Journal of Statistics on Health Decision

EXTENDED ABSTRACT

P23

Genetic characterization of AMD patients – Preliminary results of the Coimbra Eye Study

Rita Coimbra[1], Cláudia Farinha[1,2], Patrícia Barreto[1], Sandrina Nunes[1], Rufino Silva[1,2,3]

[1] Association for Innovation and Biomedical Research on Light and Image, Coimbra, Portugal (AIBILI).

[2] Ophthalmology Department, Centro Hospitalar e Universitário de Coimbra (CHUC); Faculty of Medicine, University of Coimbra.

[3] Coimbra Institute for Clinical and Biomedical Research, Faculty of Medicine, University of Coimbra (iCBR-FMUC).

Introduction

Age-related macular degeneration (AMD) is a degenerative disease of the macula, which is a small area in the center of the retina, responsible for detailed central vision. AMD is a leading cause of vision loss in developed countries in people aged over 55 years [1], and due to global population ageing, by 2040 the number of individuals affected by AMD is expected to be 288 million [2]. The early stages of the disease are mainly asymptomatic but when the progression to the late stage of the disease occurs, it causes severe vison loss with a great impact on daily tasks, such as recognizing faces, reading and driving. Treatment options, based on anti-VEGF therapy, are only available for approximately 10% of these patients, the ones with the neovascular form of AMD, leaving 90% of them without any possible treatment.

AMD is a multifactorial disease, with both genetic and environmental factors (such as age, smoking, lifestyle, diet) playing an important role. Genetic factors account for 45% to 70% of the onset of the disease [3]. In 2016 the largest genome-wide association study (GWAS) in AMD was published and 52 associated common and rare genetic variants were identified distributed across 34 loci [4]. Although complement factor H (CFH) gene variants and ARMS2/HTRA1 genes play a major role in the genetic susceptibility to AMD, also additional genetic variants in or near genes of the complement system (CFH, CFB, CFI, C2, C3), cholesterol metabolism (ABCA1, APOE, CETP, LIPC), extracellular matrix remodeling (COL8A1 ,TIMP3) and other genes in other undefined pathways have been associated with the disease.

Recently the first results of AMD prevalence and incidence, in the central region of Portugal, Mira, were reported [5,6]. Unexpectedly, late AMD incidence is lower than the one reported in major epidemiologic studies of European-descent populations. This might be due to multiple interacting factors, mainly environmental, lifestyle/diet related, and possibly genetic background.

Our purpose is to genetically characterize a Portuguese sample from a central region of Portugal (Mira), identify possible variants associated with AMD and to characterize their effect as protective or as risk factors of disease in this population. The metabolic/clinical pathways affected will also be explored.

Methods

An epidemiologic study, the Coimbra Eye Study, was conducted between 2009 and 2011 to estimate the AMD prevalence and risk factors in a Portuguese sample from the central region of Portugal, recruited from a coastal (Mira) and an inland town (Lousã). An AMD incidence study (5-year follow-up), was conducted in Mira with a sample of 1,616 patients. The presence/absence of AMD was classified according to Rotterdam Classification, using a multimodal evaluation and blood was collected for genetic analysis [6].

A total of 948 DNA samples, of 243 AMD cases (Rotterdam Classification stage 2, 3,4) and 705 control individuals (Rotterdam Classification stage 0, 1), were genotyped under the collaboration with the European Eye Epidemiology Consortium (E3). The genotyping assay was based on single molecule molecular inversion probes (smMIPs) and next-generation sequencing (NGS) covering 85 single nucleotide polymorphisms (SNPS).

69 SNPS were successfully genotyped and quality control filters were performed. Samples with more than 5% missing rate were removed from the dataset. Snps with a call rate < 95%, MAF < 1% (common snps) or showing deviations from Hardy-Weinberg Equilibrium were removed from further statistical analysis.

Keywords:
Age-related Macular Degeneration (AMD), genetics, epidemiology.

Corresponding author:
Rita Coimbra
racoimbra@aibili.pt

First published: 23 OCT 2020

A total of 656 samples and 40 snps were tested for association under an additive model, using the presence of AMD as the binary outcome. A logistic regression analysis was conducted to estimate the odds ratio (ORs) at 95 % CI for each variant. Statistical analysis was performed using R (package SNPassoc). Statistical significance was set to value <0.05.

