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Editorial

Molecular genetics of age-related macular degeneration: current status

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ABSTRACT: Age-related macular degeneration (AMD), a multifactorial human disorder, is the most common cause of acquired visual impairment in people over the age 60. It is estimated to affect millions of individuals worldwide. Prevalence increases with age; among persons 75 years and older, mild, or early forms occur in nearly 30% and advanced forms in about 7% of the population. AMD has been associated both with environmental and genetic factors. However, the clinical heterogeneity, late age at onset, and complex etiology have confounded genetic studies of the disorder. Methods applicable to the study of single-gene and some complex disorders (i.e., linkage analysis, sib-pair analysis, transmission disequilibrium test, etc.) have had limited utility in elucidating the genetic components of the complex AMD trait.

Recently, substantial progress has been made in determining the genetic basis of monogenic eye disorders. On a monthly basis mutations are identified in new genes responsible for some form of retinal degeneration. Most, if not all, of these genes become candidates for potential involvement in multifactorial disorders especially if the phenotypes of the early-onset Mendelian diseases they cause resemble later onset complex traits. Unfortunately, to date mutational analyses of the candidate genes in AMD patients to date have not yielded the highly anticipated information: statistically significant association of sequence variants with AMD. Whether this is due to the unsuccessful selection of the right candidate genes for the analysis, or the methods employed, or both, has to be elucidated.

This review summarizes current knowledge of genetic research aimed at delineating the molecular genetic basis of age-related macular degeneration. Moreover, it attempts to offer some approaches for the future studies directed towards understanding the genetic components of this complex disorder. (Eur J Ophthalmol 1999; 9: 255-65)

KEY WORDS: Age-related macular degeneration, Complex trait, Genetic predisposition, Candidate genes, Association, ABCR

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AMD is a complex, multifactorial trait

The fact that age-related macular degeneration represents a multifactorial disorder involving both environmental and genetic factors is generally accepted amongst vision researchers. Environmental influences include smoking, diet, hypertension, exposure to sunlight, and other factors that have been associated with the disorder (1-6). However, even in the case of tobacco, which has been consistently associated with AMD (2,3), the results of different studies contradict each other (7). Unfortunately, the current studies do not readily explain the apparent increase in prevalence of AMD, suggesting that the most impor-

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tant environmental factors, or their potential synergistic combination, have yet to be elucidated.

Familial aggregation, segregation, and twin studies have clearly established that genetic predisposition plays a major role in etiology of AMD (8-12). However, the magnitude of the genetic component varies significantly between studies, the familial risk estimates ranging from 2.4 (10) to 19.3 (11). The most recent population-based familial aggregation study estimates the relative risk at 4.2 and the population-attributable risk of late AMD at 23% (12). Thus, siblings of AMD patients are four times more likely to develop the disorder, and the proportion of AMD specifically due to genetic factors has been estimated as approximately 1/4 of all cases. It is generally acknowledged that multiple genes are involved, although the approximate number of those has not been reliably estimated. Furthermore, as in other complex traits the extent of genetic heterogeneity cannot be estimated so that influences of many genes and their interactions in a particular individual family are difficult to ascertain. In summary, AMD is yet another example of a complex trait where the genetic studies are complicated by the very late onset of the disorder, decreased penetrance, and potential genetic heterogeneity.

Prevalence and clinical characteristics of AMD

AMD is the leading cause of vision loss among the elderly, with about 30% of the human population over the age of 75 manifesting some form of maculopathy (13). The prevalence of clinically detectable AMD is especially high in western industrial societies (13-15), and it seems to be increasing (16). AMD affects all racial groups, but the rates of progression and therefore documented late stages of the disorder, are reportedly lower in non-white populations (17,18).

