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The Association Between Variants of Receptor for Advanced Glycation End Products (RAGE) Gene Polymorphisms and Age-Related Macular Degeneration

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Background: Age-related macular degeneration (AMD) is the leading cause of blindness in people aged 65 years and older in developed countries. The pathogenesis of AMD has been linked to mechanisms involving inflammation, oxidative stress, and basal laminar deposit formation between retinal pigment epithelium (RPE) cells and the basal membrane, caused by advanced glycation end products (AGEs). AGEs are implicated in the pathogenesis of AMD through the AGE-and receptor for AGE (RAGE) interaction, which can be altered by polymorphisms of the *RAGE* gene. We examined *RAGE* rs1800624 and rs1800625 gene polymorphisms contributing to AMD development.

Material/Methods: The study enrolled 300 patients with early AMD, 300 patients with exudative AMD, and 800 healthy controls. The genotyping was carried out using the RT-PCR method.

Results: The analysis of two single nucleotide polymorphisms (SNPs) in the *RAGE* gene showed that rs1800624 was associated with a 1.6-fold decreased risk for exudative AMD under the dominant model after adjustment for age (OR=0.616; 95% CI: 0.394–0.963; p=0.034) and each copy of allele T at rs1800624 was associated with a 1.4-fold decreased risk for exudative AMD development under the additive model after adjustment for age (OR=0.701; 95% CI: 0.510–0.962; p=0.028). Analysis revealed that the rs1800625 allele G at rs1800625 was associated with a 1.5-fold increased risk for exudative AMD after adjustment for age (OR=1.545; 95% CI: 1.003–2.379; p=0.048). These results suggested that the allele G at rs1800625 was a risk-allele for exudative AMD development. In haplotype analysis, A-G haplotype was significantly more frequently observed in exudative AMD patients compared to healthy controls (3.3% versus 1.4%, p=0.035).

Conclusions: We revealed a significant association between *RAGE* gene rs1800624 and rs1800625 polymorphisms and AMD risk. We considered T allele at rs1800624 to be protective against AMD development, while allele G at rs1800625 was considered to be a marker of poor prognosis in AMD development.

MeSH Keywords: Glycosylation End Products, Advanced • Macular Degeneration • Polymorphism, Genetic

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Background

Age-related macular degeneration (AMD) is the leading cause of vision loss among adults aged 65 years and older in developed countries [1], and there is no treatment to halt it. Many risk factors have been identified: age, gender, genetic factors, and others [2]. AMD is characterized by progressive damage to the macula and photoreceptor cell loss [3]. The basal laminar deposit formation between retinal pigment epithelium (RPE) cells and the basal membrane is characterized as part of the normal aging process until it becomes thick and contains debris consisting of lipids, inflammatory proteins, and membranous structures [2–6]. When the basal linear deposits are formed in the inner collagenous layer of Bruch's membrane, it becomes a pathological condition called AMD. Basal deposits contain not only inflammatory proteins, lipids, and cellular debris but a complex of advanced glycation end products (AGEs) as well [7]. Although drusen formation within Bruch's membrane, basal laminar deposits, and the accumulation of lipofuscin between the RPE cells and the basement membrane are part of normal aging, thickening of Bruch's membrane, choriovasculature and RPE cell loss, and photoreceptor cell death are all specific for AMD [2–6], although the specific mechanisms of disease onset and progression remains unclear.

