases as described by Ferris 3rd et al. (Ophthalmology 2013; 120(4):844-51). Blood sampling in patients with choroidal neovascularization were performed after stabilization of the lesion with anti-vascular endothelial growth factor agents (Supplementary Table S3). IRD cases were diagnosed on a clinical basis by three retinal specialists with subspecialty expertise on IRD (LAY and CB in New York and CB and FKC in Perth), and confirmed by further multimodal imaging, electrophysiology and genetic analysis through Molecular Vision Laboratory, Oregon, US, where available (Supplementary Table S4). These non-MacTel-2 retinal diseases were selected given their known association with BRB dysfunction. An additional cohort consisting of healthy control subjects without retinal disease (Supplementary Table S5) were also included on basis of normal retinal examination and multimodal imaging to provide a background ARA frequency and profile. For all participants, age, sex and medical history were recorded.

2.4. Blood collection

Patient blood samples were collected in BD Vacutainer® Plus Plastic Serum Tubes (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and allowed to clot at room temperature for 30–60 min. Blood samples were centrifuged at 2000g for 15 min at 4 °C to separate serum from red blood cells. Serum was removed and stored at −80 °C prior to analysis.

2.5. Retinal protein extraction

Whole retinal tissues (excluding retinal pigment epithelium) were dissected from posterior eye cups obtained from human donors (aged 28–61) and lysed in RIPA lysis buffer (Sigma-Aldrich, St. Louis, MO, USA) with 1% Protein Inhibitor Cocktail (Sigma) followed by shaking at 4 °C for 1 h. Lysates were then centrifuged at 15,700g for 30 min at 4 °C and the supernatant collected and stored at −20 °C. Protein concentration was determined by Bio-Rad Protein Assay (Bio-Rad, Hercules, CA, USA).

2.6. Anti-retinal antibody screening

For western blotting, human retinal protein was mixed with 4x NuPAGE LDS Sample Buffer (Invitrogen, Thermo Fisher, Scientific, Waltham MA, USA) and heated at 70 °C for 10 min before loading into a 57 mm well spanning the NuPAGE 4–12% Bis-Tris gel (Invitrogen). A total of 228 μg (4 μg/mm) of retinal protein was used on each blot. Chameleon Duo Pre-stained Protein Ladder (LI-COR Biosciences, Lincoln, Nebraska USA) was loaded into separate wells flanking the gel. Proteins were separated by gel electrophoresis in MES buffer (Invitrogen), and then transferred onto a PVDF membrane (Immobilon-FL, Merck Millipore, Billerica, MA, USA) at 80V for 90 min in transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol, pH 8.3). After transfer, the membrane was incubated with blocking buffer (5% BSA in TBS) at room temperature for 1 h. Membranes were then loaded onto Mini-PROTEAN® II Multiscreen Apparatus (Biorad) and incubated with patient serum (1:200) or rabbit anti-RP1 antibody (1:1000, HPA042257,

Sigma) diluted in blocking buffer at 4 °C overnight. Blots were then washed in TBST 3 times and the membrane incubated with IRDye® 800CW Goat anti-Human IgG (1:10,000; LI-COR) secondary antibodies diluted in 5% BSA in TBST for 1 h at room temperature. IRDye® 800CW Goat anti-Rabbit IgG (1:10,000; LI-COR) secondary antibody was used for anti-RP1 blotting. The membrane was washed then 3 times in TBST, and once with TBS without Tween20. The membrane was imaged at 800 nm wavelength using the Odyssey Infrared Imager (Model 9120, LI-COR).

Western blots included lanes probed with positive control serum from a single patient with paraneoplastic retinopathy associated with a high-grade small cell neuroendocrine tumour in the prostate (Chen et al., 2017). Additionally, to provide a reproducible reference band for retinal protein western blots we included lanes probed with a commercial antibody targeting RP1, a retinal protein expressed in photoreceptors. Labelling of bands in the RP1 positive control lane was used as a reference to standardise imaging intensities between different blots. Densitometry profiles were generated for each lane using the Image J64 software (version 1.44°, National Institutes of Health, USA). Distance migrated and labelling intensities were measured for each band. Band intensities were normalized against the labelling of the 200 kDa RP1 antigen. Band intensities greater than 50% of the RP1 control band were scored positive. Protein marker size was plotted against distance migrated to generate standard curves for determination of antigen sizes. Using this method, protein sizes could be determined from 8 to 260 kDa. To measure the errors associated with band size measurement, we compared size measurements for the 100 kDa band and 45 kDa bands labeled by an AIR patient used as a positive control across 21 western blots. Errors associated with size measurements were lower for the smaller band (SD = 1.04 kDa), than the larger band (SD = 6.36 kDa) (Supplementary Fig. S1).