Results

A total of 980 participants (controls and cases) were included in the study. The controls were younger (70.9 [66.5 – 75.8] vs 75.1 [69.5 – 80.6] years, p ≤ 0.001), with a higher proportion of males participants (42.8 % vs 38.7 %, p=0.258), presenting a higher prevalence of diabetes (20.1% vs 12.8%, p=0.031) and a lower prevalence of hyperlipidemia (1.7 % vs 4.9 %, p=0.011) (Table 1).

Table 1 - Demographics and clinical characteristics of the study population

Characteristics	Controls (N=705)	Cases (N=243)	p-value
Age (years)	70.9 (66.5 – 75.8)	75.1 (69.5 – 80.6)	**<0.001**[1]
Gender			0.258
Male	302 (42.8)	94 (38.7)	
Female	403 (57.2)	149 (61.3)	
BCVA, letters (worst eye)	80 (74 - 84)	75 (68 - 81)	**<0.001**[1]
Smoking	11 (1.6)	5 (2.1)	0.282
Co-morbidities			
Diabetes	142 (20.1)	31 (12.8)	**0.031**
Hypertension	366 (51.9)	136 (56.0)	0.140
Hypercholesterolemia	314 (44.5)	103 (42.4)	0.839
Hyperlipidemia	12 (1.7)	12 (4.9)	**0.011**

Data is presented in Median (IQR)/frequency (%)
[1]Mann-Whitney test, Statistical significance set at < 0.05 (CI 95%)

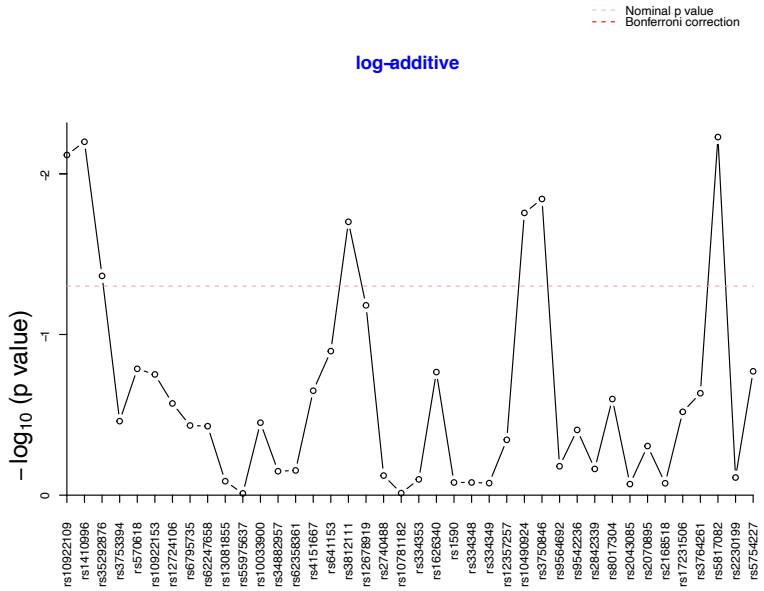

Figure 1 - log (p values) for the association analysis of the 40 variants assuming a log-additive genetic model. The statistically significant associations at nominal level (pink line) are indicated. No variant reached the Bonferroni corrected level (not shown in the figure).

A single-variant logistic regression analysis was performed for the 40 variants. None of the variants reached a Bonferroni significance, as indicate in the Figure 1. For each one of the variants who showed a suggestive association with AMD, the Minor Allele Frequency (MAF) was compared between cases and controls (Table 2).

We identified four common, intronic variants that were associated with reduced risk of AMD in our sample, suggesting a potential protective effect: rs10922109 (OR 0.72; CI 95% 0.56, 0.92, p=0.0076) in CFH gene; rs1410996 (OR 0.71; CI 95% 0.55, 0.91, p=0.0063) in CFH gene; rs5817082 (OR 0.66; CI 95% 0.49, 0.89, p=0.0059) in CETP gene and rs3812111(OR 0.75; CI 95% 0.58, 0.96, p=0.0199) in COL10A1 gene.