Some studies that have looked into the processes involved in AMD pathogenesis, have suggested that AGEs can be strongly associated with AMD pathogenesis [7–22]. AGEs are protein or lipid products of nonenzymatic glycation that is one of the most important post-translational modifications in cells [23,24]. AGEs affect a variety of cells and tissues in the body [23]. AGEs have been linked to a wide number of adverse age-related diseases such as atherosclerosis, Alzheimer disease, cataracts, osteoarthritis, cardiovascular diseases, diabetes mellitus, osteoporosis, and sarcopenia [25–32]. The actions of AGEs are mainly dependent on their specific cell-surface receptor, the receptor for AGE (RAGE) [33]. RAGE is a multi-ligand transmembrane receptor of the immunoglobulin superfamily. The receptor binds to AGEs released from dying cells such as nuclear high mobility group box 1 protein (HMGB1) and calcium-binding S100 protein [34,35], amphotericin, advanced oxidation protein products, and amyloid β-sheet fibrils [36,37]. Also, it is interesting that RAGE can be activated by β-amyloid, which is a compound closely associated with the development of neurodegenerative disorders, such as Alzheimer disease and AMD [34,35], although in normal homeostasis RAGE binds and degrades AGEs to maintain decreased levels of AGEs. The interaction between RAGE and AGEs results in various cellular effects such as inflammation, oxidative stress, and altered gene expression [38] with activation of signal transduction cascades and transcription factors such as nuclear factor-(NF-)κB and apoptosis [36,39]. RAGE can be upregulated with aging and disease, and induces a pathologic response through several signal transduction pathways [38].

RAGE is presented on the plasma membrane, the same as Toll-like receptors [40], so it forms a complex on the plasma membrane to endocytose AGE-modified proteins as a protective response. On the other hand, increased RPE exposure with AGEs can damage retinal tissue causing the pathological angiogenesis process [41] because AGEs can induce upregulation of one of the proangiogenic cytokines (i.e., vascular endothelial growth factor, VEGF) secreted by RPE cells [42]. While AGEs are removed by macrophages [43], any changes in macrophage recruitment may lead to pathological angiogenesis [43]. The main mechanism of AGE-induced age-related changes and upregulation of inflammation in the eyes is through interaction with AGE receptors, including RAGE and the AGE receptor complex (R1–R3) [44,45]. Chen M et al. demonstrated that RAGE activation by S100B contributed to choroidal neovascularization by regulating angiogenic activity, infiltration of immune cells to the lesion site, and upregulation of pro-inflammatory cytokines [46] like transcription factor NF-κB, which can be altered by the genetic polymorphism in *RAGE* [47]. Our study aimed to investigate *RAGE* polymorphisms (rs1800624 and rs1800625) as candidate markers of AMD.

Material and Methods

Permission to undertake the study was obtained from the Ethics Committee for Biomedical Research. The study was conducted in the Department of Ophthalmology, the Neuroscience Institute, Ophthalmology Laboratory, Hospital of Lithuanian University of Health Sciences (LUHS) (Number – BE-2-/13).

Our study enrolled 300 patients with a diagnosis of early AMD, 300 patients with exudative AMD, and 800 healthy controls.

Control group formation

The control group consisted of patients who had no ophthalmologic pathology on examination and who agreed to take part in this study. The control group involved 800 participants according to their gender considering the early and exudative AMD group age structure. Since the averages of ages were significantly different between the groups, age was included as a confounding factor in further logistic regression analysis of genotyping results (Table 1).

Ophthalmological evaluation

All study participants were evaluated by slit-lamp biomicroscopy to assess corneal and lenticular transparency. Classification and grading of lens opacities was performed according to the Lens Opacities Classification System III. At each examination, intraocular pressure was measured. Pupils were dilated with tropicamide 1%, after which fundoscopy, using a direct

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Table 1. Demographic characteristics of the study population.

Characteristic	Group						*p* Value
	Early AMD n=300		Exudative AMD n=300		Control n=800		
Men, n (%)	98	(32.7)	114	(38.0)	282	(35.2)	0.393
Women, n (%)	202	(67.3)	186	(62.0)	518	(64.8)	
Age, mean (SD)	71.88	(10.3)	75.5	(7.7)	50.83	(14.1)	<0.001

SD – standard deviation.

monocular ophthalmoscope, and slit-lamp biomicroscopy with a double aspheric lens of +78 diopters. Results of eye examinations were recorded on special standardized forms. For detailed analysis of the macula, stereoscopic color fundus photographs of the macula, centered at 45° and 30° to the fovea, were obtained with a Visucam NM Digital camera (Carl Zeiss Meditec AG, Germany).

The classification system of AMD formulated by the Age-Related Eye Disease Study [48] was used: early AMD consisted of a combination of multiple small and several intermediate drusen (63–124 μm in diameter), or retinal pigment epithelial abnormalities; intermediate AMD was characterized by the presence of extensive intermediate drusen and at least one large druse (≥125 μm in diameter), or geographic atrophy (GA) not involving the center of the fovea; and advanced AMD was characterized by GA involving the fovea and/or any of the features of neovascular AMD [49].

