IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2021; 10(2): 20-31 Arıkan Söylemez ES et all.

RORA, *ROBO1*, *CFH* and *HTRA1* GENE POLYMORPHISMS and NEOVASCULAR AGE-RELATED MACULAR DEGENERATION in a TURKISH COHORT STUDY

Evrim Suna ARIKAN SÖYLEMEZ[1], Mustafa SOLAK[2]

Saliha Handan YILDIZ[3], Müjgan ÖZDEMİR ERDOĞAN[3], Ümit Ubeyt İNAN[4]

Correspondence: [1]Assist. Prof.Dr. Evrim Suna ARIKAN SÖYLEMEZ, Department of Medical Biology, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey, arikanmt@gmail.com
[2]Prof.Dr. Mustafa SOLAK, Department of Medical Genetic, Faculty of Medicine, Biruni University, İstanbul, Turkey, msolak@gmail.com
[3]Prof.Dr. Saliha Handan YILDIZ, Department of Medical Genetic, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey, handanyildizus@gmail.com
[3]Prof.Dr. Müjgan ÖZDEMİR ERDOĞAN, Department of Medical Genetic, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Turkey, mozdemir1977@gmail.com
[4]Prof.Dr. Ümit Übeyt İNAN, Department of Ophthalmology, Park Hayat Hospital, Turkey, uuinan@gmail.com

ABSTRACT

The aim of this study is to investigate whether there are any associations between rs8034864 (*RORA*), rs1387665 (*ROBO1*), rs1329424 (*CFH*), rs3793917 (*HTRA1*) polymorphisms and Neovascular Age Related Macular Degeneration (nAMD).

We conducted a case-control study of the related polymorphisms in Turkish patients. We compared genotype and allele frequencies of 125 patients diagnosed nAMD and 108 healthy controls in terms of related polymorphisms. Genotyping was performed with Real-time Polymerase Chain Reaction.

We found significant association between rs1329424 (*CFH*), rs3793917 (*HTRA1*) polymorphisms and nAMD. There were significant differences between cases and controls in terms of genotype and allele frequencies of rs1329424 (*CFH*), rs3793917 (*HTRA1*) (P=0,0017, OR= 1,82; P<0,0001, OR=3,26; respectively). On contrary to these findings, there were no significant associations between rs8034864 (*RORA*), rs1387665 (*ROBO1*) polymorphisms and nAMD. There were no significant differences between cases and controls in terms of genotype and allele frequencies of rs8034864 (*RORA*) and rs1387665 (*ROBO1*) (P=0,110995, OR= 0,72; P=0,236724, OR= 1,25; respectively).

Significant association was verified between rs1329424 (*CFH*), rs3793917 (*HTRA1*) polymorphisms and nAMD in our study group as reported in literature before. However, further studies in a larger sample are needed for providing information about the rs8034864 (*RORA*), rs1387665 (*ROBO1*) polymorphisms and nAMD.

Key Words: Age-related macular degeneration, *RORA*, *ROBO1*, *CFH*, *HTRA1*, polymorphism.

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2021; 10(2): 20-31 Arıkan Söylemez ES et all.

INTRODUCTION

Age-related macular degeneration (AMD), the leading cause of irreversible central vision loss in elderly populations in developed countries (1), is a clinically heterogeneous and genetically complex disease with genetic and environmental risk factors (2). The number of affected individuals is expected to increase to 288 million by 2040 (3). There are two types of AMD, the "dry" and "wet" forms. Specially, an individual with wet AMD, also called neovascular AMD, may experience sudden loss of vision when a choroidal neovascular membrane leaks fluid or blood (4).