2.7. Statistical analysis

Proportion, mean and standard deviation (SD) were calculated. Chi-square and independent sample t-tests were used to compare the proportions of ARA positive cases between the cohorts and the mean age of ARA positive versus negative cases. Odds ratios (OR) with 95% confidence intervals (CI) of females developing ARA were calculated. A p-value of <0.05 was considered statistically significant. Linear regression analysis was performed using Excel (Microsoft, USA).

3. Results

3.1. Patient demographics and diagnosis

A total of 129 blood samples were collected from 42 patients with MacTel-2, 35 patients with non-Mactel-2 retinal disease (AMD; n = 15 and IRDs; n = 20) and 52 subjects with no retinal disease (healthy controls). The mean (SD) ages for MacTel-2 and non-MacTel-2 retinal disease groups; 62.4 (11.1), 59.1 (23.9) were significantly greater than the healthy controls; 50.2 (13.6) years ($p < 0.001$ and $p = 0.03$, respectively, Table 1). Seventy percent of the MacTel-2 cohort was female, compared with 51% and 42% of the cohorts with non-Mactel-2 retinal disease and healthy controls respectively (χ^2 (2, N = 129) = 5.92, $p = 0.05$, Table 1). There was no relationship between disease stage and age in the MacTel-2 cohort (Fig. 1A). The MacTel-2 grading scale and the clinical and genetic diagnoses for the cohort with retinal disease are provided in Supplementary Tables S2, S3 and S4.

3.2. Anti-retinal antibody frequency in the three disease groups

There were no significant differences between the frequencies of positive IgG ARA in subjects with MacTel-2 (36%) and healthy controls (25%, χ^2 (1, N = 94) = 1.275, $p = 0.26$). However, the frequency of ARA was higher in the non-MacTel-2 retinal disease cohort (AMD or IRDs, 46%) than those in the healthy control group (25%, χ^2 (1, N = 87) = 4.039, $p = 0.04$, Fig. 1B). A subgroup analysis showed that only AMD patients, but not IRDs patients, early MacTel-2 or advanced MacTel-2 cohorts had a higher frequency of ARA compared to those in the healthy control group (χ^2 (1, N = 67) = 11.83, $p = 0.0006$) (Fig. 1B). Reducing the cut off thresholds from 50% to 33% of control band intensity values for positive ARA-calling led to an increase in ARA frequency across all groups, while the relationships between groups remained unchanged (Supplementary Fig. S2). We further applied a logistic regression model to determine if ARA band intensity is a discriminator of the groups, after adjusting for gender and age. Using this model, the presence of ARA remained a significant discriminator for AMD patients ($p = 0.02$) but not for Mactel-2 or IRD patients ($p > 0.05$, Table 2), compared with healthy controls.

Table 1
Patient demographics.

Group	n	Female: Male	Mean age (range, years)
MacTel-2	42	29:13	62 (38–88)
Early	18	12:6	64 (38–77)
Advanced	24	17:7	62 (39–88)
Healthy Control (no retinal disease)	52	23:29	50 (23–75)
Retinal disease	35	18:17	59 (11–99)
AMD	15	8:7	79 (58–99)
IRDs	20	10:10	44 (11–80)
Whole group	129	70:59	62 (11–99)

AMD, age-related macular degeneration; IRDs, inherited retinal diseases; MacTel-2, type 2 macular telangiectasia.

