Genetics of AMD

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Introduction:

Age-related macular degeneration (AMD) is a complex disease with demographic and environmental risk factors (age, diet, and smoking) but also genetic risk factors.

In fact, instead of having a single contributory gene, there are multiple genes of variable effects that seem to be involved turning the issue of genetics of AMD a complex one: AMD involves environmental factors and varying susceptibilities to these external factors based upon diferent genetic backgrounds(1).

The genetic component of the disease has been suspected from family, twin and sibling studies. According to several family studies, patients with a family history of AMD have an increased risk for developing AMD(2,3).

The concordance for the presence of the disease is greater among homozigous twins than among heterozygous twins.

In 2008, Luo et al.(4) estimated the magnitude of familial risks in a population–based cross-sectional and case–control study.

Recurrence risk in relatives indicate increased relative risks in siblings (2.95), first cousins (1.29), second cousins (1.13) and parents (5.66) of affected elements.

Many linkage and association studies have showed that chromosomes 1q (1q25-31) and 10q (10q26) had genes involved in this pathology(5-7).

It was the completion of the Human Genoma Project, 5 years ago, resulting in the knowledge of the sequencing of the human genome that allowed improved DNA sequencing and mapping technologies and consequently, identification of Single Nucleotide Polymorphism (SNPs).

There are several types of genetic sequences variations (polymorphisms) in the human genome: repeated polymorphisms, insertions and deletions. However the majority of the DNA sequence variations in the human genome is in the form of SNPs which are persistent single changes, substitutions or variants of a single base in at least a population and with a frequency of more than 1% referred as alleles and representing altered forms of a gene: different alleles may produce variations in inherited characteristics(8,9).

These variants are also important because they serve as genetic markers and in this way they can help in determining those which confer increased or decreased risk of several diseases including AMD.

Dissection of the genetic background of AMD has undergone tremendous progress in the last 2 years. We know, now, some polymorphisms which modulate AMD risk.

Complement system factors and AMD

Complement factor H (CFH)

CFH is a negative regulator of alternative pathway of the complement system which means that in normal conditions, it inhibits the alternative pathway complement system.

It is encoded by a gene localized in 1q23-32 and its dysfunction may lead to excessive inflammation and tissue damage(10).

Complement activity is very important for the imune responses against pathogens and dying cells but, over-activation can result in complement-mediated damage to nearby healthy tissue cells.

It is now accepted that CFH gene is an important susceptibility gene, harbouring variants and haplotypes (short DNA sequences containing alleles) associated with increased and reduced risk of AMD.

Six CFH gene variants have been reported in AMD association studies as major genetic factors for developing AMD in Caucasians(11-15): rs1061170, (CFH Y402H); rs3753394; rs800292; rs1061147; rs380390; rs1329428.

However in the Chinese and Japanese populations only three of these CFH SNPs (rs1329498, rs800292 and rs3753394) were associated with risk of AMD(16,17).

So it is possible that CFH could play a central role in AMD pathogenesis and that multiple SNPs that alter CFH function might contribute to the development of AMD.

Their importance varies among the race of the population.

In the variant (polymorphism) CFH Y402H of the CFH gene, there is a substitution on the nucleotide in exon 9 (1277) where thymine (T) is changed for cytosine (C) (rs1061170) which is the allele risk.

This change leads to the substitution of the aminoacid in the position 402 in the protein, from tyrosine (Y) to histidine (H).

Homozygote CC or heterozygote TC can account for 50% of AMD cases.

The risk attributable for a disease is the rate of disease among individuals with a given characteristic minus the rate of the disease among indivuals without that characteristic.

The population attributable risk (PAR) in individuals with this polymorphism for developing AMD is 43% to 50%(11,12,18).

When compared with those with no risk allele TT, one copy of the Tyr402His polimorphysm (heterozygous for the risk allele TC), increases the risk of AMD by a factor of 2.2 to 4.6 (these individuals are at least twice and half more likely to develop AMD) and two copies of the risk allele (homozygous for the risk allele CC) increases the risk by a factor of 3.3 to 7.4 in Whites(19).