Different genomic regions and a variety of candidate genes have been shown to impact AMD susceptibility. For example, *ABCR* (5), *CFH* (6-9), *FBLN5* (10), *APOE* (11), *C3* (12), *HMCN1* (13), *TLR3* (14). *ARMS2* (15-16), *VEGF* (17), *HTRA1* (15, 18, 19), *RORA* (20), *ROBO1* (21). The first major gene to be associated in AMD was the complement factor H (*CFH*). *CFH* is a member of the *RCA* (regulators of complement activation) gene cluster on human chromosome 1q32. CFH is the main inhibitor of the alternative pathway of the complement system (22). Bok et al. (23) suggested that dysfunction of the complement system results in local tissue damage, particularly at vulnerable locations such as the retinal macula. SNPs in *ARMS2* (Age related maculopathy susceptibility 2) and *HTRA1* (HtrA serine peptidase 1), two genes in strong linkage disequbrium on chromosome 10q26 related to extrascellular matrix function, are also associated with AMD susceptibility (24). *RORA* is known to be involved in a number of biological processes with potential relevance to AMD, including immune and lipid metabolism pathways (25). Silveira et al. (26) reported that *RORA* and *ROBO1* are down regulated at least two-fold among individuals with AMD compared with their unaffected siblings. Although, *CFH* and *HTRA1* have been studied more commonly in the literature, *RORA* and *ROBO1* have been rarely studied in AMD and not verified yet.

To determine if the *RORA* and *ROBO1* polymorphisms are genetic risk factors for developing AMD in the studied group, we analyzed rs8034864 (*RORA*) and rs1387665 (*ROBO1*) polymorphisms, as well as rs1329424 (*CFH*) and rs3793917 (*HTRA1*) polymorphisms in patients with nAMD and healthy controls in Turkish population in the western region of Turkey.

MATERIALS and METHODS

Sample Collection

Patients with nAMD (n=125, mean age at the time of the study was 74.34 ± 6.75) attending Department of Ophthalmology were asked to participate in this case-control study. Patients attending the department for other reasons were included as control subjects (n=108, mean age at the time of the study was 74.46 ± 8.57). All the cases and controls were underwent a clinical examination by a retina specialist. If they were revealed as having neovascular AMD by funduscopy, the patients would be examined further with optical coherence tomography

(OCT) (Zeiss, Cirrus^{TM} HD-OCT 4000, Jena, Germany). When confirmed by OCT, the neovascular AMD patients would be included if they were 50 years or older and had no signs of other retinal diseases. None of the individuals had diabet or diabetic retinopaty. Patients attending the department for other reasons were taken as controls if they were 50 years or older, confirmed negative for any type of AMD with fundus examination, had no signs of other retinal diseases.

Ethics Committee Approval

This study was approved by Afyon Kocatepe University Medical Ethic Committee (11.04.2013/57) and informed consent was obtained from all the participants.

DNA Isolation and Genotype Analyses

2ml aliquots of peripheral blood samples were collected from the participants and stored in EDTA-coated vacutainers. Genomic DNA was extracted from a 200µl peripheral blood sample by using a EZ1 DNA Blood 200 µl kit (Qiagen, GmbH, Germany). Then, DNA amount and DNA purity were quantified for each DNA sample by Nanodrop ND-1000 spectrophotometer V 3.7 (Thermo Fisher Scientific; DE, USA). DNA samples were stored at -20°C until use.

Each genomic DNA sample was analysed for rs8034864 (*RORA*), rs1387665 (*ROBO1*), rs1329424 (*CFH*), rs3793917 (*HTRA1*) polymorphisms. rs8034864 (*RORA*), rs1329424 (*CFH*) and rs3793917 (*HTRA1*) genotyping were carried out by snipsig kit (Qiagen, California) and rs1387665 (*ROBO1*) genotyping was carried out by biomers.net (Germany). Genotyping was performed by real-time polymerase chain reaction (Rotor Gene-Q, Qiagen, Hilden, Germany).

Genotyping for rs8034864 (*RORA*), rs1329424 (*CFH*) and rs3793917 (*HTRA1*): 15 µl reaction mixture was prepared. The reaction mixture contained 10 µl 2X precision master mix, 1 µl genotyping primer/probe mix, 4 µl RNAse/DNAse free water, 5µl genomic diluted DNA in water according to positive control.

System under the following thermocycling conditions for rs8034864 (*RORA*) and rs3793917 (*HTRA1*) : 8 minutes at 95°C for qPCR enzyme activation followed by 10 cycles of PCR (10 seconds denaturation at 95°C, 60 seconds extension at 60°C) and followed by 35 cycles of PCR [10 seconds denaturation at 95°C, 60 seconds extension (Data collection) at 68°C].

