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The Role of SNPs in *IL1RL1* and *IL1RAP* Genes in Age-related Macular Degeneration Development and Treatment Efficacy

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Abstract. *Background: Age-related macular degeneration (AMD) affects the central part of the retina and causes blindness. In developed countries, AMD occurs in people over 50 years old. Important factors for AMD pathogenesis are an immune response, inflammation, and genetic factors. This study aimed to determine the impact of IL1RL1 rs1041973 and IL1RAP rs4624606 single nucleotide polymorphisms (SNPs) on the occurrence of AMD and the outcome of treatment with aflibercept and bevacizumab. Patients and Methods: 563 patients with AMD and 281 healthy candidates were evaluated. Patients with exudative AMD were treated with intravitreal bevacizumab and aflibercept and, after 6 months based on the changes in best-corrected visual acuity and central macular thickness, were classified as 'responders' or 'poor-responders'. Genotyping of IL1RL1 rs1041973 and IL1RAP rs4624606 was accomplished using real-time PCR. Age was compared using the Mann-Whitney U-test. Categorical data (gender, genotype, and allele distributions) compared between groups using the χ^2 test or the Fisher's exact test. Associations of gene polymorphisms were calculated using logistic regression analysis with adjustment for age in exudative and atrophic AMD analysis. An adjusted significance threshold for multiple comparisons $\alpha=0.025$ was applied. Results: Statistically significant differences in the distribution of IL1RAP rs4624606 genotypes (TT, TA and AA) were found between males with atrophic AMD and controls: 50%, 42.9% and 7.1% vs. 69.7%, 30.3% and 0%, respectively, p=0.015. Moreover, we found that 'responders' had a significantly better best-corrected visual acuity than 'poor-responders' before treatment (p=0.032). The central macular thickness was significantly lower in exudative AMD patients with IL1RL1 rs1041973 AA genotype than in wild type and heterozygous (CC+CA) genotype carriers before treatment (p=0.017). Conclusion: IL1RAP rs4624606 may be associated with atrophic AMD in males while IL1RL1 rs1041973 may play a protective role against macular thickening in exudative AMD patients.*

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Age-related macular degeneration (AMD), the leading cause of blindness in developed countries, is a complex disease caused entirely by environmental, demographic, and genetic factors (1, 2). AMD is classified into early or intermediate stages with the formation of drusen, an amorphous extracellular deposit in the retina, and characteristic pigmentary changes, and late stages, which may either be atrophic (geographic atrophy) or wet (neovascular type) (3). Early stages of the disease are often clinically asymptomatic. It progresses slowly and evolves into late AMD only in 10-20% of patients, of which two-thirds have neovascular and one-third atrophic AMD type (4). Late forms are characterized by the irreversible loss of central vision. Treatment in clinical practice is currently available only for the neovascular type: intraocular injections with VEGF inhibitors; there are no clinically approved treatment options for atrophic AMD and/or the possibility to stop early AMD forms developing to advanced AMD forms. Currently, an estimated 10.1 million people are living with AMD – projected global cases are 11.3, 14.9, and 18.6 million for 2020, 2030, and 2040, respectively (5).

In recent years, there has been growing evidence pointing to inflammatory processes and genetic components as having strong effects on AMD development (6-8). Knowledge of the molecular genetics of AMD has emerged mainly from analyses of DNA sequence and structural variations, with the

Table I. *Demographic data of the studied groups.*

	Early AMD group n=270	Exudative AMD group n=293	Atrophic AMD group n=53	Control group n=281	*p*-Value
Males, n (%)	82 (30.4)	105 (35.8)	14 (26.4)	99 (35.2)	0.225* 0.880** 0.213***
Females, n (%)	188 (69.6)	188 (64.2)	39 (73.6)	182 (64.8)	
Age median/min/max	74/59/94	77/61/95	80/61/97	73/59/94	0.065* **<0.001**** **<0.001****
Responders, n (%)	–	75 (83.3)			
Poor-responders, n (%)		15 (16.7)	–	–	–

*Early AMD *vs*. control group, **exudative AMD *vs*. control group, ***atrophic AMD *vs*. control groups; min.: the lowest value; max.: the highest value. Bold values show statistical significance.

