Eye (2018) 32, 446–450

www.nature.com/eye

LABORATORY STUDY

Assessing individual risk for AMD with genetic counseling, family history, and genetic testing

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Abstract

Purpose **The goal was to develop a simple model for predicting the individual risk profile for age-related macular degeneration (AMD) on the basis of genetic information, disease family history, and smoking habits.**
Patients and methods **The study enrolled 151 AMD patients following specific clinical and environmental inclusion criteria: age >55 years, positive family history for AMD, presence of at least one first-degree relative affected by AMD, and smoking habits. All of the samples were genotyped for rs1061170 (*CFH*) and rs10490924 (*ARMS2*) with a TaqMan assay, using a 7500 Fast Real Time PCR device. Statistical analysis was subsequently employed to calculate the real individual risk (OR) based on the genetic data (ORgn), family history (ORf), and smoking habits (ORsm).**
Results and conclusion **The combination of ORgn, ORf, and ORsm allowed the calculation of the Ort that represented the realistic individual risk for developing AMD. In this report, we present a computational model for the estimation of the individual risk for AMD. Moreover, we show that the average distribution of risk alleles in the general population and the knowledge of parents' genotype can be decisive to assess the real disease risk. In this contest, genetic counseling is crucial to provide the patients with an understanding of their individual risk and the availability for preventive actions.**
Eye (2018) **32,** 446–450; doi:10.1038/eye.2017.192; published online 15 September 2017

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Received: 26 January 2017
Accepted in revised form: 11 July 2017
Published online: 15 September 2017

Introduction

To date, tens of millions of common sequence variants are known to be frequent enough (minor allele frequency >5%) to be considered polymorphic in our species; therefore, any given individual is estimated to carry 4–5 million of these polymorphisms.[1] On the basis of this observation, genomic medicine has been attempting to elucidate the impact of variations in the sequence and/or expression of the individual genome on the likelihood, natural history, and management of diseases.

In this context, genome-wide association studies (GWASs) have uncovered thousands of genome variants associated with several complex disease traits and provided substantial insights into the genetic architecture and biological scenery of human diseases. Although these discoveries have been crucial for an understanding of the molecular basis of human multifactorial diseases, they are only able to explain a relatively small portion of the higher disease susceptibility that is apparent from epidemiological studies. The reason for this discrepancy lies in the fact that the large majority of diseases are determined by the interaction of a large number of genetic and nongenetic factors that typically present odds ratios (ORs) in the range of 1.1 to 2.0 and thereby add small/very small effects to the disease.[2] Moreover, the ORs extrapolated from GWAS represent an overestimation/underestimation of their real predictive values, and hence they are not suitable for the calculation of individual risk.[3] In fact, these ORs are calculated utilizing the lowest risk allele as a reference (ie, OR = 1), without considering that all of the possible genotypic classes of genetic variants (homozygous wild type, homozygous variant, and heterozygous) are normally distributed with their own frequencies within the general population. On this subject, the configuration of a reliable and clinically useful 'risk profile' should combine genomic profiles adjusted for the real risk in the general population with the environmental risk

factors (smoking and lifestyle) and prevalence and heritability of the disease.

In this context, age-related macular degeneration (AMD, OMIM #610149) represents a good model in which genetic and nongenetic data can be combined to assess the individual risk of developing the disease and/or progression of the disease. AMD is the progressive degeneration of the central part of the retina (*macula*) characterized by drusen and retina pigment epithelium changes (early and intermediate stage) or by abnormal choroidal neovessels (late stage/wet type) or central geographic atrophy (late stage/dry type) that ultimately results in disruption of the cytoarchitectonics of central retina. AMD affects ~8.7% of the elderly population worldwide (>55 years old), leading to the progressive loss of central vision and strong impairment of quality of life.[4] Different contributing factors act in synergy to trigger the onset of the disease:

- Aging: that is directly correlated to the disease prevalence;
- Smoking: the oxidative compounds contained in cigarettes essentially damage the RPE;
- Diet: absence of antioxidant elements in the diet cause the progression of AMD to more severe forms;
- Family history: is estimated to be 11% in the presence of affected first-relatives.[5–7]