System under the following thermocycling conditions for rs1329424 (*CFH*): 8 minutes at 95°C for qPCR enzyme activation followed by 10 cycles of PCR (10 seconds denaturation at 95°C, 60 seconds extension at 60°C) and followed by 35 cycles of PCR [10 seconds denaturation at 95°C, 60 seconds extension (Data collection) at 66°C].

Genotyping for rs1387665 (*ROBO1*) : 18 µl reaction mixture was performed. The reaction mixture contained 12.5µl 2X Type-it fast SNP probe PCR master mix, 1.25µl 20X primer-probe mix, 4.25µl RNAse/DNAse free water, 2µl genomic DNA.

System under the following thermocycling conditions rs1387665 (*ROBO1*): 10 minutes at 95°C for qPCR enzyme activation followed by 10 cycles of PCR (20 seconds denaturation at 95°C, 30 seconds extension at 55°C, 20 seconds extension at 72°C) and followed by 35 cycles of PCR [20 seconds denaturation at 95°C, 60 seconds extension (Data collection) at 55°C, 20 seconds extension at 72°C].

Allelic Discrimination Analyse was performed for detection all the polymorphisms.

Statistical Analysis

Statistical analysis was performed using the SPSS 18.0 program. Odds ratio (OR) was calculated by logistic regression analysis at a 95% confidence interval to determine the relationship between disease and genotype. Allele and genotype frequencies of the studied gene polymorphism and its relationship with nAMD were analyzed with the “chi-square” test. The deviation from the Hardy-Weinberg Equilibrium was tested with the "chi-square test". $P<0.05$ was considered significant.

RESULTS

All polymorphisms were studied in 125 patients with nAMD, but the number of the control group was 104, 108, 102 and 107 healthy subjects for *RORA*, *ROBO1*, *CFH* and *HTRA1* polymorphisms, respectively. The risk allele frequencies distribution of rs8034864, rs1387665, rs1329424 ve rs3793917 polymorphisms in cases and controls and the general information about the genes were presented at Table 1.

Table 1: Risk allele frequencies distribution of rs8034864, rs1387665, rs1329424 and rs3793917 polymorphisms and the general information about the genes

						Risk allele frequency	
SNP	**Gene**	**Chromosome**	**Position**	**Risk allele**	**Wild allele**	**nAMD patients**	**Control**
rs8034864	*RORA*	15	Intron	T	G	26,8%	33,65%
rs1387665	*ROBO1*	3	Intron	A	G	47,6%	42,11%
rs1329424	*CFH*	1	Intron	T	G	54%	39,2%
rs3793917	*HTRA1/ARMS*	10	Intergeni	G	C	53,6%	26,17%

rs8034864 (*RORA*) polymorphism has three possible genotypes GG, GT or TT. The distrubition of genotype frequencies of rs8034864 polymorphism in the controls was 46,2% for GG, 40,4% for GT and 13,5% for TT, and in the case group the distribution of genotype frequencies was 54,4% for GG, %37,6 for GT and 8% for TT. There were no significant differences between cases and controls in terms of genotype freguencies (P>0.05) (Table 2).

Table 2: Genotype frequencies of *RORA* gene rs8034864 polymorphism

Genotype	Control n=104	nAMD n=125	P
GG	48 (%46,2)	68 (%54,4)	
GT	42 (%40,4)	47 (%37,6)	0,288
TT	14 (%13,5)	10 (%8)	

The distribution of allele frequencies of rs8034864 polymorphism in the controls was 66,35% for G and 33,65% for T, and in the case group the distribution of allelic frequencies was 73.2% for G and 26.8% for T. There were no significant differences between cases and controls in terms of allele freguencies (P>0.05) (Table 3).

Table 3: Allele frequencies of *RORA* gene rs8034864 polymorphism

Allele	Control n=208	nAMD n=250	P	OR (95% CI)
G	138 (%66,35)	183 (%73.2)	0.113	0.7218
T	68 (%33.65)	67 (%26.8)		(0.4832-1.782)

rs1387665 (*ROBO1*) polymorphism has three possible genotypes GG, GA and AA. The distrubition of genotype frequencies of rs1387665 polymorphism in the controls was 34,3% for GG, 47,2% for GA and 18,5% for AA, and in the case group the distribution of genotype frequencies was 25,6% for GG, 53,6% for GA and 20,8% for AA. There were no significant differences between cases and controls in terms of genotype freguencies (P>0.05) (Table 4).