3.3. Demographic features of anti-retinal antibody positive cases

Overall, ARA positive cases were significantly older than ARA negative cases (64 ± 15 *vs* 53 ± 17 years, $p < 0.001$). For the MacTel-2 and healthy retina groups, there were no significant differences in the mean age between ARA positive and negative cases (Table 3). However, in the non-MacTel-2 retinal disease group, age was significantly higher in ARA positive compared to ARA negative cases (71 ± 18 years *vs* 49 ± 24 years, $p = 0.004$, Table 3). Overall, IgG ARAs were more frequently detected in female (43%) than in male individuals (24%, χ^2 (1, N = 129) = 5.212, $p = 0.02$, Supplementary Fig. S3). Conversely, there were more females in ARA positive cases than in ARA negative cases (68% *vs* 47%). Females were more likely than males to develop IgG ARA (odds ratio [OR] 2.41 [95% Confidence interval [CI, 1.12–5.18]). This association was driven by the healthy control group, with 39% of healthy female subjects showing ARA positivity, compared with 14% of healthy male controls ([OR] 4.02 [CI, 1.04–15.46], Supplementary Fig. S3). The female:male odds ratios of IgG ARA (95% CI) for the MacTel-2 and retinal disease groups were only 2.35 (0.53–10.41) and 1.43 (0.38–5.44), respectively.

3.4. Anti-retinal antibody profile

The sizes of retinal antigens labeled by IgG ARA varied considerably, with most patients displaying a unique pattern of immunoreactivity. To identify potential disease specific antigen profiles, we plotted the frequencies of ARA-labeled bands against antigen size for the healthy control cohort (n = 13 ARA positive patients), the MacTel-2 cohort (n = 15) and the AMD group (n = 11) (Fig. 1B). In the healthy control group, the most commonly detected antigens were a 34–36 kDa antigen, a 39–41 kDa antigen and a 45–47 kDa antigen in 31%, 23% and 23% of the ARA positive subjects, respectively. In the MacTel-2 group, the most common antigens detected were 45–47 kDa and 75–79 kDa in size; present in 27% of ARA positive cases for both. In the AMD group, the most commonly detected antigens were 45–47 kDa and 48–50 kDa in