In addition to the common risk haplotype carrying the C allele of CFH Y402H, haplotype analysis of CFH has revealed two common protective haplotypes: homozygous deletions CFHR1 or CFHR3.

The gene cluster of CFH includes other “CFH-related genes“: CFHR1, CFHR2, CFHR3, CFHR4 and CFHR5. This means that the CFH gene resides within the region of complement activation (RCA), which includes also five “CFH–related” genes.

While the function of the CFH related genes is largely unknown, the high degree of sequence similarity between these genes and the suggestion that they arose out of duplication events with CFH, suggest an overlapping function of the CFH-related genes in immune system function and /or regulation.

There is a common and widespread (commonly found in many different populations in the world) deletion within the RCA locus that encompasses the CFHR1 and CFHR3 genes.

However the frequency of homozygous CFHR1 or CFHR3 deletion shows considerable variation between ethnic groups and occurs in 17.3% of African populations, 15.9% of African-American, 6.8% of Hispanic, 4.7% of Caucasian and 2.2% of Chinese cohorts(20).

This is in agreement with AMD less frequency among African-Americans compared with Caucasians and Chinese populations.

CFH1 and CFH3 protein may compete with CFH for C3 binding and therefore interfere with normal regulation of the complement system.

Those individuals, who are homozygous for the CFHR1/CFHR3 deletions and, therefore do not express the respective proteins, are highly protected from developing AMD(20).

Complement factor B (BF), complement component 2 (C2)

Complement factor B (BF) is involved in the activation of the complement alternative pathway and complement component 2 (C2) is involved in the activation of the classical pathway of the complement and both have adjacent genes located 500 base pair apart on chromosome 6p21.3 within the major histocompatibility complex class III region(21).

Haplotypes in BF and C2 have been linked to AMD. In particular, the L9H in BF and the E318D in C2 and also the R32Q in BF and a variant in intron 10 of C2 have been showed to be protective for AMD by Gold et al.(21).

They hypothesized that the significance of the haplotypes is due largely to the BF variants, which are in strong linkage disequilibrium with C2.

BF is a complement activating factor and studies have demonstrated that at least one of the two variants associated with AMD (R32Q BF) leads to an impairment in the complement activation function of BF.

This means that the absence of these variants C2/BF can predispose patients to AMD(21).

Thus, much like impaired CFH-mediated complement inhibition confers AMD risk, decreased complement activation by BF might serve to protect against AMD risk.

Complement component 3 (C3)

C3 is the central element of the complement cascade and a candidate gene to be involved in AMD, since its cleavage product, C3a, not only was found in drusen but also was proved to induce vascular endothelial growth factor expression and promote choroidal neovascularization in both in vitro and in vivo(22-24).

The variants R102G and P314L of the C3 gene significantly increase the risk of early and all subtypes of AMD and this risk seems to be independent of CFH Y402H, LOC387715 A69S and smoking(25).

LOC387715/ARMS2 and HTRA1

In 2003 Majewski et al. suggested that chromosome 10q26 might contain an AMD gene(7).

Later this finding has been replicated by other genome-wide linkage studies(26) and supported by a genome–scan meta-analysis(5).

This locus contains three tightly linked genes: PLEKHA1, LOC387715/ARMS2 (age related maculopathy suceptibility gene 2) and HTRA1, a secreted heat shock serine protease.

In 2005 Jakobsdottir et al.(27) found that the strongest association was over LOC387715/ARMS2 and HTRA1, which share an extensive linkage disequilibrium (LD) block harbouring the high risk haplotype.

There has been more dispute than agreement between the studies in what concerns this locus.

All initial genetic studies, about ten years ago, lacked statistical power (because small samples were used), used cumbersome genotyping technologies and poorly defined cohort.

In recent years, there are some publications of preliminary and unconfirmed genetic associations of the genes in this locus to AMD(28).

