

A genetic approach to stratification of risk for age-related macular degeneration

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ABSTRACT • RÉSUMÉ

The genetic determinants of age-related macular degeneration (AMD) are reviewed and a novel approach to risk determination based upon inherited genetic polymorphisms and smoking history is presented. Although AMD was long thought to have primarily an environmental etiology, genetic variation is now known to account for the majority of the disease risk, with variations in the genes of the complement pathways playing a prominent role. Independent and validated clinical studies have implicated the C3 gene and its regulator, complement factor H (1q31.1), complement component 2 (6q21.33), and complement factor B (6q21.33). Subtle variations in complement activity increase the risk of symptomatic macular inflammation with age. A second group of AMD-associated genetic markers may aggravate complement-mediated inflammation by permitting retinal oxidative damage. Variation within the chromosomal site (10q26) coding a mitochondrial-associated protein (age-related maculopathy susceptibility 2) and an independent variation within the mitochondrial genome itself (A4917G) suggest a contributing pathophysiological role of retinal oxidative stress. A genetic panel of disease-susceptibility markers and smoking history can identify a group of individuals with greater than 65% lifetime risk of AMD. The introduction of genetic marker testing into clinical practice may identify patients with early disease who may be aided by presymptomatic monitoring or inclusion into trials of newer prophylactic agents.

L'étude examine les déterminants de la dégénérescence maculaire liée à l'âge (DMLA) et présente une nouvelle approche pour établir le risque à partir des polymorphismes génétiques héréditaires et des antécédents de tabagisme. Si l'on a longtemps pensé que l'étiologie de la DMLA était avant tout environnementale, on sait maintenant que la variation génétique explique en majorité le risque de maladie par la diversité des gènes des voies d'activation des compléments, qui y jouent un rôle de tout premier plan. Des études cliniques, indépendantes et validées, ont impliqué le gène C3 et son régulateur, le facteur complémentaire H (1q31.1), la composante complémentaire 2 (6q21.33) et le facteur complémentaire B (6q21.33). De subtiles variations dans l'activité des compléments augmentent le risque d'inflammation maculaire symptomatique avec l'âge. Un deuxième groupe de marqueurs génétiques associés à la DMLA peut aggraver l'inflammation par la médiation des compléments qui permettent le dommage oxydatif de la rétine. La variation dans le site chromosomique (10q26), qui code une protéine associée aux mitochondries (susceptibilité de maculopathie 2 liée à l'âge), et une variation indépendante dans le génome mitochondrial lui-même (A4917G) suggèrent la contribution d'un rôle pathophysiologique du stress oxydatif de la rétine. Une série de marqueurs génétiques de sensibilité de la maladie et l'antécédent de tabagisme peuvent aider à identifier un groupe de personnes qui courent un risque de plus de 65 % de DMLA pendant leur vie.

Age-related macular degeneration (AMD) is the leading cause of blindness in Canada, affecting primarily older individuals. Aging of the “baby boomers” is expected to more than double the number of Canadians 65 years of age or older to just over 9 million in 2031. This trend is also expected to double the number of Canadians with AMD from the population affected currently: 1 million with early AMD, 250 000 with advanced AMD, and 64 200 with blindness due to AMD.¹

The progressive and largely irreversible nature of AMD, its substantial societal and personal impact, and its high prevalence make it essential to develop clinical strategies to reduce the impact of this devastating disease. Evidence now supports close monitoring of patients with AMD to allow

early detection and treatment of neovascular lesions and preserve vision.^{2,3} Awareness of symptoms, use of the Amsler grid, and simple clinical monitoring by an ophthalmologist are not satisfactory means for early detection of AMD,⁴ suggesting the need for more intensive surveillance of individuals at risk and the development of targeted prophylactic measures.

Recent advances in the discovery of genetic predictors of AMD allow the identification of individuals at heightened risk of this disease, laying the foundation for a clinical approach to early detection, management, and sight preservation. Published work indicates that inherited genetic features extend conventional parameters, such as smoking history and stage of the disease, and improve the ability to

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predict progression to visual impairment among those with early disease. Using published risk factors, their population frequency, and the strength of risk, we present a predictive tool that extends earlier work.⁵ The identification of those at heightened risk of disease could aid secondary prevention strategies when proven, such as early institution of intravitreal, antivascular endothelial growth factor treatment and emerging primary prevention techniques involving complement-specific anti-inflammatory molecules. These first steps in risk-stratified therapy have the potential for a significant impact on this major form of acquired blindness.