Clinically, AMD has been divided into two subgroups: the majority of patients are diagnosed with the “dry” form(s) including macular drusen, retinal pigment epithelial (RPE) pigmentary irregularities and/or geographic atrophy, while the remaining patients manifest an exudative or “wet” form characterized by serous or hemorrhagic detachment of the RPE and/or choroidal neovascularization (19). Distinction between the dry and wet phenotypes can be ambiguous. The ambiguity results from lack of uniformity in clinical classification, in which the term AMD may be used to denote any age-change regardless of whether or not it is accompanied by visual loss, or to those with visual loss as a consequence. In the first case the term dry refers to individuals with drusen only, as well as those with visual loss so that patients classified as dry represent 90% of cases (19). In the second definition, the term dry refers to those with geographic atrophy only (late AMD), which represent a minority of cases (20). Whether the two clinical manifestations have different genetic risk factors remains to be determined. Occasionally, individuals with the dry form in one eye may develop choroidal neovascularization later in life either in the same or fellow eye (21). Several distinct grading systems have been developed and are simultaneously used by physicians (20,22-25). The grading systems are very similar to each other but the distinction results in certain difficulties for comparing analogous studies that exceed semantic problems. Dissection of the current status of diagnostics of AMD goes beyond the scope of this review, nevertheless the importance of establishing unified criteria of universally accepted guidelines for AMD diagnosis can not be overemphasized. Genetic research of any disorder is stymied without initial specific phenotypic characterization of the patient population.

Genetic approaches to AMD

A high prevalence of AMD is registered among panmictic white populations of European descent. A higher frequency of the disorder has not been described in any population isolates, complicating the application of several powerful strategies for disease gene mapping such as homozygosity mapping and linkage disequilibrium. According to the prevailing hypothesis, the majority of AMD cases are not a collection of multiple single-gene disorders but instead represent a quantitative phenotype, an expression of interaction of multiple susceptibility loci. Unfortunately, the number of loci involved, the attributable risk conferred by each locus, and the degree of interaction between loci, remain obscure. AMD resembles other complex disorders such as schizophrenia, wherein parametric linkage analysis in large pedigrees (a method of choice in Mendelian and some complex disorders) has not yielded robust results (for review see (26)). In the case of AMD, reasons for limitations of traditional genetic methods may include the difficulty of collecting large

pedigrees segregating the phenotype due to the very late onset of the disease, and the imprecise definition of diagnostic criteria. To date, linkage of one AMD phenotype (ARMD1; MIM 603075) to a specific chromosomal region, 1q25-q31, has been documented in only one study (27). Because the members of this pedigree manifest a specific phenotype and no additional pedigrees have been linked to this locus, it is feasible that this represents an isolated case of a single major gene causing this particular, albeit rare, form of the disorder. Whether the gene from 1q plays a role in other AMD patients has to be determined following the isolation and analysis of the gene.

What approaches remain to unravel the genetics of AMD? In their seminal paper, Risch and Merikangas suggested candidate gene-based association studies as an alternative to linkage to identify genes that contribute a modest effect to a complex trait (28). They defined the term genotypic relative risk (GRR, the increased chance that an individual with a particular genotype has the disease) as a measure of contribution of a genotype to the heritability of the disorder, or a gene effect (28). If a variant in a candidate gene is available and its frequency (as the possible disease allele, or at least the one in strong disequilibrium) is determined, it is possible to calculate the number of families or individuals needed for linkage or association analysis to obtain sufficient power to unequivocally demonstrate involvement of the variant in the phenotype. For genes of moderate effect the candidate gene association studies are clearly more feasible than those employing linkage (28).

The approach to a complex trait consisting of identifying a reasonable candidate gene, finding a single nucleotide polymorphism (SNP) in its sequence (preferentially a potentially disease-causing one), and screening sufficiently large cohorts of patients and controls for the presence of the variant, appears simple. The subsequent statistical analysis could classify the variant as associated with the disease, showing no association, or in fact suggesting protection from disease effects. In reality, association studies, especially of the case-control variety, are prone to produce false positives for a multitude of reasons. If there is no evidence of the functional effect of a variant the proof of association has to be evaluated rigorously by statistical analysis, especially if multiple variants in a single gene are studied. Population stratification, a situation in which diseased individuals and comparison groups are derived from genetically different subpopulations, can be a confounding factor and a source of spurious results. The latter could be potentially overcome by family-based methods with internal controls, such as the transmission disequilibrium test (TDT, 29). However, application of these methods is difficult in AMD because the parents of patients with the late onset disease are usually deceased and thus not available for genotyping. The sample size becomes another important variable especially in studies where negative results are reported.