The following participant exclusion criteria were used: 1) unrelated eye disorders, e.g., high refractive error, cloudy cornea, lens opacity (nuclear, cortical, or posterior subcapsular cataract) except minor opacities, keratitis, acute or chronic uveitis, glaucoma, or diseases of the optic nerve; 2) systemic illnesses, e.g., diabetes mellitus, malignant tumors, systemic connective tissue disorders, chronic infectious diseases, or conditions following organ or tissue transplantation; 3) ungraded color fundus photographs resulting from obscuration of the ocular optic system or because of fundus photograph quality.

Single nucleotide polymorphisms (SNP) selection

In our study, we selected two well-characterized single nucleotide polymorphisms (SNPs): rs1800624 (T-374A) and rs1800625 (T-429C), which are located in the promoter region of the *RAGE* gene (http://www.ncbi.nlm.nih.gov/projects/SNP/). Both SNPs have already been associated with several types of medical conditions such as diabetes mellitus [50,51] and coronary artery disease [52] which are strongly associated with AMD [53,54]; studies have also confirmed a minor allele frequency of >5% [50–52]. Based on these criteria, the two SNPs were selected.

DNA extraction and genotyping

The DNA extraction and analysis of the polymorphisms of the *RAGE* gene (rs1800624 and rs1800625) were carried out in the Laboratory of Ophthalmology at the Institute of Neuroscience of LUHS. DNA was extracted from white blood cells using the silica-based membrane technology utilizing a genomic DNA extraction kit (GeneJET Genomic DNA Purification Kit, Thermo Scientific) according to the manufacturer's recommendations.

The genotyping of *RAGE* gene polymorphisms (rs1800624 and rs1800625) was carried out using real-time polymerase chain reaction (RT-PCR) method. Both SNPs were determined using TaqMan® Genotyping assays (Applied Biosystems; Thermo Fisher Scientific, Inc.), C___3293837_1_(rs1800624) and C___8848033_1_ (rs1800625) according to manufacturer's protocols by a Rotor-Gene Q RT-PCR quantification system (Qiagen, USA).

Genotyping quality control

For quality control, 5% of randomly chosen samples for each of the two SNPs were selected for repetitive analysis. Replication experiments revealed a 100% concordance rate of genotypes and alleles with the initial genotyping results.

Statistical analysis

The data are presented as absolute numbers with percentages in brackets and average of ages. The frequencies of genotypes and alleles (in percentages) are presented in Table 2.

Hardy-Weinberg analysis was performed to compare the observed and expected frequencies of polymorphisms (rs1800624 and rs1800625) using the χ^2 test in all groups. The distribution of the *RAGE* (rs1800624 and rs1800625) SNPs in the early and exudative AMD and control groups were compared using the χ^2 test or the Fisher's exact test. To reduce the possibility of type I error due to multiple testing, Bonferroni correction, and a $p>0.05/2$ (since we analysed two different SNPs) were used to confirm statistical significance. Risk prediction for early

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Table 2. The genotype distributions and allele frequencies of polymorphisms in RAGE gene in early and exudative AMD patients and controls.

SNP	Genotype/ alleles	Controls, n (%)	HWE p value	Early AMD, n (%)	HWE p value	Exudative AMD, n (%)	HWE p value	p value
rs1800624	AA	320 (40.0)	0.075	129 (43.0)	0.169	135 (45.0)	0.439	0.474
	AT	353 (44.1)		127 (42.3)		128 (42.7)		
	TT	127 (15.9)		44 (14.7)		37 (12.3)		
	A	993 (62.06)		385 (64.17)		398 (66.33)		
	T	607 (37.94)		215 (35.83)		202 (33.67)		
rs1800625	AA	580 (72.5)	0.378	202 (67.3)	0.382	200 (66.7)	0.779	0.122
	AG	206 (25.8)		91 (30.3)		89 (29.7)		
	GG	14 (1.8)		7 (2.3)		11 (3.7)		
	A	1366 (85.38)		495 (82.5)		489 (81.5)		
	G	234 (14.63)*		105 (17.5)		111 (18.5)*		

* p=0.026; ** Bonferroni-corrected significance threshold p=0.05/2.

and exudative AMD with *RAGE* gene polymorphisms was calculated by logistic regression analysis after controlling for age. Adjustments for age as adjusted odds ratios (aOR) and its 95% confidence interval (95% CI) are presented in Tables 3 and 4.