ARMS2 (LOC387715) SNP and AMD

The association of ARMS2 gene and AMD has been now replicated in various independent studies especially the advanced form of the disease, that means to say, the “wet” or with choroidal neovascularization and the “dry” or geographic atrophy form of the disease(29-32).

The risk confering polymorphism consists in a change in the 69 position aminoacid alanine (A) to serine (S).

According to Ross(31), heterozygosis at the ARMS2/LOC387715 (A69A/A69S) is associated with odds ratio (OR) of 1.69-3.0 for advanced AMD while homozygozity for the risk conferring allele (A69S/A69S) results in a OR of 2.20-12.1.

The frequency of the risk allele is higher in patients with advanced AMD than in those with early or intermediate AMD(27,33).

Later two studies, based on semiquantitavive expression data of allele associated differences in HTRA1 mRNA or protein levels, suggested a diferent variant (rs11200638) in the same LD block, in the promoter of HTRA1 gene as the functional variant(34,35).

HTRA1 (high temperature required factor A-1) SNP and AMD

HTRA1 gene is located on chromosome 10q26.3, extremely close to the locus of the ARMS2 gene (10q26.13) and because of its role in extracellular matrix homeostasis (its extracellular protease activity may favour neovascularization) and in cellular growth or survival (it is an inhibitor of TGF-ß family member(36) and it could play a critical role in controlling TGF-ß dependent neuronal survival(37) it seems a possible functional candidate gene.

Four significant SNPs have been reported in the promoter and the first exon of HTRA1: G625A (rs11200638); T487C (rs2672598); C102T, A34A (rs1049331); G108T, G36G (rs2293870). However the most well documented, statistically significant AMD associated SNP is rs11200638 (G625A) in the promotor region.

Caucasians, Chinese and Japanese heterozygous for the risk allele (G/A) have a high OR of 1.60-2.61 and Caucasians, Chinese and Japanese homozygous for the risk allele (A/A), 6.56-10.0(34,35,38-40).

According to Tam et al.(40), there is an increase in population attributable risk (about 5.5 fold increase) by the joint effect of smoking and HTRA1 allele.

This means that smokers homozygous for the risk allele had a substantially higher risk of developing wet AMD than non smokers with the risk allele.

However Deangelis et al(41), in 2008 reported no interaction between this SNP and smoking.

In what concerns the studies which relate HRTA1 promoter polymorphisms to risk factors for developing AMD, three problems arise according to Allikmets and Dean(28).

The variant encoding the A69S (rs10490924) in ARMS2 and the rs11200638 variant in HTRA1 are almost in complete LD, so it is impossible to assign causality on the basis of allele frequency alone.

10q26 locus doesn’t harbour a wet AMD gene as the authors claimed but a late AMD gene as showned by Weber and colleagues in 2005(30).

All subsequent studies have failed to replicate the functional data(32,42).

This basically means that, as there is strong linkage disequilibrium (LD) across ARMS2-HTRA1 region, genetic association studies alone are insufficient to distinguish between the two candidates.

It is also necessary not only the characterization of the extent of the variants associated to the disease but also the analysis of their possible functional relevance in the disease process(42). Doing this, Fritsche et al.(42) claimed that the functional variant in this locus is the deletion-insertion polymorphism variant 372-815delins54 in the ARMS2 gene.

The deletion removes the polyadenilation signal sequence at position 395-400 exclusively used for the addition of a poly A tract 19 bp downstream.

The insertion introduces a 64 bp AU-rich element, known for its properties to control mRNA decay in many transcripts that encode a wide variety of proteins(43,45).

They demonstrated that it is a major risk factor for AMD: individuals carrying a single copy of the risk allele deletion-insertion in ARMS2 gene have a 2.8–fold increased risk compared with an 8.1–fold increased risk in homozigous individuals.

Their work, also revealed that in homozygous for the deletion-insertion variant, expression of ARMS2 is absent.

They localized the ARMS2 protein within the photoreceptor layer namely, to the mitochondria–enriched ellipsoid region of the inner segments and in accordance; they proposed a functional role of ARMS2 in mitochondrial homeostasis.

