

Received: 2010.0226
Accepted: 2011.09.13
Published: 2012.02.01

A69S and R38X ARMS2 and Y402H CFH gene polymorphisms as risk factors for neovascular age-related macular degeneration in Poland – a brief report

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
- F** Literature Search
- G** Funds Collection

Sławomir J. Teper^{1ABCDEF}, Anna Nowińska^{1BF}, Edward Wylęgała^{1AFG}

Department of Ophthalmology, OSK, Katowice, Poland

The study was presented (in part) during the EURETINA meeting in Nice, France, 2009 and the EVER meeting in Portoroz, Slovenia, 2009

Source of support: This study was supported by the Medical University of Silesia in Katowice, Poland (Department of Nursing and Social Medical Issues)

Summary

Background:

The wet form of age-related macular degeneration (ARMD) is a leading cause of irreversible blindness in Caucasians. Our purpose was to assess influence of gene polymorphisms A69S (rs10490924) and R38X (rs2736911) ARMS2 and Y402 (rs1061170) CFH on wet ARMD risk in a Polish population.

Material/Methods:

130 unrelated patients (90 with wet ARMD and 40 controls) took part in the study. Dry blood was used for DNA isolation. PCR amplification and gene sequencing were performed. In subjects with R38X and A69S, SNP gene cloning was used to exclude the possible combined variant.

Results:

Homozygous Y402H and A69S conferred a significance risk of wet ARMD in Poland: Y402H odds ratio (OR) was 5.57 (95% confidence interval: 1.58–19.6), $p=0.002$; and A69S OR was 7.72 (95% confidence interval: 1.73–34.36), $p=0.001$. R38X is probably more common in healthy subjects: OR was 0.45 (95% confidence interval: 0.19–1.05), $p=0.053$.

Conclusions:

The etiologic role in ARMD of A69S ARMS2 and Y402H CFH gene variants were confirmed in a Polish population for the first time. R38X variant of ARMS2 seems to be protective from wet ARMD.

key words:

age-related macular susceptibility 2 (ARMS2) • complement factor H (CFH) • age-related macular degeneration • gene polymorphism

Full-text PDF:

<http://www.medscimonit.com/fulltxt.php?ICID=882447>

Word count:

1646

Tables:

–

Figures:

–

References:

11

Author's address:

Sławomir Teper, Department of Ophthalmology, OSK in Katowice, Panewnicka 65 St., 40-760 Katowice, Poland, e-mail: s_teper@wp.pl

BACKGROUND

Since 2003 scientists have been using data from the HapMap Project. One of the main purposes of the database is to facilitate haplotype association and present human genome recombination patterns in correlation with linkage disequilibrium [1]. It is very difficult to achieve statistical significance when searching for gene associations in familial studies in late-onset multifactorial and multilocus diseases such as age-related macular degeneration (ARMD). Thus single nucleotide polymorphism map has become a crucial tool in ARMD studies.

Association studies often present data that differ substantially among populations. Therefore it is important to check whether the role of a particular SNPs is observed worldwide – the more studies there are to compare, the higher the confidence achieved. In many multifactorial diseases there are many SNPs increasing/decreasing risk, with irregular geographic distribution.

This is the first study of the most common genetic linkage in ARMD in Poland and one of few that considered R38X ARMS2 and its protective effect.

MATERIAL AND METHODS

Patients

Ninety patients with non-familial ARMD and 40 unrelated control individuals took part in the study. The ARMD group consisted of patients qualified for intravitreal VEGF inhibition due to CNV subsequent to ARMD confirmed by fluorescein angiography and optical coherence tomography. Patients with high myopia (over -8D) were excluded from both groups. Control subjects were randomly selected from a pool of patients undergoing cataract surgery. Inclusion criteria in this group were age over 75, macula without pathologic changes in biomicroscopy, and OCT in both eyes.

All the study subjects provided written consent. The research followed the Tenets of the Declaration of Helsinki. The Ethics Committee of the Medical University of Silesia (Katowice, Poland) approved the study protocol.