We identified also three variants associated with AMD presence in the sample: intronic variant rs3750846 (OR 1.49; CI 95% 1.09, 2.03, p=0.0143) in ARMS2/HTRA1gene, missense variant

Table 2 - Genotype frequency and Association variants in a Portuguese population

Gene	SNP	Allele (Ref/Alt)	Chr	Position	MAF cases (n=243)	MAF controls (n=705)	OR (95% CI)	p-value
CFH	rs10922109	C/A	1	196704632	0.361	0.442	0.72 (0.56 - 0.92)	0.0076
CFH	rs1410996	G/A	1	196696933	0.360	0.441	0.71 (0.55 - 0.91)	0.0063
CETP	rs5817082	C/CA	16	56997349	0.236	0.288	0.66 (0.49 -0.89)	0.0059
COL10A1	rs3812111	T/A	6	116443735	0.415	0.457	0.75 (0.58 -0.96)	0.0199
ARMS2/HTRA1	rs3750846	T/C	10	124215565	0.197	0.140	1.49 (1.09 – 2.03)	0.0143
ARMS2	rs10490924	G/T	10	124214448	0.201	0.142	1.46 (1.07 – 2.00)	0.0175
CFH	rs35292876	C/T	1	196706642	0.023	0.011	2.64 (1.05-6.61)	0.0432

rs10490924 (OR 1.46; CI 95% 1.07, 2.00, p=0.0175) in ARMS2 and synonymous variant rs35292876 (OR 2.64; CI 95% 1.05, 6.61, p=0.0432) in CFH gene.

Discussion and conclusions

Our preliminary analysis showed similar results with published European larger studies (4,7), despite of the limited number of participants and common variants. Of all known AMD variants, the CFH locus variant rs10922109 is the strongest AMD-associated, reported as having protective effect, but with a lower OR: 0.38 (7). In our results, CFH rs35292876 variant showed the higher fold increase risk (OR 2.64; CI 95% 1.05, 6.61, p=0.0432), similar to the one previously reported in a GWAS study (7): OR = 2.42.

We also identified 7 variants possibly associated with AMD, within genes of the complement system (CFH), cholesterol metabolism (CETP and COL10A1) and also Age-Related Maculopathy Susceptibility Protein 2 (ARMS2) and High-Temperature Requirement A Serine Peptidase 1 (HTRA1) genes. We know from previous studies that the lipid metabolism is involved in AMD pathogenesis and in our results the cases showed a significant higher prevalence of hyperlipidemia.

Further analyses are needed to better characterize genetically our population. The variants that showed a possible association with AMD will be selected for inclusion in a multivariable logistic regression model, adjusted for age, sex and smoking habits and also nutritional and/or phenotypic factors. We also plan to explore and perform a rare variant association analysis, since several studies suggest that rare variants can have larger effect sizes than the common ones

Acknowledgements:

The authors gratefully acknowledge the financing from Novartis Pharma AG that made this study possible and the collaboration and dedication from the personnel of primary healthcare unit of Mira.

References

1. Tomany SC, Wang JJ, Van leeuwen R, et al. Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. Optlamology. 2004; 111(7):1280-7. https://doi.org/10.1016/j.ophtha.2003.11.010
2. 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-analyis. Lancet Glob Health. 2014; 2(2):e106-16. https://doi.org/10.1016/S2214-109X(13)70145-1
3. Seddon, J.M.; Cote, J.; Page, W.F.; Aggen, S.H.; Neale, M.C. The US twin study of age-related macular degeneration: Relative roles of genetic and environmental influences. Arch. Ophthalmol. 2005, 123, 32. https://doi.org/10.1001/archopht.123.3.321
4. Fritsche LG, Igl W, Bailey JNC, Grassman F, Sengupta S, Bragg-Gresham JL, et al. A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. Nat Genet. 2016. https://dx.doi.org/10.1038%2Fng.3448
5. Cachulo M da L, Laíns I, Lobo C, Figueira J, Ribeiro L, Marques JP, et al. Age-related macular degeneration in Portugal: prevalence and risk factors in a coastal and an inland town. The Coimbra Eye Study – Report 2. Acta Ophthalmol. 2016. https://doi.org/10.1111/aos.12950
6. Farinha C, Cachulo M da L, et al. Age-Related Macular Degeneration Staging by Color Fundus Photography vs. Multimodal Imaging—Epidemiological Implications (The Coimbra Eye Study—Report 6). J Clin Med. 2020 May 2;9(5):1329. https://doi.org/10.3390/jcm9051329
7. de Breuk A, Acar IE, Kersten E, et al. Development of a Genotype Assay for Age-Related Macular Degeneration: The EYE-RISK Consortium. Ophthalmology. 2020; S0161-6420(20)30725-9. https://doi.org/10.1016/j.ophtha.2020.07.037