The genetic picture of AMD includes several susceptibility loci (*CFH*, *ARMS2*, *IL-8*, *TIMP3*, *VEGFA*, *COL8A1*, *SLC16A8*, *RAD51B*, *ADAM*, *LIPC*, *APOE*, *COL10A1*, *IER3-DDR1*, *B3GALTL*, *TGFBR1*, and *CETP*), among which *CFH* (1q31) and *ARMS2* (10q26.16) are the major contributors to genetic susceptibility in the Italian population.[8–10]

CFH codifies for complement factor H that is essentially involved in the inhibition of complement cascade activity. In particular, the SNP rs1061170 (T/C) located in *CFH* was one of the first polymorphisms to be significantly associated with high susceptibility to AMD (P-value $= 1.1 \times 10^{-13}$; OR = 13.06 for the CC genotype, 95% CI = 6.27–27.19).[11] *ARMS2*, instead, encodes for the age-related maculopathy susceptibility protein and its function is still unclear. In 2008, an InDel polymorphism at the 3′UTR of *ARMS2* (composed of a 443 bp deletion followed by a 54 bp insertion; del443ins54) was found to be significantly associated with AMD susceptibility (P-value $= 2.5 \times 10^{-16}$; OR = 20.61 in the case of homozygosity for the InDel, 95% CI = 8.83–48.11).[12] The InDel polymorphism has been observed to be in strong linkage disequilibrium (LD) with the SNP rs10490924 (G/T) located in *ARMS2*. In particular, G is in LD with the wild-type (wt) *ARMS2* sequence, whereas T is in LD with del443ins54 in the gene. The rs10490924 SNP has also been related to AMD susceptibility (P-value $= 2.32 \times 10^{-54}$, OR: 9.17 for the TT genotype, 95% CI = 6.52–12.9).[13]

Interestingly, multivariate analysis shows that *CFH* and *ARMS2* substantially affect up to 20% of the genetic susceptibility to AMD, with respect to the 3% accounted for by other associated genes. Given the penetrance of *CFH* and *ARMS2* related to the onset of AMD, here we report a novel predictive model for drawing an individual risk profile based on the combination of genetic and familial information with well-established environmental risk factors.

Materials and methods

This study enrolled 151 w-AMD patients selected from the UOSD Retinal Diseases at PTV Foundation 'Tor Vergata' according to specific clinical and environmental criteria:

- Age >55 years;
- positive family history for AMD;
- presence of at least one first-degree relative affected by AMD; and
- presence of a smoking habit.

The study was previously approved by the ethics committee of the University of Rome 'Tor Vergata' (reference number: 16.15, approved on 23 January 2015).

During genetic counseling, all of the participants were required to provide written informed consent before the collection of two buccal swab samples. Genomic DNA was extracted from buccal swabs using an EZ1 DNA Investigator Kit and an EZ1 Advanced XL automated extractor (Qiagen, Valencia, CA, USA). All of the samples were genotyped for rs1061170 (*CFH*) and rs10490924 (*ARMS2*) with a TaqMan assay using a 7500 Fast Real Time PCR device according to the manufacturer's instructions (Applied Biosystems, Warrington, UK). The genotyping results were interpreted using Sequence Detection System 2.1 software (Applied Biosystems). Each Real Time PCR run was performed using a negative control and three positive control samples previously confirmed by direct sequencing (BigDye Terminator v3.1, BigDyeXTerminator and ABI3130xl, Applied Biosystems).

The genotyping results were evaluated by statistical analysis. First, the OR value corresponding to the individual genetic risk (ORg) for AMD was calculated for all of the patients through a classical case–control study. Second, we calculated the average risk of the general population by multiplying the ORs of the three classes of each polymorphism (risk allele homozygous and

protective allele homozygous and heterozygous) for their relative frequencies in the general population. We then used these values as references to calculate the normalized OR (ORgn) of *CFH* and *ARMS2*.