GENETIC DIVERSITY

Types of naturally occurring DNA variation

While largely identical in sequence, humans differ genetically in a nonrandom fashion at specific sites within the human genome. Much of the variation within the human genome occurs at the single base level (single nucleotide polymorphisms or SNP), reflecting mutations introduced earlier in human population expansion and resulting in their representation in a significant proportion of modern populations (Fig. 1). These occur at millions of sites throughout the 3 billion bases constituting the human genome.⁶ Genetic variation can also occur in copy number of blocks of several hundred thousand base pairs, or insertions or deletions of smaller sequences (“indels”).⁷ Given the ease of analysis, recent genetic research has focused on SNP variability associated with common human diseases. Diseases associated with specific SNP variants include malignant diseases (colon, breast), inflammatory disease (Crohn’s disease, asthma), metabolic disease (osteoporosis, diabetes, coronary artery disease), and many others.⁸ Such discoveries have the potential to ultimately change risk-stratified approaches to human health maintenance.

Genetic risk for AMD

AMD is perhaps the most inheritable human multigenic disease identified to date, providing an opportunity to

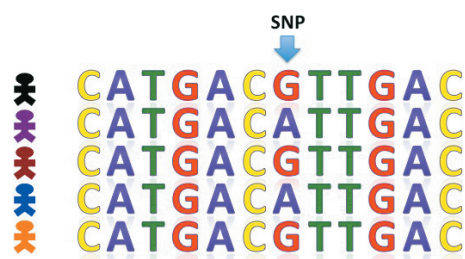


Fig. 1—Simulated primary sequence segments of 5 individuals (represented iconically). While all share sequence similarity, a naturally occurring single nucleotide polymorphism occurs at the site indicated. In this example, 60% of individuals have a guanine at this site, whereas 40% have an adenine. The relative prevalence of individual forms varies among noninterbreeding populations.

identify those who are at risk while still presymptomatic.⁹ Susceptibility genes are found in complement-mediated inflammation and oxidative stress pathways, consistent with our current understanding of the biology of this disease—specifically, the importance of immune-mediated retinal inflammation and the role of oxygen free radicals and oxidative damage.

The complement system and AMD

Patients with AMD have increased baseline systemic complement activation consistent with heightened non-specific immune activation.¹⁰ The complement system is a primitive enzymatic cascade that has evolved to eliminate pathogens from the bloodstream by disrupting their membranes by pore formation (Fig. 2).^{11,12} It functions both in an antibody-directed fashion (the classical pathway) and independent of specific antigenic recognition (the alternative pathway). States of complement dysfunction result in susceptibility to bacterial infections, such as those due to *Streptococcus pneumoniae*,¹³ or autoimmune diseases, such as systemic lupus erythematosus.¹⁴ Subtle functional differences in the DNA sequence of some complement components found in patients with AMD promote macular degeneration and are described below.

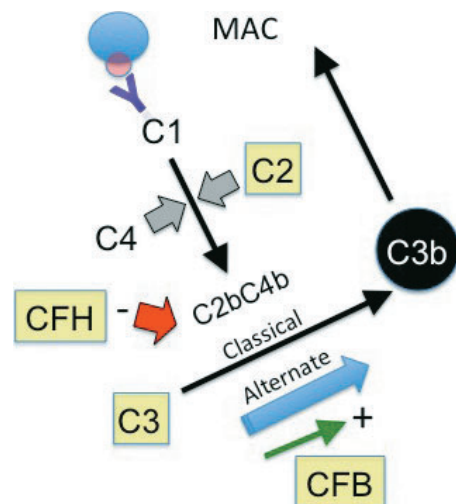


Fig. 2—Components of the complement cascade implicated in the pathogenesis of AMD are presented in context. Complement component 3 is a point of convergence of the classical and the alternative pathways. CFH is an inhibitor of C3 convertase. Complement component 2 functions in the classical pathway to help form the C3 activating C2bC4b complex. CFB facilitates the spontaneous conversion of C3 into C3b, its active form. The MAC is formed as the result of C3b acting with late complement components to disrupt pathogen membrane integrity. (AMD, age-related macular degeneration; CFH, complement factor H; C3, component 3; CFB, complement factor B; MAC, membrane attack complex.)