Linkage disequilibrium analysis and haplotype-based case-control analysis were performed using the expectation maximization algorithm and free PLINK software (version 1.07) [55]. Statistical analysis was performed using the SPSS/W 20.0 software (Statistical Package for the Social Sciences for Windows, Inc., Chicago, IL, USA).

Differences were considered statistically significant when $p<0.05$.

Results

Statistical analysis revealed that rs1800624 and rs1800625 genotype distributions did not deviate from Hardy-Weinberg equilibrium in all groups at a significance level of 5%.

Further analysis did not show statistically significant differences ($p>0.05$) in the genotype distributions of rs1800624 and rs1800625 between the AMD and control groups, but analysis of rs1800625 polymorphism in the *RAGE* gene showed statistically higher frequency of allele G in exudative AMD patients than in the control patients (18.5% versus 14.63%; $p=0.026$) (Table 2). Unfortunately, these results did not survive the Bonferroni correction ($p>0.05/2$).

Binomial logistic regression analysis was performed to evaluate the risk prediction of rs1800624 and rs1800625 for early and exudative AMD development. Analysis in early AMD did not show any statistically significant variables. On the other hand, analysis in exudative AMD showed significant variables after adjustment for age: rs1800624 AT+TT genotypes together were associated with a 1.6-fold decreased risk for exudative AMD under the dominant model after adjustment for age (OR=0.616; 95% CI: 0.394–0.963; $p=0.034$) (Table 3). Results showed that each copy of allele T at rs1800624 was associated with a 1.4-fold decreased risk for exudative AMD development under the additive model after adjustment for age (OR=0.701; 95% CI: 0.510–0.962; $p=0.028$) (Table 3). Results suggested that allele T at rs1800624 was protective against AMD development.

The same analysis was performed on rs1800625 SNP and revealed that allele G at rs1800625 was associated with a 1.5-fold increased risk for exudative AMD after adjustment for age (OR=1.545; 95% CI: 1.003–2.379; $p=0.048$) (Table 3). Results suggested that allele G at rs1800625 was a risk-allele for exudative AMD development.

In the haplotype analysis, after assigning the most common haplotype A-A as a reference, we found that haplotype A-G was significantly more frequently observed in exudative AMD patients compared to healthy controls (3.3% versus 1.4%, $p=0.035$) (Table 4) but there were no associations after adjustment for age.

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Table 3. The risk prediction of two single nucleotide polymorphisms (SNPs) in RAGE gene for early and exudative AMD under the genetic models.

Polymorphisms	Model	Early AMD	Exudative AMD
		*aOR; 95% CI; p	
Codominant			
rs1800624	AT	1.052; 0.677–1.634; 0.823	0.655; 0.406–1.056; 0.082
	TT	0.726; 0.386–1.364; 0.319	0.515; 0.261–1.017; 0.056
rs1800625	AG	1.457; 0.921–2.304; 0.108	1.372; 0.830–2.268; 0.217
	GG	1.819; 0.462–7.163; 0.393	4.078; 0.976–17.048; 0.054
Dominant			
rs1800624	AT+TT	0.960; 0.634–1.455; 0.849	**0.616; 0.394–0.963; 0.034**
rs1800625	AG+GG	1.481; 0.949–2.312; 0.084	1.499; 0.922–2.437; 0.102
Recessive			
rs1800624	TT	0.707; 0.394–1.267; 0.244	0.634; 0.336–1.198; 0.161
rs1800625	GG	1.639; 0.422–6.6376; 0.476	3.726; 0.902–15.397; 0.069
Overdominant			
rs1800624	AT	1.148; 0.763–1.726; 0.508	0.770; 0.493–1.204; 0.252
rs1800625	AG	1.431; 0.907–2.258; 0.124	1.309; 0.795–2.154; 0.0290
Additive			
rs1800624	T	0.896; 0.670–1.200; 0.462	**0.701; 0.510–0.962; 0.028**
rs1800625	G	1.424; 0.958–2.118; 0.081	**1.545; 1.003–2.379; 0.048**

* Adjusted for age.