Afterwards, the ORgn was integrated with the OR of familiarity of AMD and smoking habits, the two main risk factors for AMD. In particular, an OR of 2.2 was attributed to the presence of first-degree familiarity (ORf) for AMD.[5]

Concerning smoking habits, three different ORs (ORsm) were used according to the status of being a smoker (ORsm = 3.1), ex-smoker (ORsm = 1.5), or nonsmoker (ORsm = 1), as reported elsewhere.[7]

Finally, the ORgn, ORf, and the ORsm were all combined in a single computational tool that calculated the total OR (ORt) that was descriptive of the individual risk profile for AMD susceptibility.

Results

The genotyping analysis was successfully performed for all of the samples, recognizing the three expected genotypic classes of *CFH* and *ARMS2* susceptibility genes. The frequencies of the *CFH* genotypes were f(CC) = 12%, f(CT) = 71%, and f(TT) = 62%, whereas the frequencies of the *ARMS2* genotypes were f(TT) = 2%, f(GT) = 58%, and f(GG) = 84%.

Statistical analysis was subsequently employed to calculate the ORs (ORg, ORgn, and ORt) and generate the computational model for assessing the risk profile for AMD.

The ORg represents a classical measure of association between markers and disease status that results from the genotyping analysis (Table 1).

Second, we calculated the average risk for the general population by multiplying the ORs of the three classes of each polymorphism (risk allele homozygous and protective allele homozygous and heterozygous) for their relative frequencies in the general population. These values represented the reference risk of the general population, and they were quite different from 1, the standard value utilized for the statistical evaluation of the association strength in patients. This analysis led to normalized ORs (ORgn) for *CFH* and *ARMS2* in relation to the average distribution of risk alleles in the general population. The ORgn are reported in Table 1. Interestingly, the ORgn was found to be strongly lower than the original ORg, suggesting that the average distribution of risk alleles in the general population significantly affected the estimation of the individual genetic risk.

Table 1 Results of the statistical analysis

ORg CFH	*ORgn CFH*
C/C = 13.06	C/C = 3.80
T/C = 2.88	T/C = 0.84
T/T = 1	T/T = 0.29
ORg *ARMS2*	ORgn *ARMS2*
T/T = 20.61	T/T = 8.31
G/T = 3.18	G/T = 1.28
G/G = 1	G/G = 0.40

The final step of the analysis allowed the generation of the computational model based on the combination of ORgn with the above reported ORs for a positive family history of the disease (ORf) and smoking habits (ORsm). The final result of these statistical calculations was the Ort that represented the actual estimation of a more realistic individual risk for developing AMD.

Discussion

In this report, we showed that the average distribution of risk alleles in the general population significantly affected the estimation of individual genetic risk. The average risk in the general population is different from 1 and depends on the penetrance of the risk alleles (strength of association) and their relative frequencies in the population. Moreover, we propose a simple model that combines three types of inputs, the genetic information, presence/absence of disease family history, and smoking habits, to provide a reliable measure of the risk to develop AMD (namely, the ORt). As expected, our results showed that the personal risk profile of single subjects could account for other well-established factors that are family history and smoking habits in this case. Moreover, the importance of genetic counseling cannot be overemphasized, especially when studying the family can affect the interpretation of a genetic test. In particular, knowledge of the parents' genotypes can be crucial for the assessment of the real disease risk in the offspring. First, it is important to consider that the average distribution of risk alleles in the cohort of patients reported here could not be representative of the genetic status of single patients. On this subject, we assessed the number of AMD patients without risk variants in our cohort by counting the number of risk variants in *CFH* and *ARMS2* carried by each patient (with a maximum of 4 risk alleles; Table 2). It is important to note that 15% of the patients did not carry risk alleles in *ARMS2* and *CFH* (0 risk alleles). On the basis of these data, let us consider two families (families A and B) with an affected parent, a healthy parent, and two children. As reported in Figure 1, if we consider the number of risk variants of the children only, we may

Table 2 Count of the number of risk variants in our patients cohort ($n=151$)

Genotypes (CFH/ARMS2)	*Number of risk alleles*	*Sample count*
CC/TT	**4**	**5** (3%)
CC/GT	**3**	**11** (7%)
CC/GG	**1**	**5** (3%)
T**C/TT**	**3**	**14** (9%)
T**C/GT**	**2**	**36** (24%)
T**C**/GG	**1**	**30** (20%)
TT/**TT**	**2**	**5** (3%)
TT/G**T**	**1**	**22** (14%)
TT/GG	**0**	**23** (15%)
		Total: 151

Risk alleles are written in bold and have been underlined.