IJBCS
ISSN: 2147-1428

Table 4: Genotype frequencies of *ROBO1* gene rs1387665 polymorphism

Genotype	Control n=108	nAMD n=125	P
GG	37 (%34,3)	32 (%25,6)	
GA	51 (%47,2)	67 (%53,6)	0,353
AA	20 (%18,5)	26 (%20,8)	

The distribution of allelele frequencies of rs1387665 polymorphism in the controls was 57,87% for G and %42,13 for T, and in the case group the distribution of allelic frequencies was 52,4% for G and 47,6% for T. There were no significant differences between cases and controls in terms of allele freguencies (P>0.05) (Table 5).

Table 5: Allele frequencies of *ROBO1* gene rs1387665 polymorphism

Allele	Control n=216	nAMD n=250	P	OR (95% CI)
G	125 (%57,87)	131 (%52,4)	0.237	1,2478
A	91 (%42,13)	119 (%47,6)		(0.8646-1.8007)

rs1329424 (*CFH*) polymorphism has three possible genotypes GG, GT or TT. The distrubition of genotype frequencies of rs1329424 polymorphism in the controls was 34,35% for GG, 52,9% for GT, and %12,7 for TT, and in the case group the distribution of genotype frequencies was 21,6%GG, 48,8% for GT and 29,6% for TT. There were significant differences between cases and controls in terms of genotype freguencies (P<0,005) (Table 6).

Table 6: Genotype frequencies of CFH gene rs1329424 polymorphism

Genotype	Control n=102	nAMD n=125	P
GG	35 (%34,3)	27 (%21,6)	
GT	54 (%52,9)	61 (%48,8)	<0,005
TT	13 (%12,7)	37 (%29,6)	

The distribution of allelele frequencies of rs1329424 polymorphism in the controls was 60,78% for G and 39,22% for T, and in the case group the distribution of allelic frequencies was 46% for G and 54% for T. There were significant differences between cases and controls in terms of allele freguencies (p:0.0017; OR= 1.82, % 95 CI= 1.25-2.65) (Table 7).

Table 7: Allele frequencies of *CFH* gene rs1329424 polymorphism

Allele	**Control n=204**	**nAMD n=250**	**P**	**OR (95% CI)**
G	124 (%60,78)	115 (%46)	0.001274	1.8196
T	80 (%39.22)	135 (%54)		(1.2502-2.6483)

rs3793917 (*HTRA1*) polymorphism has three possible genotypes CC, CG or GG. The distrubition of genotype frequencies of rs3793917 polymorphism in the controls was 57,9% for CC, 31,8% for CG and 10,3% for GG, and in the case group the distribution of genotype frequencies was 26,4% for CC, 40% for CG and 33,6% for GG. There were significant differences between cases and controls in terms of genotype freguencies (P<0,005) (Table 8).

Tablo 8: Genotype frequencies of HTRA1 gene rs3793917 polymorphism

Genotype	**Control n=107**	**nAMD n=125**	**P**
CC	62 (%57,9)	33 (%26,4)	
CG	34 (%31,8)	50 (%40,0)	<0,005
GG	11 (%10,3)	42 (%33,6)	

The distribution of allele frequencies of rs37939917 polymorphism in the controls was 73,83% for C and 26,17% for G, and in the case group the distribution of allelic frequencies was 46,4% for C and 53,6% for G. There were significant differences between cases and controls in terms of allele frequencies (P<0,0001, OR=3,26, % 95 CI= 2.20-4.83) (Table 9).

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2021; 10(2): 20-31 Arıkan Söylemez ES et all.

Tablo 9: Allele frequencies of *HTRA1* gene rs3793917 polymorphism

Allele	Control n=214	nAMD n=250	P	OR (95% CI)
C	158 (%73.83)	116 (%46.4)	<0.0001	3.2592
G	56 (%26.17)	134 (%53.6)		(2.1994-4.8298)