large-scale genome-wide association studies (GWAS) reporting findings on the polygenic nature and incomplete penetrance of many common DNA variants associated with the disease (9, 10). Interleukin-1 receptor type I (IL1R1), also known as cluster of differentiation 121a (CD121a), is an interleukin receptor. IL1R1 also denotes its human gene. IL1RL1 has gained recognition due to its involvement in immune/inflammatory disorders (11). Interleukin-1 receptor accessory protein in humans is encoded by the *IL1RAP* gene. It has been shown that interleukin 1 induces synthesis of the acute phase and proinflammatory proteins during infection, tissue damage, or stress by forming a complex at the cell membrane with an interleukin 1 receptor and an accessory protein. The protein is a necessary part of the interleukin-1 receptor complex, which initiates signaling events that result in the activation of interleukin-1 responsive genes. Alternative splicing of this gene results in two transcript variants encoding two different isoforms, one membrane-bound and one soluble. The ratio of soluble to membrane-bound forms increases during acute-phase induction or stress (12). Madore *et al*. have found that the strongest gene-gene statistical interaction is between the *IL1RAP* and *IL1R1* genes and it has the most biological relevance. These genes encode proteins that are associated with the regulation of the IL-1 receptor and the functionality of IL-1 (13). Indeed, IL-1R1 and IL-1RAP have been shown to form a protein complex with IL-1, and the interplay between these proteins may serve to unbalance the interaction between IL-1 and its signaling receptor (IL-1R1 and IL-1RAP) and its decoy receptor complex (IL-1R2 and IL-1RAP). Furthermore, it has been shown that inhibition of IL1R1 in mice with increased VEGF-A expression reduces severe degenerative and atrophic changes of the retinal pigment epithelium (RPE), thick basal laminar sub-RPE deposits, shortening of photoreceptor outer and inner segments, and attenuation of the photoreceptor outer nuclear layer (14). Also, we hypothesized that each individual's genetic variations can determine the exudative AMD treatment response to intravitreal injections and that identified genetic markers, predicting the differentiated response, may improve personalized medicine.

Our research aimed to elucidate the possible involvement of *IL1RL1* rs1041973 and *IL1RAP* rs4624606 gene polymorphisms in early and exudative AMD development in the Lithuanian population and analyze the outcome of exudative AMD treatment.

Patients and Methods

Patients and study design. The study was approved by the Ethics Committee for Biomedical Research, Lithuanian University of Health Sciences (No. BE-2-/13). A total of 897 individuals participated in this study: 270 patients with early AMD, 293 patients with exudative AMD, 53 patients with atrophic AMD, and the healthy control group consisted of 281 individuals (Table I). Participants were involved in our study according to previously published criteria (15). Study subjects underwent ophthalmological evaluation and general examination (15). DNA extraction and genotyping were performed as described in our previous publication (15). The distribution of males and females among the early, exudative and atrophic AMD and control groups did not differ statistically significantly (p=0.225, p=0.880 and p=0.213, respectively) (Table I). The age of controls matched the age of early AMD patients (p=0.065), but controls were younger than patients with exudative and atrophic AMD (p<0.001 for both) (Table I).

Exudative AMD treatment efficacy evaluation. Exudative AMD patients were treated with intraocular injections with VEGF inhibitors. Every 8 weeks, 2 mg of aflibercept and every 4 weeks 1.25 mg of bevacizumab (after three initial monthly injections) were given until choroidal neovascularization (CNV) was no longer active. Signs of active neovascularization were defined as fluid on OCT, new or persistent hemorrhage, decreased visual acuity as compared with the previous examination, or dye leakage or increased lesion size on fluorescein angiography. Best-corrected visual acuity (BCVA) and the central macular thickness were evaluated in 90 patients treated for exudative AMD. We chose to evaluate BCVA and

Table II. *The impact of IL1RL1 rs1041973 and IL1RAP rs4624606 on early age-related macular degeneration (AMD) development.*