Nevertheless, case-control studies represent an important approach for defining associations in complex traits. Several guidelines need to be adhered to in order to obtain meaningful results from a case-control association study. These include: 1) Unified diagnostic criteria for all patient and control collections; 2) Very large sample sizes of both cohorts; 3) Increased efficiency and throughput of mutation detection methods; and 4) Data pooling from various studies (meta-analysis), or large multicenter-based studies.

Candidate genes for AMD

The number of potential candidate genes for AMD is large and limited only by the preferences of a researcher. Subtle defects in many genes, whether involved in the phototransduction cascade, photoreceptor metabolism, structural components of the retina, or visual cycle, can potentially increase (or decrease) susceptibility to this complex disorder. Genes involved in diseases with Mendelian inheritance, especially those with phenotypes resembling AMD, may be considered as primary candidates (Tab. I A/B). More candidate genes include those expressed exclusively, or primarily, in the retina even if the function of these remains obscure. There is no reason to eliminate any of the genes suggested above as candidates for association with AMD since the limitations of current knowledge on the functional significance of the vast majority of genetic variation does not enable exclusions. The current status of the association studies for four genes, implicated in various Mendelian macular dystrophies (Tab. I A) is summarized below. A list of genes and disease loci relevant to retinal pathology can be found at:

http://www. sph.uth.tmc.edu/ Retnet/disease.htm

Genes implicated in macular disorders showing Mendelian inheritance, that have been analyzed in AMD patients include: the tissue inhibitor of metalloproteinases-3 (TIMP3) mutated in patients with Sorsby fundus dystrophy; the Best disease (vitelliform macular dystrophy, VMD2) gene; ABCR, involved in several recessive retinal dystrophies; and the fibrillin-like extracellular matrix protein (EFEMP1), mutated in Doyne honeycomb retinal dystrophy (Tab. I A). Each of these diseases has phenotypic similarities with AMD specifically because the macula is affected at some point during disease progression.

Sorsby Fundus Dystrophy (MIM 136900) is an autosomal dominant disorder that resembles the neovascular (wet) form of AMD in that its hallmarks include subretinal neovascularization and hemorrhage evolving into disciform scar in some instances. The causal gene,TIMP3, was identified as a positional candidate on chromosome 22q12-q13. Heterozygous mutations that appear to affect the proper folding of the TIMP3 gene product are associated with the disorder (30). Tissue inhibitors of metalloproteinases are Zn-binding endopeptidases involved in degradation of the extracellular matrix and are characterized as growth repressors (31). Delineating the role of TIMP3 in Sorsby dystrophy focused attention on the metabolism of the extracellular matrix in other macular dystrophies. Unfortunately, although seemingly a good model for neovascular AMD, no mutations were detected in TIMP3 in patients diagnosed with macular degeneration (32) and the TIMP3 locus was thus excluded as a gene potentially involved in AMD (33).

Vitelliform Macular Dystrophy (VMD2, Best disease, MIM 153700) represents another autosomal dominant form of macular dystrophy characterized by deposition of lipofuscin-like material within and below

TABLE IA - GENES CAUSING MENDELIAN RETINAL DISEASES EVALUATED IN AMD

Monogenic Disease(s)	MIM #	Gene	Locus	Inheritance	Mutations in AMD patients	References
Stargardt (STGD1) (also FFM, CRD, RP19)	248200	ABCR	1p22	Recessive	8-16%	(39,42)
Doyne Honeycomb Dystrophy/ Malattia Leventinese	126600	EFEMP1	2p16-p21	Dominant	<1%	(37)
Best Vitelliform Dystrophy	153700	VMD2	11q12-q13	Dominant	<1%	(34-36)
Sorsby Fundus Dystrophy	136900	TIMP3	22q12-q13	Dominant	Not found	(30,32)