Table 4. The frequencies of gene-based haplotypes and their risk prediction of early and exudative AMD.

Haplotype	Patients, n (%)	Controls, n (%)	p	*aOR; 95% CI; p
RAGE gene: rs1800624-rs18006025				
Early AMD				
A-A	252 (84.0)	662 (82.5)	0.622	Reference
A-G	4 (1.3)	11 (1.4)	0.958	1.857; 0.368–9.363; 0.453
T-A	41 (13.7)	124 (15.5)	0.448	0.684; 0.375–1.248; 0.216
T-G	3 (1.0)	3 (0.4)	0.210	1.250; 0.122–12.783; 0.851
Exudative AMD				
A-A	253 (84.3)	662 (82.5)	0.532	Reference
A-G	10 (3.3)	11 (1.4)	**0.035**	3.347; 0.672–16.670; 0.140
T-A	36 (12.0)	124 (15.5)	0.143	0.588; 0.308–1.120; 0.106
T-G	1 (0.3)	3 (0.4)	0.919	4.991; 0.289–86.224; 0.269

* Adjusted for age.

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Discussion

The impact of two *RAGE* gene polymorphisms on the development of early and exudative AMD was analyzed in our study. To our knowledge, no studies analyzing the impact of genes' polymorphisms on the development of early or exudative AMD have been carried out. Previous studies on the morphogenesis of AMD drew attention to the role of advanced glycation end products (AGEs) formation in Bruch's membrane overlying deposits [8–21] as well as in retinal pigment epithelium (RPE) [22] but not genetic predisposition.

In our study, we examined the genetic predisposition of polymorphisms in *RAGE* gene to test the hypothesis that these two polymorphisms (rs1800624 and rs1800625) may contribute to formation of pathogenic deposits of AGEs causing AMD.

In our study, single locus analysis did not show statistically significant differences in the genotype distributions of rs1800624 and rs1800625 between the AMD and the control groups.

Further analysis showed that rs1800624 AT+TT genotypes together were associated with a 1.6-fold decreased risk for exudative AMD under the dominant model after adjustment for age (OR=0.616; 95% CI: 0.394–0.963; p=0.034) and each copy of allele T at rs1800624 was associated with a 1.4-fold decreased risk for exudative AMD development under the additive model after adjustment for age (OR=0.701; 95% CI: 0.510–0.962; p=0.028).

Analysis of rs1800625 revealed that allele G at rs1800625 was associated with a 1.5-fold increased risk for exudative AMD after adjustment for age (OR=1.545; 95% CI: 1.003–2.379; p=0.048). As AGE receptors are presented on the plasma membrane, the same as Toll-like receptors [40], and form a complex on the plasma membrane to endocytose AGE-modified proteins, any SNP in gene coding AGE receptors can alter the function of the receptor and it's availability to bind the AGEs and inhibit the protective function. Also, increased RPE exposure with AGEs can damage retinal tissue causing the pathological angiogenesis process [41] because AGEs can induce upregulation of one of the proangiogenic cytokines (i.e., VEGF) secreted by RPE cells [42] and cause the exudative AMD.

Because a single gene polymorphism may not show a significant association with the disease because of its small effect, a haplotype analysis was performed. In the haplotype analysis, after assigning the most common haplotype A-A as a reference, we found that haplotype A-G was significantly more frequently observed in exudative AMD patients compared to healthy controls (3.3% versus 1.4%, p=0.035) but no associations were found after adjustment for age.