Early AMD				
IL1RL1 rs1041973				
Model	Genotype/allele	OR (95% CI)	*p*-Value	AIC
Codominant	CA *vs*. CC	1.423 (1.001-2.002)	0.050	763.479
	AA *vs*. CC	0.978 (0.503-1.900)	0.948	
Dominant	CA+AA *vs*. CC	1.342 (0.960-1.878)	0.085	762.661
Recessive	AA *vs*. CA+CC	0.841 (0.440-1.605)	0.599	428.377
Overdominant	CA *vs*. CC+AA	1.426 (1.013-2.009)	**0.042**	761.483
Additive	A	1.170 (0.896-1.528)	0.249	764.299
IL1RAP rs4624606				
Codominant	TA *vs*. TT	1.163 (0.801-1.687)	0.428	765.443
	AA *vs*. TT	2.209 (0.654-7.458)	0.202	
Dominant	TA+AA *vs*. TT	1.215 (0.845-1.747)	0.294	764.524
Recessive	AA *vs*. TA+TT	2.115 (0.629-7.106)	0.226	764.072
Overdominant	TA *vs*. TT+AA	1.136 (0.784-1.646)	0.501	765.176
Additive	A	1.240 (0.895-1.719)	0.196	763.952

OR: Odds ratio; CI: confidence interval; AIC: Akaike information criterion. Bold values show statistical significance.

central macular thickness changes before treatment and at 3 and 6 months after treatment. According to these data, we divided patients with exudative AMD into two subgroups: responders and poor-responders (Table I). 'Poor-responders' had at least two of the three signs: persistence of submacular fluid, decreased visual acuity or increased central macular thickness (≥100 micrometers). 'Responders' did not have any of the mentioned signs.

Statistical analysis. Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, Illinois, USA). Age is presented as median, minimum (min.) and maximum (max.) values and compared using the Mann-Whitney *U*-test. Categorical data (gender, genotype, and allele distributions) are presented as absolute numbers with percentages in brackets and compared between the early, exudative, and atrophic AMD and control groups using the χ^2 test or the Fisher exact test.

Associations of gene polymorphisms were calculated using logistic regression analysis and present odds ratios (OR) with 95% confidence intervals (CI). Adjustment for age was done in exudative and atrophic AMD analysis. Due to multiple association calculations, we introduced an adjusted significance threshold for multiple comparisons α=0.025 (0.05/2, since we analyzed 2 different SNPs). The impact of genotypes is expressed as genetic models (codominant: heterozygotes *versus* wild type homozygotes and minor allele homozygotes *versus* wild type homozygotes; dominant: minor allele homozygotes and heterozygotes *versus* wild type homozygotes; recessive: minor allele homozygotes *versus* wild type homozygotes and heterozygotes; overdominant: heterozygotes *versus* wild type homozygotes and minor allele homozygotes; the additive model was used to evaluate the impact of each minor allele on AMD development). The selection of the best genetic model was based on the Akaike Information Criterion (AIC); therefore, the best genetic models were those with the lowest AIC values.

Results

Hardy-Weinberg equilibrium. We estimated the distributions of rs1041973 and rs4624606 genotypes in the control group using Hardy-Weinberg equilibrium (HWE). Both SNPs were in HWE (p>0.05) (Supplementary material).

IL1RL1 rs1041973 and IL1RAP rs4624606 analysis in AMD. We analyzed *IL1RL1* rs1041973 and *IL1RAP* rs4624606 polymorphisms in the early, exudative, and atrophic AMD, and control groups. The analysis results did not reveal any differences between *IL1RL1* rs1041973 and *IL1RAP* rs4624606 genotype and allele distributions in the early, exudative, and atrophic AMD groups (Supplementary material).

The binary logistic regression analysis showed that *IL1RL1* rs1041973 CA genotype was associated with 1.4-fold increased odds of early AMD development (OR=1.426. CI=1.013-2.009, p=0.042) (Table II) and 1.9-fold increased odds of atrophic AMD development (OR=1.883. CI=1.011-3.507, p=0.046) (Table III); unfortunately, the results were not statistically significant according to the Bonferroni correction (p>0.025). No statistically significant associations were found in the exudative AMD group (Supplementary material).