TABLE IB - SELECTED CANDIDATE GENES AND DISEASE LOCI FOR ASSOCIATION WITH AMD

Monogenic Disease(s)	MIM #	Gene	Locus	Inheritance	Mutations in AMD patients	References
Leber Congenital Amaurosis	204100	RPE65	1p31	Recessive	?	(59,60)
Stargardt-like Dystrophy (STGD4)	603786	?	4p15	Dominant	N/A	(61)
Retinal Degeneration Slow MD, Pattern dystrophy, AVMD	179605	RDS	6p21.2-cen	Dominant	Not found (?)	(62-64)
Stargardt-like Dystrophy (STGD2) Cone-Rod Dystrophy	600110 603649	?	6q11-q15	Dominant	N/A	(58,65,66)
Bifocal Chorioretinal Atrophy/ North Carolina Macular Dystrophy	600790 136550	?	6q14-q16	Dominant	N/A	(67,68)
Stargardt-like Dystrophy (STGD3)	153900	?	13q34	Dominant	N/A	(58)
Cone Dystrophy (CORD5, RCD2)	600977 601251	?	17p13-p12	Dominant	N/A	(69)

N/A – not analyzed (causal gene not yet cloned)

the RPE forming a distinctive “egg yolk cyst” in the foveal area. This accumulation is associated with degeneration of the RPE and overlying photoreceptors, and may culminate in geographic atrophy of the macula and/or choroidal neovascularization. The gene for Best disease, VMD2, was independently cloned in two laboratories (34,35) and a wide variety of heterozygous mutations were associated with the VMD phenotype - see the VMD2 web site at:
http://www.uniwuerzburg.de/humangenetics/vmd2.html

The VMD2 gene is expressed in the RPE of both human and rodent eyes and encodes for a transmembrane protein. Although the function of the VMD2 protein bestrophin remains unknown, it has been suggested that it may be involved in the transport or the metabolism of polyunsaturated fatty acids in the retina through regulation of the iron storage protein ferritin (34). Because of the phenotypic overlap between Best disease and AMD phenotypes the gene was considered as a primary candidate for involvement in the latter. Several laboratories have independently screened hundreds of AMD patients for mutations in VMD2 and the results are in good agreement. The only published study to date (36) found amino acid-changing VMD2 variants in about 1% of patients with AMD. Similar results were documented in the laboratories of Drs B.H.F. Weber and E.M. Stone, and have been presented at several meetings. Together, these data provide no statistically significant evidence that variants in the Best disease gene are associated with the AMD phenotype. However, the analyses do not exclude the possibility that some VMD2 variants might be involved in selected sporadic AMD cases. Unambiguous classification of rare VMD2 variants as disease causing in AMD is complicated for two reasons: 1) the lack of any functional test to evaluate the consequences of VMD2 alterations, and 2) the diagnostic difficulty to unequivocally segregate patients with the late onset Best disease from those with AMD. The latter complication is one of the most difficult problems to overcome in any study involving a multifactorial disorder.

Doyne Honeycomb Retinal Dystrophy (DHRD, also known as autosomal dominant radial macular drusen, or Malattia Leventinese, MIM 126600) was always considered one of the best single-gene disease models for AMD. The hallmark of Doyne is extensive distinct drusen in the foveal and macular areas, which closely resembles the “early” or dry subtype of AMD. Not surprisingly, cloning of the responsible gene and its analysis in AMD was highly anticipated by ophthalmologists and ophthalmic geneticists. Recently, Stone et al (37) presented an extensive analysis of a positional candidate on 2p15-p16, the previously characterized S1-5 gene (38), in patients diagnosed with DHRD and AMD. Surprisingly, screening of the gene now known as EFEMP1 (for EGF-containing fibulin-like extracellular matrix protein 1) revealed only one sequence change resulting in a missense amino acid substitution (Arg345Trp) in all DHRD patients. No EFEMP1 sequence variants have been reported in individuals diagnosed with AMD (37). The data suggest that the founder allele, Arg345Trp, is the cause of all familial DHRD. However, it has not been entirely ruled out that the discovered non-conservative missense mutation Arg345Trp is a genetic marker (SNP) that is in linkage disequilibrium with the real disease-causing mutation.