DISCUSSION

RORA and *ROBO1* genes were suggested as candidate genes for Age Related Macular Degeneration (AMD) (20, 21, 26). *RORA* has been implicated in the pathology of circadian rhythms, bone growth, angiogenesis, development of cones, cellular metabolism and a mediator in the immune and lipid metabolism pathways (25). Also, it is reported that cholesterol/lipid metabolism has an importand role in the development of AMD (27-29). Additionally, *RORA* gene has been shown to be included in the pathopysiology of AMD (26). In our study, there were no significant associations between rs8034864 (*RORA*), rs1387665 (*ROBO1*) polymorphisms and neovascular AMD. Schaumberg et al. (20) measured genotypes for 18 variants including rs8034864 in intron 1 of the RORA gene among cases who developed nAMD and controls. They identified rs8034864 as a significant part of a haplotype block. However, they noted that rs8034864 was not significantly associated with the risk of nAMD. Beside, Silveria et al. (26) reported that *RORA* could affect transcription of another genes that in turn would influence the development and progression of AMD. Also they noted rs80348640 was present in the database but was not significantly associated with transcript expression. Schaumberg et al. (20) reported that *RORA* influences the development of nAMD through the genes it regulates, or indirectly through the genes that regulate *RORA*.

Jun et al. (21) evaluated association of 19 single nucleotide polymorphisms (SNPs) in *ROBO1* with wet and dry stages of AMD in a sibling cohort and a Greek case-control cohort. They reported that the most significantly associated *ROBO1* SNPs was rs1387665 under an additive model for nAMD. On contrary to these results, there were no significant differences between nAMD patients and controls in terms of allele and genotype frequencies of rs1387665 in our study. Some of the possible reasons for these different results may be different ethnic groups, different numbers of patients or regional differences. Silveira et al. (26) suggested that in order to determine whether or not *RORA* expression is truly correlated with nAMD, further studies need to be conducted on a greater number of patient samples examining not only the levels of RORA but also the specific transcripts of *RORA* expressed in the various cell types of the retina and cells involved in the process of neovascularization. Also Jun et al. (21) suggested that distinct *ROBO1* variants may influence the risk of wet and dry AMD, and the effects of *ROBO1* on AMD risk may be modulated by *RORA* variants.

We found significant association between rs1329424 (*CFH*), rs3793917 (*HTRA1*) polymorphisms and nAMD. *CFH* encodes a protein involved in the body's first line of immune defense against infection by bacteria and other microbes (23). Variation in multiple complement system genes is one of the most consistent statistical associations with AMD risk (30, 31). Hageman et al. (32) reported that a variation in the *CFH* gene dramatically increases the likelihood of developing AMD. *CFH* has been identified by various studies as a major AMD susceptible gene in some populations (33, 34) which is consistent with our study. Chen et al. (35) reported that there was a strong association between rs1329424 (*CFH*) and AMD. Tian et al. (36) reported that some genetic variants in *CFH*, including rs1329424 were associated with AMD.

A region on chromosome 10q26 (*ARMS2*, Age related maculopathy susceptibility 2) and the promoter of high-temperature requirement A1 (*HTRA1*) is also a major genetic risk for AMD (37, 38). Holliday et al. (39) detected highly significant association of numerous SNPs including rs 1329424 within *CFH* and rs3793917 within *ARMS2/HTRA1* loci with risk of early AMD. In our study, T allele frequency for rs1329424 in nAMD group was significantly higher. Additionally, there were significant differences between cases and controls in terms of genotype freguencies. These results shows that rs1329424 polymorphism is a risk for nAMD cases. Also, Tian et al. (36) reported that rs3793917 in *HTRA1* conferred strong susceptibility for AMD. Richardson et al. (40) reported that multiple tSNPs across the region showed association with AMD with the tSNP rs3793917 having the highest association with AMD. However, Haddad et al. (5) reported that, AMD is a disease with complex inheritance patterns, it may be difficult to discover each individual susceptibility gene due to multiple genetic and environmental effects and interactions. Development and severity of complex diseases like AMD are influenced by many factors (41).

To our knowledge, this is the first study to examine the association on the genetic variants of rs8034864 (*RORA*), rs1387665 (*ROBO1*) to AMD in the Turkish cohort. In conclusion, we found that *RORA* rs8034864 and *ROBO* rs1387665 gene polymorphisims are not risk factor for nAMD in our study group. We also confirmed that rs1329424 (*CFH*) and rs3793917 (*HTRA1*) polymorphisms are related to risk of AMD in a Turkish cohort. Despite the small sample size, finding significant association between studied polymorphisms and AMD shows that these polymorphisms have potential risk for nAMD. However, further studies in a larger sample are needed for providing information about the rs8034864 (*RORA*), rs1387665 (*ROBO1*) polymorphisms and AMD.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

ACKNOWLEDGEMENTS

This study was supported by the Afyon Kocatepe University Scientific Research Projects Commission with a projected numbered as 13.SAG.BIL.02.