IL1RL1 rs1041973 and IL1RAP rs4624606 analysis in AMD by gender. SNPs analysis performed by gender did not reveal any statistically significant associations in the early and exudative AMD groups (Supplementary material). Statistically significant differences in the distribution of *IL1RAP* rs4624606

Table III. *The impact of IL1RL1 rs1041973 and IL1RAP rs4624606 on atrophic age-related macular degeneration (AMD) development.*

Atrophic AMD				
IL1RL1 rs1041973				
Model	Genotype/allele	OR* (95% CI)	*p*-Value	AIC
Codominant	CA *vs*. CC	1.829 (0.968-3.455)	0.063	262.581
	AA *vs*. CC	0.734 (0.154-3.502)	0.698	
Dominant	CA+AA *vs*. CC	1.658 (0.890-3.087)	0.111	262.154
Recessive	AA *vs*. CA+CC	0.553 (0.120-2.554)	0.448	264.060
Overdominant	CA *vs*. CC+AA	1.883 (1.011-3.507)	**0.046**	260.741
Additive	A	1.285 (0.787-2.099)	0.317	263.728
IL1RAP rs4624606				
Codominant	TA *vs*. TT	1.371 (0.706-2.664)	0.352	264.674
	AA *vs*. TT	3.350 (0.490-22.881)	0.218	
Dominant	TA+AA *vs*. TT	1.457 (0.765-2.777)	0.253	263.429
Recessive	AA *vs*. TA+TT	3.031 (0.449-20.478)	0.255	263.527
Overdominant	TA *vs*. TT+AA	1.316 (0.681-2.543)	0.414	264.058
Additive	A	1.491 (0.836-2.659)	0.176	262.934

*Adjusted for age; OR: odds ratio; CI: confidence interval; AIC: Akaike information criterion. Bold values show statistical significance.

Table IV. *Distributions of IL1RL1 rs1041973 and IL1RAP rs4624606 genotypes and alleles in control and atrophic age-related macular degeneration (AMD) groups by gender.*

SNP	Genotypes/ alleles	Males		*p*-Value	Females		*p*-Value
		Control n (%) (n=99)	Atrophic AMD n (%) (n=14)		Control n (%) (n=182)	Atrophic AMD n (%) (n=39)	
IL1RL1 rs1041973	CC	57 (57.6)	7 (50.0)	0.575	102 (56.0)	16 (41.0)	0.064
	CA	38 (38.4)	7 (50.0)		62 (34.1)	21 (53.8)	
	AA	4 (4.0)	0 (0)		18 (9.9)	2 (5.1)	
	C	152 (76.8)	21 (75.0)	0.814	266 (73.1)	53 (67.9)	0.404
	A	46 (23.2)	7 (25.0)		98 (26.9)	25 (32.1)	
IL1RAP rs4624606	TT	69 (69.7)	7 (50.0)	**0.015**	132 (72.5)	26 (66.7)	0.762
	TA	30 (30.3)	6 (42.9)		46 (25.3)	12 (30.8)	
	AA	0 (0)	1 (7.1)		4 (2.2)	1 (2.6)	
	T	168 (84.8)	20 (71.4)	0.102	310 (85.2)	64 (82.1)	0.491
	A	30 (15.2)	8 (28.6)		54 (14.8)	14 (17.9)	

Bold values show statistical significance.

genotypes (TT, TA an AA) were found between males with atrophic AMD and controls: 50%, 42.9% and 7.1% *vs*. 69.7%, 30.3% and 0%, respectively (p=0.015) (Table IV).

Exudative AMD treatment efficacy. We evaluated the treatment response of exudative AMD and differentiated patients into 'responders' and 'poor-responders'. Best-corrected visual acuity (BCVA) and the macular thickness before treatment and after 3 and 6 months, as well as macular thickness changes after 6 months, were compared between 'responders' and 'poor-responders' (Table V). We found that before treatment, 'responders' had significantly better BCVA than 'poor-responders' (p=0.032) (Table V). None of the other parameters reached significant differences between the groups.