Complement factor H

The first genetic association of a complement pathway gene variation with AMD appeared in 2005 when the complement factor H (CFH) gene on chromosome 1q31 was linked to this disease.¹⁵ CFH is a natural inhibitor of complement component 3 (C3) convertase and impedes the activation of the alternative complement pathway (Fig. 2). It is synthesized in the retinal pigment epithelium and is present within drusen.¹⁶ The CFH single nucleotide polymorphism (SNP) rs1061170 (T/C) alters a tyrosine (Y) codon at position 402 (TAC) to histidine (H) (CAC), producing a Y402H polymorphism.^{16,17} Further refinement of the association of the broader CFH locus with AMD has shown that the Y402H polymorphism may not itself be etiologically important but is linked to yet-to-be-identified elements that affect the function of CFH or closely associated genes.¹⁸ Extended CFH haplotypes (multiple polymorphic markers found on the same strand of DNA) that are independent of the Y402H polymorphism are superior predictors of AMD risk.¹⁸ This presumably results from the ability of many markers to interrogate a larger part of this gene and similar adjacent genes. One set of researchers has grouped CFH polymorphisms to specify 2 common risk haplotypes (H1 and H3), several rare risk haplotypes (H5–H8), and 2 that specify a reduced risk (H2 and H4).¹⁸ The odds ratio (OR) for having AMD in individuals with a risk haplotype on each copy of chromosome 1 compared with 2 low-risk haplotypes is approximately 18.

Complement component 3

C3 is a convergence point of all complement pathways. Polymorphisms in the C3 gene predict increased risk of AMD development.^{19,20} A study of Caucasian subjects from the United Kingdom and other groups first identified the rs2230199 (C/G) polymorphism as conferring risk.^{19–21} This base is found in the third exon of the C3 gene in codon 102 and causes a nonsynonymous substitution of risk-associated glycine (GGC) for a protective arginine (CGC). The OR for individuals having the heterozygous form is 1.7, and for those having the risk allele in the homozygous form (G/G), it is 2.6.¹⁹

Other complement components

Several polymorphisms in other complement factors have been tentatively identified and await confirmation. Polymorphisms on chromosome 6 within the adjacent complement factor B and complement component 2 genes are associated with AMD in some reports,²² though some of these sites have not been confirmed.²³ A polymorphism within the Serping 1 gene, a naturally occurring complement component 1 inhibitor, is reported by 1 group to occur more often in patients with AMD,²⁴ a result that has not been confirmed by others.²⁵

Oxidative phosphorylation and AMD

Chronic, unopposed oxidative stress may contribute to AMD-associated retinal damage. All cells maintain a reducing environment that is preserved by enzymes that require metabolic energy. Disturbances in this balance can cause toxic effects through the production of peroxides and free radicals, which damage cellular components such as proteins, lipids, and DNA.²⁶ Most of these oxygen-derived free radicals are constantly generated by normal aerobic metabolism, and the damage they cause to cells must be continuously mitigated.²⁷ For cells that are renewed slowly or not at all, such as neurons and retinal photoreceptors, these processes may lead to degenerative diseases such as AMD.²⁸

Predisposition to oxidative cell injury may result from the inheritance of functional variants of molecules that participate in the initiation or repair of oxidative stress. The AMD-associated locus on chromosome 10q26 contains the age-related maculopathy susceptibility 2 (ARMS2) gene, which is expressed in retinal mitochondria and may mediate oxidative stress through yet unknown mechanisms.²⁹ The rs10490924 G/T polymorphism in this site tags a large linkage block associated with AMD.³⁰ It is in absolute linkage disequilibrium with a small insertion/deletion polymorphism (del443ins54), affecting the polyadenylation signal of the ARMS2 gene, which results in instability of its messenger RNA and reduced production of its gene product.³¹ Early reports examining 1166 cases and 945 controls identified an OR for developing AMD of 2.7 for the TG diplotype and 8.2 for the TT diplotype relative to the low risk GG diplotype.³² This single locus risk assessment agrees with the risks associated with the pathologic del443ins54 form, which confers a 2.9 OR in heterozygous form and 8.1 in the homozygous form.³¹