There are no studies analyzing *RAGE* (rs1800624, rs1800625) gene polymorphisms, but immunohistochemical studies analyzing RAGE in patients with AMD are in agreement and state that RAGE plays an important role in the development of AMD. Glenn et al. found in their study that RAGE was important in the retinal aging process [56]. Schmidt et al. reported agreement with the previous study by Glenn et al. and stated that RAGE can be upregulated with aging and disease, and induce a pathologic response through several signal transduction pathways [57]. Another study, performed by Howes et al., immunolocalized RAGE and AGEs in the RPE and photoreceptors in early AMD and geographic atrophy, and hypothesized that RAGE mediates a local inflammatory response which is important in AMD pathogenic process [9]. Yamada et al. showed that RAGE is expressed on RPE and the levels increased during age-related pathology, especially in cells near drusen [58]. It was found that in areas of basal deposits, the RPE had more intense staining for RAGE and AGER1 compared to regions of healthy Bruch's membrane. The study authors suggested that AGE receptors could influence the formation of basal deposits during aging and AMD development as well [58]. Research by Tian et al. stated that AGEs were increased in RPE, drusen, and Bruch's membrane in ageing eyes and in patients with AMD, and that this process was associated with chronic inflammation in the outer retina [59]. It should be noted that findings in AGE linkage to RPE-BM-choroid microenvironment may be strongly associated with other age-related conditions such as atherosclerosis or AMD [59]. In an AMD experimental model, McFarlane et al. characterized the AGE receptor complex in human cultured RPE [60], and in an experimental diabetic retinopathy model also found that transgenic expression of RAGE augmented blood-retinal barrier breakdown and leukostasis, accompanied by increased expression of VEGF and ICAM-1 in the retina [61]. RAGE was found to be significantly elevated in the Müller glia in the diabetic retina as well [62] and Chen et al. showed that RAGE could play an essential role in immune cell activation within CNV lesions. Their study showed that RAGE expression was significantly increased in the retina during CNV of WT mice and RAGE-/- mice exhibited significantly reduced CNV lesion size when compared to WT controls [63].

In the normal retina, RAGE expression occurs predominantly in the Müller glia. AGEs were localized primarily in the vitreous cavity and internal limiting membrane of the retina, where they were intimately associated with the footplates of RAGE-expressing Müller cells [64].

Many researchers focus their attention on detection of genes involved in lipid metabolism that play a strong role in the pathogenesis and progression of AMD; for example, two common SNPs (rs493258 and rs10468017) in LIPC gene were found to be associated with decreased risk for advanced AMD development (ORhom=0.74; 95% CI 0.63, 0.87; $p=1.21\times10^{-4}$ and

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Table 5. Characteristics of studies of the RAGE rs1800624 and rs1800625 polymorphisms in control groups.

Study	Race	No. of controls	Control, N (%)			P HWE
			rs18000624			
			TT (AA)	TA (AT)	AA (TT)	
Yue L et al.,2016 [66]	Asian	518	341 (65.8)	152 (29.3)	25 (4.8)	0.008
Su S-C et al., 2015 [67]	Asian	300	220 (73.3)	72 (24)	8 (2.7)	0.476
Su S et al., 2015 [68]	Asian	592	435 (73.5)	136 (23.0)	21 (3.5)	0.014
Pan H et al., 2014 [69]	Asian	504	354 (70.3)	143 (28.3)	7 (1.4)	0.077
Wang ZT et al., 2014 [70]	Asian	479	343 (71.6)	123 (25.7)	13 (2.7)	0.623
Cohena CR et al., 2012 [71]	Caucasian-Brazilians	260	121 (46.5)	100 (38.5)	33 (12.7)	0.094
Cohena CR et al., 2012 [71]	African-Brazilians	151	38 (51.3)	21 (28.4)	6 (8.1)	0.236
Cunha C et al., 2011 [7]	Caucasian	468	215 (45.9)	253 (54.1)		0.340
Lindholm E et al., 2008 [73]	Scandinavian	206	127 (62.0)	67 (32.7)	11 (5.4)	0.582
Study	**Race**	**No. of controls**	**Control, N (%)**			**P HWE**
			rs18000625			
			TT (AA)	TC (AG)	CC (GG)	
Yue L et al., 2016 [66]	Asian	518	360 (69.4)	143 (27.7)	15 (2.9)	0.861
Su S-C et al., 2015 [67]	Asian	300	277 (92.3)	22 (7.4)	1 (0.3)	0.434
Su S et al., 2015 [68]	Asian	592	532 (89.9)	57 (9.6)	3 (0.5)	0.280
Pan H et al., 2014 [69]	Asian	504	365 (72.4)	130 (25.7)	9 (1.8)	0.507
Wang ZT et al., 2014 [70]	Asian	479	353 (73.7)	118 (24.63)	8 (1.7)	0.602
Cohena CR et al., 2012 [71]	Caucasian-Brazilians	257	190 (74.0)	60 (23.3)	7 (2.7)	0.397
Cohena CR et al., 2012 [71]	African-Brazilians	74	61 (82.4)	12 (16.2)	1 (1.4)	0.647
Prasad P et al., 2010 [74]	Asian-Indians	225	173 (77.0)	50 (22.0)	2 (1.0)	0.434