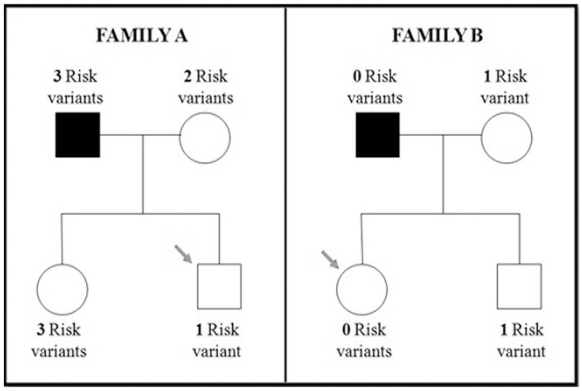

Figure 1 Genetic pedigrees of two families with a positive history for AMD. The risk variants that were evaluated are related to the *CFH* and *ARMS2* susceptibility genes. The two families reported in the figure highlight the importance of knowing the parents' genotype/family history to understand the meaning of the results of the genetic tests for AMD. At first glance, if we consider only the genetic risk of the two subjects indicated by the arrows, we may conclude that the child in family A has a higher risk than the child of family B. However, looking at the risk variants carried by the parents and the disease status of one of them, the subject of the family B (indicated with the arrow) has higher risk for the disease because the disease in the family was not explained by the *ARMS2* and *CFH* gene variants that were considered.

conclude that the child with one risk variant in family A has a higher risk for AMD with respect to the child with 0 risk variants in family B. However, if we look at the risk variants carried by the parents (Figure 1), the real disease risk of the two children may be very different. In fact, the child with 0 risk variants in family B actually has a higher risk of developing AMD with respect to that suggested by the genetic test results. In fact, his parent is affected by AMD without carrying disease-associated variants in *CFH* and *ARMS2*. This information is crucial for interpreting the meaning of genetic tests in every single family because we can observe a positive family history of the disease in the absence of risk alleles. This is probably because of the presence of other susceptibility genes, although their penetrance is significantly lower with respect to *CFH* and *ARMS2* as well as to possible nongenetically related clinical manifestations of AMD.[14]

Given the complex relationship between genotypes and other factors in the AMD etiopathogenesis, the proposed predictive model described above should be accomplished by accurate genetic counseling before and after the genetic test. The genetic counseling is essentially based on a series of key elements, such as: anamnesis (diagnostic and clinical data about the patient and family documentation), pedigree information, description of the available genetic tests, subscription of the informed consent, explanation of the results and implications of genetic testing, and support in the decision-making process. Genetic counseling is essentially oriented to discriminate between 'high-risk' and 'low-risk' subjects, according to the results of our model. It is therefore extremely important to provide the patient and clinicians with full comprehension of the meaning of the genetic test. In addition, collecting clinical information about the family history of disease can be sometimes really difficult because of the lack of adequate diagnostic information on close relatives affected by the same disease. Most part of family documentation can be usually provided by patients undergoing the counseling, and thus it is essential to achieve the best relationship with the patient in order to obtain a detailed picture of his clinical and family history.

Our predictive model is fundamentally based on the measurement of ORs, and it should be noted that the final result is an accurate estimation of the risk for developing AMD, without providing certain information on the disease onset of single patients and their offspring.

In conclusion, we presented a 'proof-of-concept' model that is able to offer a realistic individual risk profile for AMD. In this context, it is crucial to note the social and scientific roles of genetic counseling in the management of genetic testing for susceptibility genes. In particular, more interactions between geneticists and clinicians should be encouraged to promote the translation of genomic medicine in routine use. The genetic counselor should interact with both patients and clinicians to explain the benefits of the genetic tests. The genetic counselor, together with the clinicians, is expected to provide the patients with an understanding of the meaning of their individual risk and the availability/opportunities for preventive actions.