Genotyping

Genomic DNA was extracted from peripheral blood leukocytes (dry blood samples collected on FTA® cards, Whatmann). A 2.0 mm disc was punched and collected into a sterile Eppendorf tube for DNA isolation. DNA was isolated by using the lysis and neutralization solutions from REExtract-N-Amp™ Blood PCR Kit (Sigma) according to the manufacturer's protocol. The following primers were used for PCR amplification of the ARMS2: 5'-ATACCCAGGACCGATGGTAAC-3' and 5'-AGAGGAAGGCTGAATTGCCTA-3' primer pair, while: 5'-TTGACTAATGCCCATTAATAGGAG-3' and 5'-TTGATATTTCTTTTTGTGCAAACC-3' primer pair was used for CFH alleles. A 25 ml PCR reaction mix from the same kit was used twice with 1ul of DNA sample and 5 pmol of each primer pair. The amplification conditions were as follow: 95°C initial denaturation for 3 min (minutes), 35 cycles of 94°C denaturation for 10 sec (seconds), 58°C (for ARMS2) or 50°C (for CFH) annealing for 20 sec and

elongation at 72°C for 40 sec. In all PCR reactions a final elongation step at 72°C for 7 min was applied. The quality and quantity of the PCR products was checked on 1% agarose gel by electrophoresis in TBE buffer. About 20 ng of each PCR product was purified with the ExoSAP-IT® enzyme mix according to the manufacturer's instructions and directly submitted for DNA sequencing.

Cloning of the ARMS2 alleles

Genomic heterozygous DNA was PCR amplified as described above and the obtained PCR product was directly ligated into the pGEM-T Easy Vector (Promega). The reaction products were transformed into the DH5a competent cells (Invitrogen). Fourteen colonies were selected for sequence analysis of cloned DNA. Small amounts of bacterial colonies were re-suspended in 20 ml of water and incubated in 98°C for 10 min prior to PCR amplification, purification and sequencing.

Statistical analysis

Significance of SNP distribution frequencies between the treated group and controls were calculated with one-sided Fisher's exact test. The level of significance was set at $p < 0.05$, and odds ratios (OR) with 95% confidence intervals (CI) were calculated for those comparisons. SPSS for Windows 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

Genetic testing results in the treatment group: 47 patients were heterozygous and 28 homozygous for Y402H; 33 were heterozygous and 26 were homozygous for A69S. In comparative group homozygous/heterozygous patients respectively: 22/3 for Y402H, and 14/2 for A69S. In addition, R38X polymorphism was found in ARMS2 – in 16 patients from the treated group and 13 in the comparative group. In 7 patients it coexisted with A69S SNP on another allele. Odds ratios for homozygous patients were significant (or close to significant, in the case of R38X): Y402H – 5.57 (95% CI: 1.58–19.6), $p=0.002$, A69S – 7.72 (95% CI: 1.73–34.36), $p=0.001$. R38X (wild type *vs.* heterozygotes) – 0.45 (95% CI: 0.19–1.05) $p=0.053$.

There were no R38X homozygotes.

DISCUSSION

Currently 2 single-nucleotide polymorphisms – rs10490924 (A69S) (Age-Related Macular Susceptibility 2, ARMS2 gene) and rs1061170 (Y402H) (Complement Factor H, CFH gene) – are the best-known genetic factors contributing to disease, which is the leading cause of irreversible blindness in Caucasians [2].

Complement and ARMS2 studies have been replicated in diverse populations. Owing to such studies we know that the SNPs distribution varies worldwide [2].

To our knowledge this is the first report of genetic association between major AMD risk factors and neovascular AMD in a Polish population.

How do these polymorphisms contribute to the disease risk? Y402H CFH variant is less effective in complement inhibition. Oxidative stress factors, in addition to CFH and other members of the alternative complement pathway, play an etiologic role, thus suggesting the inflammatory nature of ARMD [3,4].

Assigning causative alleles through genotyping experiments alone is impossible in many diseases due to strong linkage disequilibrium. In such cases we must determine gene expression and function, as well as transcript localization, to find the right gene. Despite many implemented methods, there are still confusing results in ARMD, related to the other locus we took into consideration.