Table V. *Best-corrected visual acuity and macular thickness in 'responders' and 'poor-responders'.*

Parameter	Measurement time	Responders Median (IQR)	Poor-responders Median (IQR)	*p*-Value
Central macular thickness (micrometres)	Before treatment	337 (97.5)	288 (106.5)	0.061
	After 3 months	279 (102.75)	286.5 (101.5)	0.730
	After 6 months	276 (87.75)	327 (111.5)	0.094
BCVA	Before treatment	0.30 (0.24)	0.046 (0.45)	**0.032**
	After 3 months	0.40 (0.30)	0.30 (0.50)	0.594
	After 6 months	0.35 (0.25)	0.30 (0.35)	0.155
Central macular thickness changes	After 6 months	28.5 (99.5)	75.5 (-)	0.387

BCVA: Best-corrected visual acuity. Bold values show statistical significance.

Table VI. *Associations between IL1RL1 rs1041973 and central macular thickness and best-corrected visual acuity.*

Parameter	Measurement time	CC+CA Median (IQR)	AA Median (IQR)	*p*-Value
Central macular thickness (micrometres)	Before treatment	338 (95)	251 (82)	**0.017**
	After 3 months	280 (115)	270 (30)	0.740
	After 6 months	283 (108)	266 (146)	0.594
Central macular thickness changes	After 6 months	33 (98)	-7 (68)	0.104
BCVA	Before treatment	0.30 (0.21)	0.45 (0.41)	0.157
	After 3 months	0.40 (0.3)	0.50 (0.51)	0.266
	After 6 months	0.34 (0.28)	0.45 (0.39)	0.297
BCVA changes	After 3 months	0.00 (0.11)	0.05 (0.13)	0.621
	After 6 months	0.04 (0.11)	0.00 (0.04)	0.447

BCVA: Best-corrected visual acuity. Bold values show statistical significance.

In further analysis, we compared the distributions of *IL1RL1* rs1041973 and *IL1RAP* rs4624606 genotypes and alleles between these two groups; however, there were no differences found (Supplementary material). On the other hand, we revealed the association between *IL1RL1* rs1041973 homozygous minor allele carriers (AA) and the central macular thickness before treatment. The macular thickness was significantly lower in exudative AMD patients with AA genotype than in wild type and heterozygous (CC+CA) genotype carriers [median (IQR): 338 (95) *vs*. 251 (82), *p*=0.017] (Table VI). No associations were found between *IL1RL1* rs1041973 and BCVA (Table VI). *IL1RAP* rs4624606 analysis showed no associations with the macular thickness or BCVA, which may be caused by a very small number of *IL1RAP* rs4624606 homozygous minor allele carriers (Supplementary material).

Discussion

To our knowledge, this is the first time that *IL1RL1* rs1041973 and *IL1RAP* rs4624606 gene polymorphisms associations with early, exudative, and atrophic AMD development and exudative AMD treatment efficacy are analyzed.

Our results revealed that the *IL1RL1* rs1041973 CA genotype was associated with 1.4-fold increased odds of early AMD development (*p*=0.042) and 1.9-fold increased odds of atrophic AMD development (*p*=0.046); however, the Bonferroni correction did not allow us to confirm this association as statistically significant. Also, we found that central macular thickness was significantly lower in exudative AMD patients with AA genotype than in wild type and heterozygous (CC+CA) genotype carriers before treatment [median (IQR): 338 (95) *vs*. 251 (82), *p*=0.017].

IL1RL1 is located in an interleukin 1 (IL1) receptor gene cluster on chromosome 2q12 (17). Human *IL1RL1* encodes the receptor for interleukin-33 (IL33) and has three isoforms, including soluble IL1RL1 (IL1RL1-a), that can modify T helper cell responses by inhibition of IL33 signaling (18). IL1RL1-a can be induced by pro-inflammatory stimuli and appears to be essential for the normal function of T helper cells (17, 19). Serum levels of IL1RL1 were found elevated in atopic asthmatic patients during acute exacerbations (20). Marneros has analyzed the VEGF-a induced aging diseases of the eye and found that targeting NLRP3 inflammasome components or Il1r1 strongly inhibited both neovascular and non-exudative AMD-like pathologies (14). Other scientist groups have also proved that *IL1RL1* genetic polymorphisms

and IL1RL1-a serum levels are associated with severe arthritis, acute heart disease, and airway disease such as asthma (21-25). Also, studies are analyzing the association of other diseases, mostly asthma, with *IL1RL1* gene polymorphisms. SNPs in *IL1RL1* are associated with asthma-related phenotypes in three studies (26-28). Given the role of eosinophils in the pathogenesis of asthma, alleles that are associated with increased eosinophil count could be detrimental in terms of asthma risk and severity. In a GWAS of eosinophil count in an Icelandic population, an SNP in *IL1RL1* (rs1420101) showed the most significant association. The A allele of rs1420101 is associated with an increased eosinophil count and, in further analyses, with increased serum IgE as well as with three asthma phenotypes (asthma, atopic asthma, nonatopic asthma) in nine European populations and one East Asian population (28).