According to Fritsche et al., this suggests, that this polimorphysm is the sought-after functional variant with relevance in AMD etiology in 10q26 locus.

However, as Fritsche et al. recognize, it is ultimately required formal exclusion of functional consequences for the remaining polymorphisms on the risk haplotype namely, A69S in ARMS2 gene and HTRA1 promoter variant.

The A69S and the InDel are in 100% LD and on the same haplotype and so the effects are not independent to each other(46).

The work of Fritsche and colleagues does not eliminate all other possibilities(28), nobody disputes the role of complement genes in AMD in spite of the functional consequences of the disease associated variants being not known for CFH, CFB/C2 and/or C3.

Apolipoprotein E gene (ApoE)

The ApoE gene, located on chromosome 19q13.2, is polymorphic and has three isoforms wich are common, E2, E3 and E4, coded by different alleles: the ancestral E3 and the SNPs, E2 and E4(47).

Most studies favour a protective role for the ApoE4 SNP and a slight risk-conferring role for ApoE2(47-51).

However other studies do not(52-55).

SNP genotype and therapeutic responses

Genotype and response to antioxidative/zinc therapeutics

One of the first works in this area was that of Michael Klein et al.(56).

These authors correlated the CFH and LOC387715 A69S genotypes with the therapeutic responses to supplementation with antioxidants and zinc.

They concluded that, in homozygous individuals for the non-risk phenotype (Y402Y/Y402Y), 34% of those treated with placebo progressed to advanced AMD, compared to 11% of those treated with antioxidants and zinc: a reduction of approximately 68%.

In homozygous individuals for the risk allele (Y402H/Y402H), 44% of those treated with placebo progressed to advanced AMD, compared with 39% of those treated with antioxidants plus zinc: a reduction of only 11%.

A similar interaction was observed in the groups taking zinc versus those not taking zinc: intake had a more protective effect in patients with non-risk alleles compared to patients with risk alleles.

These results suggest that the zinc plus antioxidative treatment seems to have less impact on those with the high-risk CFH variant.

These authors found no association between LOC387715/ARMS2 A69S and the response to AREDS treatment.

Genotype and response to intravitreous bevacizumab

Brantley et al(57) investigated 86 wet AMD patients for the association between CFH and LOC387715/ARMS2 genotypes and the response to treatment with bevacizumab.

For the CFH genotypes results show that only 10.5% of patients homozigous for the risk - conferring allele Y402H/Y402H genotype demonstrated improved vision with treatment compared with 53.7% of patients homozigous for the non-risk allele Y402Y/Y402Y and the heterozigous for the non-risk allele

Y402Y/Y402H variants.

They found that the CFH variants are associated to the responses to bevacizumab but that LOC387715/ARMS2 are not.

Genotype and response to photodynamic therapy

Goverdhan et al(58) and Brantley et al(59) studied the association of the CFH and LOC387715 genotypes with the response to PDT.

They found no statistical association with the LOC387715 genotype but a statistical association with CFH genotype: risk allele genotypes have better results than non-risk allele genotypes.

However more studies are warranted before any definitive conclusions.

Conclusions

1. Genetics variants at two chromosomal loci, 1q31 and 10q26, confer major disease risks, together accounting for more than 50% of AMD pathology(11-14, 27,30).

At present SNPs are the best available markers of AMD risk: SNPs in complementt factor H and ARMS2/HTRA1 capture a substancial fraction of AMD risk and permit the identification of individuals at high risk of developing AMD.

Genetic markers can successefully identify individuals whose lifetime risk of age-related macular degeneration ranges from 1% to greater than 50%.

2. Understanding the genetic basis of AMD has important implications for the ophthalmologists as it allows the identification of the biochemical pathway for a large proportion of AMD patients, raises the possibility to perform pre-symptomatic diagnostic testing of risk genotypes and to stratify the response to therapy based on genetic risks and supports the development of new therapies being the inhibition of complement a potential one among others that are already being tested.

>> References

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