ORhom=0.74; 95% CI 0.60, 0.92; $p=1.67\times10^{-3}$) [65]. A significant association ($p=4.95\times10^{-}10$) was found with the gene encoding CFH, and the tyrosine-402 → histidine-402 protein polymorphism was associated with a 2.7-fold greater risk of AMD [66]. In a study by Haines et al., Y402H, a common coding variant in the CFH gene, was found to increase the risk of AMD by 2.45–5.57 times [67]. In 2009, Bergeron-Sawitzke et al. examined 424 patients with AMD and 215 control individuals who were genotyped for SNPs. The study revealed that the GG genotype (rs1410996) of the CFH gene was associated with the greatest risk of AMD (OR=6.6, 95% CI, 3.5–12; $p=8.7\times10^{-11}$) [68].

Other researchers are looking for associations between AMD risk alleles and response to anti-angiogenic treatment; one of the studies showed that anti-VEGF treatment was much more effective in patients with nAMD having rs833061 (CC versus TT: OR=2.222, 95% CI 1.252; 3.944, $p=0.006$; CT versus TT: OR=2.537, 95% CI 1.478; 4.356, $p=0.001$ and CC versus CT+TT: OR=2.362, 95% CI 1.414; 3.946, $p=0.001$), [69]. In addition, newer technologies allow researchers to consider other insights into iPSC-RPE AMD modelling, by adding or eliminating the risk alleles for better response to the treatment [70].

To the best of our knowledge, there are no studies analyzing the *RAGE* rs1800624 and rs1800625 gene polymorphisms in patients with AMD and, therefore, we can only compare the results of our control group with different studies that also analyzed these gene polymorphisms in control groups (Table 5).

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Our results showed that *RAGE* rs1800624 TT (15.9%) genotype in the control group was more frequent compared to others studies [65-73]. But most studies were done on Asians populations; with only two studies, Cunha et al. (2011) [74] and Cohena et al. (2012) [70], that analyzed Caucasians and revealed quite similar results for genotype distribution. *RAGE* rs1800625 GG (1.8%) genotype prevalence in our study was very similar to other studies that varied from 0.3% to 2.7% (Table 5).

One strength of our study was the thorough clinical assessment of the patients. The patients with ischemic heart disease, neurological disorders, malignant tumors, rheumatoid diseases, and end-stage liver or renal diseases were excluded.

Results for the *RAGE* rs18006255 were largely consistent with previous studies, but *RAGE* rs1800624 results were reverse compared to other studies. Of course, we can explain the difference of the results from previous studies by different genotype distribution in different populations. While one genotype may be protective in one population, the same genotype may increase the possibility of disease development in another population.

To the best of our knowledge, for the first time, both of our study gene polymorphisms were investigated for the association with AMD.

Patients with early AMD have to be followed in order to find out which form of AMD (wet or dry) will manifest in later years.

Conclusions

In conclusion, the rs1800624 and rs1800625 polymorphisms in the *RAGE* gene were associated with the susceptibility of early and exudative AMD patients. However, to our knowledge, this is the first study aimed to investigate the association of *RAGE* gene polymorphisms with AMD, so their role as a biomarker for prognosis of AMD development cannot yet be confirmed. For this reason, further studies are needed to explore and confirm these associations.

Conflicts of interests

None.

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