Two (rs1041973 and rs10206753) of the four coding non-synonymous *IL1RL1* SNPs, associated with asthma in the Mexican population, were also examined in a Dutch population where they showed no associations (26).

Another gene we evaluated in patients with AMD was *IL1RAP* rs4624606. IL1RAP is commonly known to be the co-receptor for the IL-1 receptor and it is required to transduce IL-1 signaling, leading to pro-inflammatory gene expression and cell survival (29). The *IL-1RAcP* gene is located on chromosome 3q28 (30).

Furthermore, Theodoropoulou *et al.* have demonstrated that IL-1 receptor accessory protein (IL1RAP) is expressed by cells in the murine retina and retinal pigment epithelium (RPE). They have sought to determine whether similar expression profiles were present in healthy human ocular tissue and revealed robust expression of IL1RAP in the nerve fiber layer of the inner retina and the RPE (31). In our study, we found statistically significant differences in the distribution of *IL1RAP* rs4624606 genotypes (TT, TA an AA) between males with atrophic AMD and controls: 50%, 42.9% and 7.1% *vs.* 69.7%, 30.3% and 0%, respectively (p=0.015).

In large genome-wide case-control studies, *IL1RAP* was found to be associated with Alzheimer's disease (32). The same gene polymorphism was analyzed in patients with cardiovascular heart disease risk by Wu and colleagues (33). The study has shown that rs46246 is nominally associated with CHD risk. The AA genotype was associated with a 1.85-fold increased risk of CHD (p=0.045) compared to the TT genotype. Further analysis showed that AA carriers also had a higher risk of CHD than TT+TA carriers [odds ratio (p =0.043)] (33).

Genetic variants in *IL-1RAcP* have been associated with prostate cancer, Kawasaki disease, and persistent hepatitis B virus (33-36). Menon *et al.* have also reported that rs4624606 was associated with higher amniotic fluid-1β concentrations (37). IL1RAP may also play a role in acute myeloid leukemia (AML) as well as in high-risk myelodysplastic syndromes, hematologic malignancies that often progress to AML (38-41).

Conclusion

Our results showed that *IL1RL1* rs1041973 and *IL1RAP* rs4624606 gene polymorphisms may not play a role in early and exudative AMD development but *IL1RAP* rs4624606 may be associated with atrophic AMD in males. Also, *IL1RL1* rs1041973 gene polymorphism possibly plays a protective role against macular thickening in exudative AMD patients. Further research should focus on determining the IL1RL1 and IL1RAP protein expression associations with AMD development.

Data Availability

The genotyping and ophthalmological test data used to support the findings of this study are available from the corresponding author upon request.

Supplementary Material

Available at: https://docs.google.com/document/d/1gTl2gNFba2TD6z5nIvtAV_TPJjTEwmHAzPrr_hQsyw0/edit

Conflicts of Interest

None of the Authors has any proprietary interests or conflicts of interest related to this submission.

Authors' Contributions

Conceptualization, A.V., N.B., L.K., R.Z., and R.L.; Methodology, A.V., N.B., R.M., D.C., L.K., and R.L.; Formal Analysis, A.V., N.B., L.K., D.C., J.B., R.Z., and R.L.; Investigation, A.V., N.B., L.K., R.M., D.C., J.B., R.Z., and R.L.; Data Curation, A.V., N.B., R.M., D.C., J.B., R.Z., and R.L.; Writing – Original Draft Preparation, A.V., N.B., D.C., R.M., L.K., R.Z., J.B., and R.L.; Writing – Review & Editing, N. B., A.V., and R.L; Supervision, R.L.

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