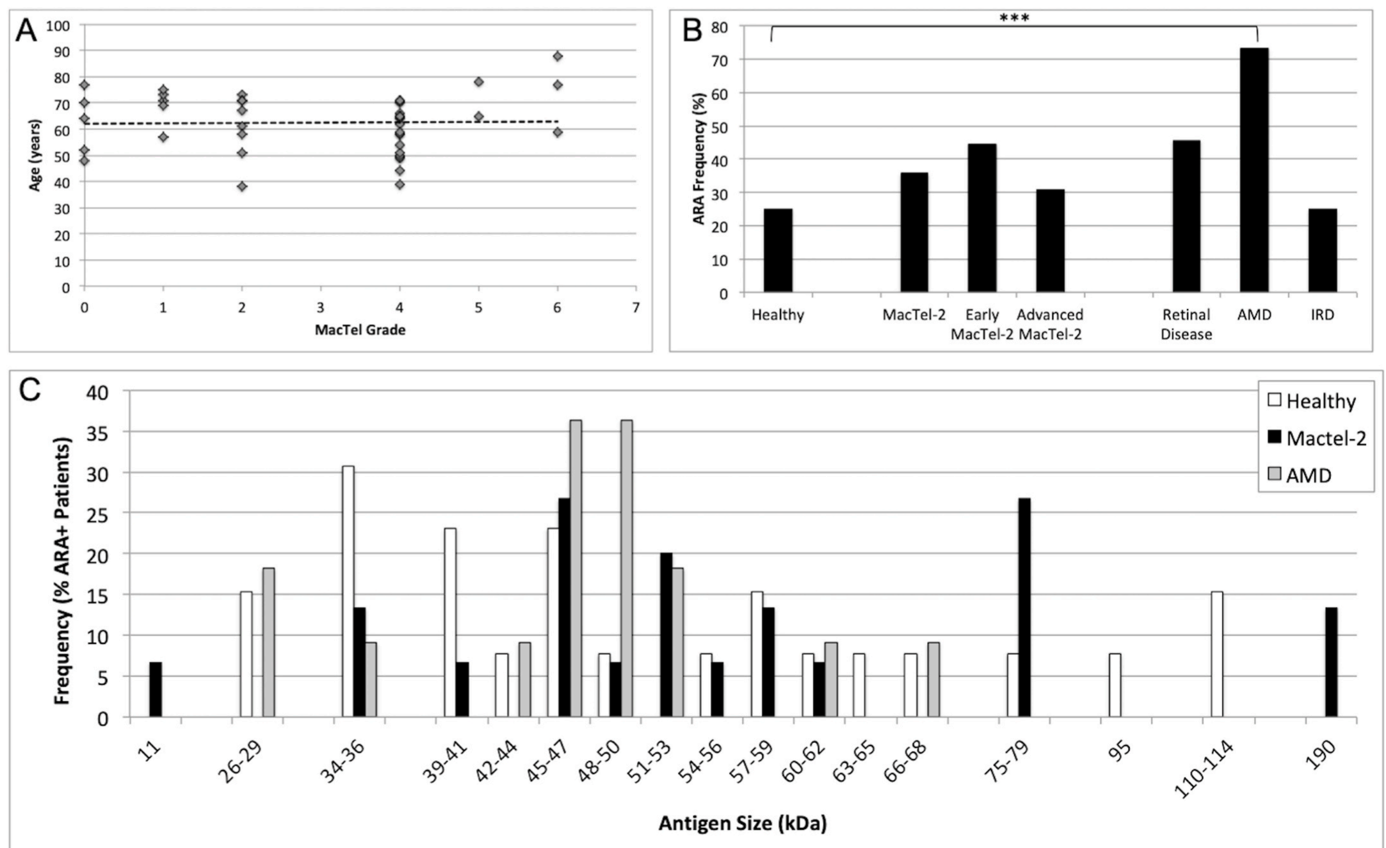

Fig. 1. **A**) Patients in the MacTel-2 cohort were plotted by age and MacTel grade. Linear regression analysis demonstrated no correlation between patient age and disease severity (dotted line, R = 0.00047). **B**) ARA frequencies were calculated for each group using a cut off threshold of 50% of the 200 kDa anti-RP1 control band. No significant differences in the frequency of ARA detection were found between healthy controls and patients with MacTel-2 (early, advanced or total) or patients with other retinal diseases or IRDs. In contrast, the AMD patient subgroup showed significantly increased rates of ARA detection ($p < 0.0001$). **C**) ARA-binding antigens detected were binned according to size and plotted against their frequencies in ARA-positive healthy controls, MacTel-2 patients and AMD patients.

Table 2
Logistic regression model estimates with healthy controls as the reference outcome level.

Predictor	Model Estimate	OR (95% CI)	*p*-Value
AMD vs Healthy			
Intercept	−26.2 (−53.4,-12.3)	0.0 (0.0,0.0)	
%RP1	4.1 (1.4,8.7)	60.2 (4.1,5774.2)	0.018[a]
Gender[b]	2.0 (−0.6,5.5)	7.1 (0.6,239.5)	0.178
Age in years	0.3 (0.1,0.7)	1.4 (1.2,2)	0.013[a]
IRD vs Healthy			
Intercept	0.9 (−1.3,3.1)	2.4 (0.3,22.8)	
%RP1	1.3 (−0.6,3.3)	3.8 (0.5,27)	0.170
Gender[b]	−0.5 (−1.7,0.6)	0.6 (0.2,1.9)	0.380
Age in years	−0.044 (−0.09,-0.004)	0.957 (0.914,0.996)	0.041[a]
Mactel-2 vs Healthy			
Intercept	−4.7 (−7.6,-2.3)	0.0 (0.0,0.1)	
%RP1	1.4 (−0.1,3.2)	4 (0.9,23.7)	0.094
Gender[b]	−0.4 (−1.4,0.6)	0.7 (0.2,1.8)	0.422
Age in years	0.1 (0,0.1)	1.1 (1,1.1)	0.001[a]

[a] Significant at 5% level.
[b] Female = reference category; OR = odds ratio, CI = confidence interval.

size, present in 36% of ARA positive cases for both.