REFERENCES

1. Gehrs KM, Anderson DH, Johnson LV, Hageman GS. Age-related macular degeneration–emerging pathogenetic and therapeutic concepts. Ann Med. 2006;38:450-471.
2. Age-Related Eye Disease Study Research Group. Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3. Ophthalmology. 2000;107:2224-2232.
3. Wong WL, Su X, Li X et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014: pp: e106–e116.
4. Haddad S, Chen CA, Santangelo SL. Seddon JM. The genetics of age-related macular degeneration: a review of progress to date. Surv Ophthalmol. 2006: **51(4)**:316-363.
5. Shroyer NF, Lewis RA, Lupski JR. Analysis of the ABCR (ABCA4) gene in 4-aminoquinoline retinopathy: is retinal toxicity by chloroquine and hydroxychloroquine related to Stargardt disease? Am J Ophthalmol. 2001 Jun;131(6):761-6.
6. Klein RJ, Zeiss C, Chew EY et al. Complement factor H polymorphism in age-related macular degeneration. Science. 2005;308: 385-389.
7. Edwards AO, Ritter R, Abel KJ, Manning A, Panhuysen C, Farrer LA. Complement factor H polymorphism and age-related macular degeneration. Science. 2005;308: 421-424.
8. Schaumberg DA, Christen WG, Buring JE, Glynn RJ, Rifai N, Ridker PM. High-sensitivity C-reactive protein, other markers of inflammation, and the incidence of macular degeneration in women. Arch Ophthalmol. 2007;125(3):300-305.
9. Huang L, Meng Q, Zhang C, et al. Gene-gene interaction of CFH, ARMS2, and ARMS2/HTRA1 on the risk of neovascular age-related macular degeneration and polypoidal choroidal vasculopathy in Chinese population. Eye (Lond). 2015 May;29(5):691-8. doi: 10.1038/eye.2015.32.
10. Lotery AJ, Baas D, Ridley C et al. Reduced secretion of fibulin 5 in age-related macular degeneration and cutis laxa. Hum Mutat. 2006;27(6):568-74.
11. Bojanowski CM, Shen D, Chew EY et al. An apolipoprotein E variant may protect against age-related macular degeneration through cytokine regulation. Environ Mol Mutagen. 2006;47:594-602

12. Francis PJ, Hamon SC, Ott J, Weleber RG, Klein ML. Polymorphisms in C2, CFB and C3 are associated with progression to advanced age related macular degeneration associated with visual loss. J Med Genet. 2009;46:300-307.
13. Thompson CL, Klein BE, Klein R et al. Complement factor H and hemicentin-1 in age-related macular degeneration and renal phenotypes. Hum. Mol Genet. 2007;16(17):2135-48.
14. Yang Z, Stratton C, Francis PJ et al. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. N Engl J Med. 2008;359(14):1456-63.
15. Dewan A, Liu M, Hartman S et al. HTRA1 promoter polymorphism in wet age-related macular degeneration. Science. 2006;314: 989-992.
16. Kanda A, Chen W, Othman M et al. A variant of mitochondrialprotein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. Proc Natl Acad Sci. USA 2007;104(41):16227-16232.
17. Lin JM, Wan L, Tsai YY et al. Vascular endothelial growth factor gene polymorphisms in age-related macular degeneration. Am J Ophthalmol. 2008;145(6):1045-1051.
18. De Angelis MM, Ji F, Adams S et al. Alleles in the HtrA serine peptidase 1 gene alter the risk of neovascular age-related macular degeneration. Ophthalmology. 2008;115(7):1209-1215.
19. Gibbs D, Yang Z, Constantine R et al. Further mapping of 10q26 supports strong association of HTRA1 polymorphisms with age-related macular degeneration. Vision Res. 2008;48(5):685-689.
20. Schaumberg DA, Chasman D, Morrison MA, et al. Prospective study of common variants in the retinoic acid receptor-related orphan receptor α gene and risk of neovascular age-related macular degeneration. Arch Ophthalmol. 2010;128(11):1462-1471.
21. Jun G, Nicolaou M, Morrison MA, et al. Influence of ROBO1 and RORA on risk of age-related macular degeneration reveals genetically distinct phenotypes in disease pathophysiology. PLoS ONE. 2011;6(10):e25775.
22. Rodríguez de Cordoba S, Esparza-Gordillo J, Goicoechea de Jorge E, Lopez-Trascasa M, Sanchez-Corral P. The human complement factor H: functional roles, genetic variations and disease associations. Mol Immunol. 2004;41:355-367.
23. Bok, D. Evidence for an inflammatory process in age-related macular degeneration gains new support. Proc Natl Acad Sci. USA. 2005; 102(20):7053-7054.
24. Wang G, Dubovy SR, Kovach JL, et al. Variants at chromosome 10q26 locus and the expression of HTRA1 in the retina. Exp Eye Res. 2013;112:102-105.
25. Jetten, AM. Retinoid-related orphan receptors (RORs): critical roles indevelopment, immunity, circadian rhythm, and cellular metabolism. Nucl.Recept.Signal. 2009;7: e003. doi: 10.1621/nrs.07003.
26. Silveira AC, Morrison MA, Ji F, et al. Convergence of linkage, gene expression and association data demonstrates the influence of the RAR-related orphan receptor alpha (RORA) gene on neovascular AMD: a systems biology based approach.Vision Res. 2010;50(7):698-715.