Summary

What was known before

- AMD mainly affects the elderly population and it is responsible for progressive loss of central vision and strong impairment of quality of life.
- Different factors (aging, cigarette smoking, diet, and family history) are known to act as triggers of disease.
- To date, several susceptibility genes have been identified, among which *CFH* and *ARMS2* are known as the major contributors to genetic susceptibility of AMD in the Italian population.

What this study adds

- The individual risk profile can be assessed on the basis of genetic and familial information together with environmental risk factors.
- A predictive model has been conceived to support clinicians and patients in the decision making concerning the possible therapeutic or prevention strategies.
- The knowledge of the parents' genotypes can be crucial for the assessment of the real disease risk in the offspring.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge the Macula Foundation Onlus that supported this work.

References

1 Ginsburg GS, Willard HF. *Genomic and Personalized Medicine*, 2nd ed. Elsevier BV: London, UK, 2013.
2 Amin N, van Duijn CM, Janssens AC. Genetic scoring analysis: a way forward in genome wide association studies? *J Epidemiol* 2009; **24**(10): 585–587.
3 Janssens AC, van Duijn CM. Genome-based prediction of common diseases: methodological considerations for future research. *Genome Med* 2009; **1**(2): 20.
4 Cascella R, Ragazzo M, Strafella C, Missiroli F, Borgiani P, Angelucci F *et al.* Age-related macular degeneration: insights into inflammatory genes. *J Ophthalmol* 2014; **2014**: 582842.
5 Hughes AE, Orr N, Patterson C, Esfandiary H, Hogg R, McConnell V *et al.* Neovascular age-related macular degeneration risk based on CFH, LOC387715/HTRA1, and smoking. *PLoS Med* 2007; **4**(12): e355.
6 Zampatti S, Ricci F, Cusumano A, Marsella LT, Novelli G, Giardina E. Review of nutrient actions on age-related macular degeneration. *Nutr Res* 2014; **34**(2): 95–105.
7 Wray NR, Yang J, Goddard ME, Visscher PM. The genetic interpretation of area under the ROC curve in genomic profiling. *PLoS Genet* 2010; **6**(2): e1000864.
8 Fritsche LG, Chen W, Schu M, Yaspan BL, Yu Y, Thorleifsson G *et al.* Seven new loci associated with age-related macular degeneration. *Nat Genet* 2013; **45**(4): 433–439e1-2.
9 Ricci F, Staurenghi G, Lepre T, Missiroli F, Zampatti S, Cascella R *et al.* Haplotypes in IL-8 gene are associated to age-related macular degeneration: a case-control study. *PLoS One* 2013; **8**(6): e66978.
10 Ricci F, Zampatti S, D'Abbruzzi F, Missiroli F, Martone C, Lepre T *et al.* Typing ofARMS2 and CFH in age-related macular degeneration: case-control study and assessment of frequency in the Italian population. *Arch Ophthalmol* 2009; **127**(10): 1368–1372.
11 Schramm EC, Clark SJ, Triebwasser MP, Raychaudhuri S, Seddon JM, Atkinson JP. Genetic variants in the complement system predisposing to age-related macular degeneration: a review. *Mol Immunol* 2014; **61**(2): 118–125.
12 Fritsche LG, Loenhardt T, Janssen A, Fisher SA, Rivera A, Keilhauer CN *et al.* Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. *Nat Genet* 2008; **40**(7): 892–896.
13 Seddon JM, Reynolds R, Maller J, Fagerness JA, Daly MJ, Rosner B. Prediction model for prevalence and incidence of advanced age-related macular degeneration based on genetic, demographic, and environmental variables. *Invest Ophthalmol Vis Sci* 2009; **50**(5): 2044–2053.
14 Fritsche LG, Igl W, Bailey JN, Grassmann 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* 2015; **48**(2): 134–143.