4. Discussion

In this study, we demonstrated no significant increase in frequency of IgG ARA amongst patients with MacTel-2 compared to healthy control subjects. Previously, Zhu et al. (2013) detected ARA in 69% of MacTel-2 patients, compared with 16% of healthy control subjects. The higher rate in their MacTel-2 cohort is likely to be related to methodological differences in ARA assay as their methodology did not discriminate between IgG, IgA or IgM isotypes. Polyreactive IgM autoantibodies that bind to self-antigens with moderate affinities and high avidities are known to occur in healthy individuals (Elkon and Casali, 2008). These IgM antibodies recognize a broad spectrum of microbial targets and play an important role in defending against invading pathogens. Additionally, recognition of self-antigens by autoantibodies contributes to the development and homeostasis of the immune system. IgM antibodies

Table 3
Mean age of anti-retinal antibody positive versus negative cases.

Group	Mean Age in those without ARA (±SD)	Mean Age in those with ARA (±SD)	Mean age (All, ±SD)
MacTel-2[a]	62 ± 11	63 ± 11	62
Early[a]	67	60	64
Advanced[a]	60	66	62
Healthy Control[a]**(no retinal disease)**	49 ± 14	55 ± 12	50
Retinal disease[b]	49 ± 24	71 ± 18	59
AMD[a]	80	79	79
IRDs[a]	41	54	44
Whole group[c]	53 ± 17	64 ± 15	62

ARA, anti-retinal antibody; AMD, age-related macular degeneration; IRDs, inherited retinal diseases; MacTel-2, type 2 macular telangiectasia.
[a] No statistically significant difference in age.
[b] Difference in age was statistically significant, $p = 0.004$.
[c] Difference in age was statistically significant, $p = 0.0007$.

may bind to multiple unrelated antigens and are present in healthy individuals, while high-affinity IgG autoantibodies are generally thought to reflect a pathological autoimmune response (Elkon and Casali, 2008) and for this reason we focused our ARA screening on IgG detection only.

IgG ARA were previously detected in 58% (64/108) of patients with AMD compared to 54% (14/26) of healthy controls (Adamus et al., 2014). The higher rate reported in this healthy control cohort may be related to differences in the cut off threshold used for band-calling. In our study, a quantitative, densitometry-based method was used for ARA detection in patient serum samples by western blotting. Labeling intensity was standardized by including a lane probed with anti-RP1 antibody in all blots, enabling normalization of signals between blots. Additionally, a positive control sample from an ARA positive patient with paraneoplastic autoimmune retinopathy was included on every blot. These controls were used to determine cut off thresholds for band positivity, which were set at 50% of the RP1 positive control band. Reducing the threshold to 33% of the RP1 reference band increased the number of positive patients detected by approximately 20% in all groups except the AMD group, which increased by 7%. Since this increase in ARA frequency occurred evenly across the MacTel-2, healthy control and the IRDs groups without affecting the AMD group, it is likely that it reflects an increase in detection of false positive bands. The significance of these low intensity bands remains uncertain, however their relative abundance in the healthy population suggests they are not pathogenic in nature.

ARA detection rates were high in AMD patients (73%), supporting previous studies indicating the development of IgG ARA is a significant feature of AMD (Adamus et al., 2014; Chen et al., 1993; Cherepanoff et al., 2006; Iannaccone et al., 2015; Kubicka-Trząska et al., 2014; Morohoshi et al., 2012; Patel et al., 2005). Adamus et al. (2014) showed that increases in IgG ARA detection rates were only significant in AMD patients with less severe disease phenotypes, suggesting IgG ARA may be a biomarker or indeed play a role in early stage disease. Although we were unable to compare AMD subgroups due to low patient numbers in the group, we observed a similar trend in the MacTel-2 cohort.

In contrast with the AMD group, the IRD group showed no significant difference in ARA frequency compared with healthy controls. ARA have previously been detected in animal models of IRDs (Adamus et al., 2012; Iannaccone and Radic, 2019; Kyger et al., 2013) as well as human patients with retinitis pigmentosa (Chant et al., 1985; Heckenlively et al., 1985, 1999, 2000). In early studies, Chant et al. reported ARA frequencies of up to 37% in retinitis pigmentosa (RP) patients (Chant et al., 1985), while later studies showed ARA were detected in up to 90% of RP patients with cystoid macular oedema (CMO), but only 13% of RP patients without CMO (Heckenlively et al., 1999). In our study, only 3/20 IRD patients had CMO and all of them were negative for IgG ARA. These ARA negative cases may reflect the young age of one CMO patient (14yo with *MERTK* mutations) or the mild disease in the other CMO patients (29yo with *RHO* mutation and 34yo with *USH2A* mutation).