A variation in the DNA found within the mitochondria is also associated with AMD. The mitochondria are key to retinal oxidative stress through their role in oxidative phosphorylation. Each cell has thousands of mitochondria with identical 16 500 bp haploid sequences. The human mitochondrion codes for 37 proteins that function largely in aerobic cellular respiration.³³ Within the mitochondrial genome 144 catalogued sites of genetic variation exist with frequency greater than 1%. A mitochondrial SNP at position 4917 (A→G) is observed more frequently in patients with vision loss from AMD than in controls, as measured in a study population of 1547.³⁴ The G polymorphism is associated with an OR of 2.16 compared with the A SNP. Additional risk-associated polymorphisms within mitochondria DNA contribute to disease risk, supporting the role of energy metabolism in the etiology of AMD.³⁵

Environmental risk factors

Population-wide surveys have identified environmental risk factors for AMD. Apart from age, tobacco smoking is the only risk factor consistently associated with any form of AMD in these large studies.^{36–38} The Beaver Dam study in Wisconsin, involving a North American population, found

smoking-related OR of 1.46 (0.71–2.97) and 3.14 (1.39–7.90) for previous and current smokers, respectively.³⁶

Practice tools: an algorithm incorporating genetic and behavioural risk

Early detection of subfoveal choroidal neovascularization resulting in early management may lead to effective secondary prevention of AMD-associated blindness.^{3,4,39} The obvious utility of identifying a group of individuals whose condition is likely to progress to choroidal neovascularization from early disease has prompted several attempts to assemble multiple genetic and environmental risk factors into indicators of progression. Individuals at highest risk can be provided with existing and future primary prevention and enrolled in surveillance programs culminating in choroidal neovascularization therapy before vision loss.

We present a mathematical model to predict the absolute risk of geographic atrophy and choroidal neovascular AMD risk based on smoking history and genetic risk factors using published data and the population prevalence of these complications in individuals of European ancestry, the most studied population. To simplify the consideration of the CFH locus as part of a genetic prediction tool, haplotypes of high, intermediate, and low risk can be used to determine the weighted average OR for each CFH diplotype (2 haplotypes).¹⁸ The risk data associated with each of the 8 commonly occurring CFH haplotypes were reparameterized so that CFH low-risk diplotypes became the reference category with OR = 1, intermediate-risk haplotypes having an OR of 4.33, and high-risk diplotypes having an OR of 17.97 in comparison.¹⁸ This approach significantly improves the dynamic range of the rs1061170 single SNP, which confers an OR for disease of 7.4 for the CC “high-risk” relative to the TT “low-risk” diplotype.¹⁷ The product of the ORs of each genetic or environmental determinant, shown in Table 1, produces a simple multifactorial risk estimation system. With knowledge of the frequency in the population of these risk factors and the overall prevalence of the disease, an iterative statistical approach can be used to estimate the amount of disease risk conferred by the test features.⁴⁰ Smoking status, rs10490924, rs2230199, and mtA4917 were used to produce an illustrative algorithm with ORs for geographic atrophy and choroidal neovascularization and frequencies as cited (Table 1). The absolute prevalence of vision loss secondary to geographic atrophy and choroidal neovascularization is estimated to be 9.5%, as determined by international prevalence surveys.⁴¹

A risk score (X) for the development of geographic atrophy or choroidal neovascularization can be derived as follows:

$$X = (\text{CFH factor})(\text{ARMS2 factor})(\text{C3 factor})(\text{mt factor})(\text{smoking factor})$$

Clinically significant groups of disease prevalence can be parsed from risk scores to produce a simple clinical tool (Table 2). We determined risk score thresholds that predict for less than average risk (<5%) of vision loss by age 80, an average risk (5%–15%), a moderate risk (15%–40%), a high risk (40%–55%), and a very high risk (>55%). Under such a grouping, 20% of the population are predicted to have an increased risk of geographic atrophy or choroidal neovascularization and 1% a very high risk. The age-specific risk estimation for each risk group is graphically represented in Fig. 3.