27. Yamada Y, Tian J, Yang Y, et al. Oxidized low density lipoproteins induce a pathologic response by retinal pigmented epithelial cells. J Neurochem. 2008;105:1187–1197.
28. Yu AL, Lorenz RL, Haritoglou C, Kampik A, Welge-Lussen U. Biological effects of native and oxidized low-density lipoproteins in cultured human retinal pigment epithelial cells. Exp Eye Res. 2009;(88), pp. 495-503.
29. Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration Prog Retin Eye Res. 2009;28(1):1-18.
30. Bird AC. Therapeutic targets in age-related macular disease. J Clin Invest. 2010;120:3033-3041.
31. Bradley DT, Zipfel PF, Hughes AE. Complement in age-related macular degeneration: a focus on function. Eye (Lond). 2011;25(6):683-693.
32. Hageman GS, Anderson DH, Johnson LV, et al. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc Natl Acad Sci USA. 2005;102:7227-7232.
33. Liu X, Zhao P, Tang S. Association study of complement factor H, C2, CFB, and C3 and age-related macular degeneration in a Han Chinese population. Retina. 2010;30:1177-1184.
34. Seddon JM, Gensler G, Rosner B. C-reactive protein and CFH, ARMS2/HTRA1 gene variants are independently associated with risk of macular degeneration. Ophthalmology. 2010;117:1560-1566.
35. Chen W, Stambolian D, Edwards AO, et al. Genetic variants near TIMP3 and high-density lipoprotein-associated loci influence susceptibility to agerelatedmacular degeneration. Proc Natl Acad Sci USA. 2010;107:7401-7406.
36. Tian J, Yu W, Qin X, et al. Association of genetic polymorphisms and age-related macular degeneration in Chinese population. Invest Ophthalmol Vis Sci. 2012;53(7):4262-4269.
37. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome10q26. Am.J.Hum.Genet. 2005;77:389-407.
38. Rivera A, Fisher SA, Fritsche LG, et al. Hypothetical LOC387715 isa second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H todisease risk. Hum. Mol. Genet. 2005;14:3227-3236.
39. Holliday EG, Smith AV, Cornes BK. Insights into the genetic architecture of early stage age-related macular degeneration: a genome-wide association study meta-analysis. PLoS One. 2013;8(1):e53830
40. Richardson AJ, Islam FM, Aung KZ, Guymer RH, Baird PN. An intergenic region between the tagSNP rs3793917 and rs11200638 in the HTRA1 gene indicates association with age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 2010;51(10):4932-4936
41. De Angelis MA, Silveira AC, Carr ES and Kim IK. Genetics of Age-Related Macular Degeneration: Current Concepts, Future Directions. Semin Ophthalmol. 2011;26(3): 77-93.