Overall, the MacTel-2 group did not show a significant increase in IgG ARA detection rates compared with healthy controls (36% vs 25%). However, when early and advanced stage disease subgroups were analysed separately, the presence of IgG ARA was further increased in patients with early stage disease (44%) while ARA detection rates in patients with advanced stage MacTel-2 (29%) were similar to healthy controls (25%). In support of an association between ARA positivity and early stage MacTel-2, the mean age of the ARA positive patients was lower than the mean age of the ARA negative cases. In contrast, the ARA positive healthy control cohort and IRDs subgroup were older than those without ARA, while ARA positive and negative AMD groups were of similar ages (Table 3). However, since these differences did not achieve statistical significance, the clinical significance of ARA in early stage MacTel-2 warrants further investigation.

Antigen profiles were found to be highly variable between ARA positive Mactel-2 patients. Zhu et al. (2013) found three commonly labeled antigens (AGL, RBP3 and CK-B) using mass spectrometry. In the present study, we found no evidence for increased frequencies of CK-B (40 kDa), RBP3 (120 kDa) or AGL (150 kDa) reactive IgG ARAs in MacTel-2 patients. Reactivity to a 39–41 kDa antigen was observed in only 1/42 MacTel-2 patients, but was found in 3/52 subjects in the healthy control group. No IgG ARA-positive antigens were found to have molecular weights of 120 kDa or 150 kDa, although 2 MacTel-2 patients showed reactivity to a 190 kDa band. Within the MacTel-2 group, the most common antigens detected were 45–47 kDa and 75–79 kDa in size, which each occurred in only four out of 42 MacTel-2 patients. Reactivity to a 45–47 kDa antigen was detected in >20% of ARA positive cases in all groups, with the highest detection rates found in the AMD group (35%). Previously, Adamus et al. identified a 46 kDa antigen as the most commonly detected ARA target in both control subjects and in patients with AMD (Adamus et al., 2014). The authors proposed this antigen was enolase based on its size.

In our study, high intensity signals (>50% of the RP1 reference band) were present in only 25% of healthy controls. It is possible that the strong IgG ARA signals in these control subjects represent polyreactivity of antibodies directed at other antigens, which bind to retinal proteins on western blots as an off-target effect or due to epitope unmasking during protein denaturation. On the other hand, in some control subjects, development of ARA may reflect smoking status (Adamus et al., 2014) or the presence of undiagnosed cancers (Adamus, 2009; Chen et al., 2017).

Female patients were more likely to present with ARA reactivities than male patients across all groups, consistent with known differences in humoral immunity between the sexes (Klein and Flanagan, 2016). Females have higher circulating immunoglobulin levels than men, as well as an increased incidence of autoimmune disease (Fink and Klein, 2018; Wilhelmson et al., 2018).

5. Study limitations

A general limitation of ARA detection by western blot is the relative abundance of specific retinal proteins presenting on the blot. Binding of ARA to low abundance retinal antigens may not produce sufficient signal for discrimination from non-specific background signals evident in densitometry traces. Future studies investigating ARA reactivities to specific retinal antigens may help to overcome this limitation. The unique retinal antigen profile in patients with MacTel-2 has not been fully characterised yet and this is an opportunity for a future study. An additional limitation is that this study utilized neuroretinal tissues as a source of retinal antigens. Further studies utilizing retinal pigment epithelium as the antigen source could be performed to investigate the potential role of anti-RPE antibodies in MacTel-2. Finally, eight of the twenty patients with IRDs did not have a confirmed causative mutation due to lack of testing or inconclusive results. Therefore, differences in ARA frequency may be confounded by misdiagnosis of non-hereditary phenocopies as IRDs.

6. Conclusions

In summary, we found the majority of MacTel-2 patients tested negative for IgG ARA and the frequency of ARA was only marginally greater than healthy controls. The finding of a trend towards higher frequency of ARA in those with early MacTel-2 may warrant further investigation, however the lack of a strong association with greater ARA frequency or specificity in the MacTel-2 cohort suggests that antibody mediated immunological mechanisms are unlikely to play a significant role in disease pathogenesis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.exer.2022.109024.

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