Clinical application

Vision loss from advanced AMD causes extensive debility and dependence for sufferers. The ability to identify normal-sighted individuals with normal retinas or early disease who are at risk of future vision loss invites attempts

Table 1—Odds ratios of individual risk factors for geographic atrophy/choroidal neovascularization in age-related macular degeneration

Risk factor	Prevalence
CFH factor ¹⁸ (H2 and H4 combinations alone)	0.302
4.33 (one of H1/H3/H5–8 and one of H2/H4)	0.495
17.97 (H1, H3, and H5–8 combinations alone)	0.203
ARMS2 factor (rs10490924) ³¹ #1130	
1 for GG	0.593
2.7 for TG	0.390
8.2 for TT	0.017
C3 factor (rs2230199) ¹⁹ #3864	
1 for CC	0.712
1.7 for GC	0.237
2.6 for GG	0.051
mt factor (mtA4917G) ³⁴ #527	
1 for A	0.910
2.16 for G	0.090
Smoking factor ³⁶ #4016, #4094	
1 for never	0.440
1.46 ex-smoker	0.370
3.14 current smoker	0.190

Table 2—Geographic atrophy and choroidal neovascularization risk categories, their frequency in the population, risk scores, and the predicted category-specific, age-adjusted prevalence of disease

Risk score (X)	Risk category					
	Low	Average	Moderate	High	Very high	
	1.00–7.90	8.0–28.9	29.0–100	101–184	185–2600	
Freq.	0.50	0.30	0.17	0.02	0.01	
Overall prevalence (%)	Category-specific predicted prevalence of GA or CNV for each age group (%)					
50–4	0.19	0.1	0.2	0.5	0.9	1.3
55–9	0.22	0.1	0.2	0.6	1.1	1.5
60–4	0.34	0.1	0.3	0.8	1.6	2.3
65–9	0.62	0.2	0.6	1.5	3.0	4.2
70–4	1.2	0.3	1.1	3.0	5.8	8.1
75–9	2.5	0.6	2.3	6.1	11.7	16.4
>80	9.7	2.4	9.2	24.1	46.5	65.0

Note: GA, geographic atrophy; CNV, choroidal neovascularization.

at intervention. Maguire et al.,² writing for the Complications of Age-related Macular Degeneration Research Group, concludes that close monitoring of at-risk eyes, such as fellow eyes from those patients with unilateral choroidal neovascularization or eyes with multiple large drusen and pigment changes, allows detection of early neovascularization prior to a loss in visual acuity. Use of antivascular endothelial growth factor agents, such as bevacizumab and ranibizumab, leads to the preservation and improvement of sight in visually impaired patients with neovascular AMD and may prevent the deterioration of sight in those with presymptomatic choroidal neovascularization.⁴²

Diet may also be an effective intervention for those at risk of the neovascular or atrophic forms of AMD. San-Giovanni et al.⁴³ writing for the Age-Related Eye Disease Study Research Group, reports that lutein or zeaxanthin dietary intake decreases the risk of choroidal neovascularization and geographic atrophy in patients with early-stage disease, prompting many eye care societies to advise use of these agents as chemoprophylactics. Daily intake of folate and vitamins B6 and B12 may provide primary prevention, though a targeted approach to individuals at highest risk of the disease will be required.⁴⁴

AMD is a genetic disease with devastating consequences. Tools that improve upon the performance of clinical predictors alone are now available for the early identification of patients at risk of geographic atrophy and choroidal neovascularization.^{5,45} We show that a simple mathematical model can identify those at increased risk of this disease using genetic variability and smoking history as primary inputs. An intensified schedule of follow-up may be indicated for those individuals at greatest risk of disease progression with the provision of nutritional primary prevention; possibly, with early detection and treatment, vision loss associated with AMD can be prevented.

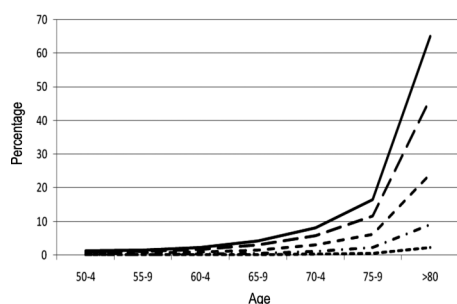


Fig. 3—Age-specific prevalence of GA and CNV AMD for each of 5 genetic/smoking risk groups (solid: very high; long dash: high; short dash: moderate; dot+dash: average; shortest dash: low) (GA, geographic atrophy; CNV, choroidal neovascularization; AMD, age-related macular degeneration.)

Brent Zanke is the Chief Scientific Officer of ArcticDx Inc, and David Chow and Steven Hawken serve as scientific consultants to ArcticDx Inc.

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