The exact function of ARMS2 (LOC387715) protein remains unknown. The ARMS2 gene has evolved exclusively in primates, which is consistent with macula evolution. The gene is in linkage disequilibrium with HTRA1, which is why both genes often are presented as ARMS2/HTRA1 complex in association studies [5,6]. Kanda et al reported in 2007 that rs10490924 in ARMS2 could explain the link between ARMD and 10q26 chromosome region [7]. HTRA1 and its SNP rs11200638 in promoter region was excluded, but further investigation did not confirm it, indicating that such studies alone are insufficient to distinguish between the 2 candidates. In addition, it was demonstrated that HTRA1 expression was upregulated in ARMD eyes and in the major risk haplotype. Such overexpression leads to fragmentation of the Bruch's membrane elastic layer. [8] Mitochondrial localization of ARMS2 protein suggested by Kanda was not confirmed. Immunofluorescence examinations revealed it is a constituent of the extracellular matrix, so it could be involved in protection against drusen formation [7]. Interestingly, A69S variant do not alternate protein function. Unstable ARMS2 mRNA corresponds with AMD risk (deletion-insertion mutation NM_001099667.1: c. (*)372_815del443ins54). It was suggested that the indel variant in the same haplotype as rs10490924 is probably responsible for crucial changes in matrix function [9]. R38X (rs2736911) results in truncated protein. Despite putative functional similarity to the above-mentioned indel polymorphism, it was considered as a non-risk variant [10]. In fact, it seems to be a protective polymorphism, which is in accordance with our data. It means that loss of gene message due to haploinsufficiency (R38X) or indel variant is probably not related to ARMD pathogenesis. Friedrich et al confirmed that ARMS2 protein deficiency is not likely to cause the disease, although they demonstrated that common risk alleles alter ARMS2 but not HTRA1 expression [11]. It should be mentioned that there has never been found any rare mutation in ARMS2 or HTRA1 leading to the disease, which is why dual causality was proposed – downregulation of ARMS2 and upregulation of HTRA1 [6]. However, this needs further investigation.

Authors of ARMD genetic studies often present both ARMS2 and HTRA1 genotyping results. However, such strong linkage disequilibrium is unlikely to distinguish which one of this pair confers to actual risk unless the number of included subjects is in the tens of thousands or above. Having

assumed that statistical significance would not be achieved, we decided to focus on A69S, which seemed at the time to be more closely related to ARMD, not only because of slightly higher correlation in association studies, but also due to the above-mentioned extracellular matrix location and function. It can probably now be assumed that both genes are involved in extracellular matrix metabolism, and that gene interactions possibly constitute an important factor. Despite many years of studies, we do not know exactly how ARMS2/HTRA1 contributes to ARMD pathogenesis.

CONCLUSIONS

The etiologic role of A69S ARMS2 and Y402H CFH gene variants in wet ARMD were confirmed, while R38X variant of ARMS2 seems to be protective in a Polish population.

Acknowledgments

We are thankful to Andrzej Palucha from Genomed Co. (Warsaw, Poland) for DNA analysis.

All study subjects provided written consent. The research followed the Tenets of the Declaration of Helsinki. The Ethics Committee of the Medical University of Silesia (Katowice, Poland) approved the study protocol. Proprietary interest: none.

REFERENCES:

1. International HapMap Consortium: The International HapMap Project. *Nature*. 2003; 426(6968): 789–96
2. Katta S, Kaur I, Chakrabarti S: The molecular genetic basis of age-related macular degeneration: an overview. *J Genet*, 2009; 88(4): 425–49
3. Hecker LA, Edwards AO, Ryu E et al: Genetic control of the alternative pathway of complement in humans and age-related macular degeneration. *Hum Mol Genet*, 2010; 19(1): 209–15
4. Kowalski M, Bielecka-Kowalska A, Oszajca K et al: Manganese superoxide dismutase (MnSOD) gene (Ala-9Val, Ile58Thr) polymorphism in patients with age-related macular degeneration (AMD). *Med Sci Monit*, 2010; 16(4): CR190–96
5. Fritsche LG, Loenhardt T, Janssen A et al: Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. *Nat Genet*, 2008; 40(7): 892–96
6. Yang Z, Tong Z, Chen Y et al: Genetic and functional dissection of HTRA1 and LOC387715 in age-related macular degeneration. *PLoS Genet*, 2010; 6(2): e1000836
7. Kanda A, Chen W, Othman M et al: A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. *Proc Natl Acad Sci USA*, 2007; 104(41): 16227–32
8. Vierkotten S, Muether PS, Fauser S: Overexpression of HTRA1 Leads to Ultrastructural Changes in the Elastic Layer of Bruch's Membrane via Cleavage of Extracellular Matrix Components. *PLoS One*, 2011; 6(8): e22959
9. Kortvely E, Hauck SM, Duetsch G et al: ARMS2 is a constituent of the extracellular matrix providing a link between familial and sporadic age-related macular degenerations. *Invest Ophthalmol Vis Sci*, 2010; 51(1): 79–88
10. Allikmets R, Dean M: Bringing age-related macular degeneration into focus. *Nat Genet*, 2008; 40(7): 820–21
11. Friedrich U, Myers CA, Fritsche LG et al: Risk- and non-risk-associated variants at the 10q26 AMD locus influence ARMS2 mRNA expression but exclude pathogenic effects due to protein deficiency. *Hum Mol Genet*, 2011; 20(7): 1387–99