Stargardt Disease (STGD1, MIM 248200, also Fundus Flavimaculatus (FFM)) is a juvenile-onset autosomal recessive disorder characterized by diminished central visual acuity, the appearance of small yellowish lesions or flecks at the level of the retinal pigment epithelium, and atrophic changes in the macula. Discovery of the gene responsible for STGD1, ABCR (39), was perhaps one of the most exciting recent events in the genetics of eye diseases for several reasons. First, mutations in this gene were subsequently described in eye diseases with very different phenotypes from STGD1, such as retinitis pigmentosa (RP19, (40)), and one form of cone-rod dystrophy (CORD3, (41)). Second, ABCR is a member of a well-characterized superfamily of proteins called ATP-binding cassette (ABC) transporters. Other members of this superfamily have been implicated in a number of inherited diseases that involve transport defects, allowing reasonable predictions as to the function of the ABCR protein. Third, ABCR became a good candidate for association with AMD since AMD and STGD1 share phenotypic similarities. Several laboratories have tested the latter hypothesis and their interpretation of the results is contradictory, which likely reflects methodological differences in studying the complex AMD trait.

In the first study, 167 patients with AMD and 220 racially matched population controls were screened for variants in all 50 exons of the ABCR gene (42). A

total of 13 alterations in 26 patients (16%) were detected that were interpreted as associated with the disease phenotype since they were found in less than 1% of controls. Most alterations resulted in rare missense mutations, some of which had also been found in STGD1 patients (42). Subsequently, three reports disputed the conclusions derived from the original data stating that they were unable to replicate our findings and, therefore, to confirm the association (43-45). Problems with replication of an association study of a complex disease are not unexpected (26). Difficulties can arise from: 1) the virtual impossibility in replicating the patient and control populations; 2) differences in methods of mutational analysis and their efficiency; 3) sample size.

In the case of AMD patient selection can be especially problematic due to broad heterogeneity of the disease phenotype and different diagnostic criteria. In our study (42), only 20% (33/167) of patients were classified as manifesting the wet, exudative, phenotype. The remaining 80% were diagnosed with the dry form of the disease. The latter classification includes both patients with geographic atrophy and those with extensive drusen and pigmentary abnormalities (19). In the three other studies the fraction of patients with exudative phenotype ranged from at least 60% (43) to more than 80% (44,45). Only 1 out of 33 patients with the wet form possessed the ABCR mutation in our study (42). This observation prompted us to conclude that: “this (exudative) form of AMD may be associated with factors other than ABCR alterations” (42). Thus, the conclusion of the three studies sustains our initial assessment that ABCR variants are rare in wet AMD, but does not imply that the ABCR variants are not associated with any AMD phenotype.

In order to make a statement about the lack of sequence variants in a gene in a cohort of patients, one must be assured that the mutation scanning method employed will detect the substantial majority of all sequence changes. For ABCR a valid standard for the efficiency of mutation screening is the fraction of mutations found in STGD1 patients, since ABCR is the gene exclusively responsible for classic recessive STGD (39). Stone et al found ABCR mutations in only 20% of STGD chromosomes (43) which is substantially less than 60% that were detected in two similar studies (46,47). The study by De La Paz et al did not find even the common ABCR polymorphisms identified by all other European and American laboratories (39,42,43,47,48), thus rendering the presented results uninterpretable.

The above further raises the most important question – what has to be done to reproduce an association? In a recent study, Long and Langley (49) tested the power of association studies to detect the contribution of candidate gene loci of modest effect to variation in complex traits. Their population genetics model simulated the processes expected in actual population samples and enabled them to derive several important conclusions: 1) To achieve a sufficient power to detect the presence of causative polymorphisms of small effect, about 500 individuals have to be sampled; 2) Greater power is achieved by increasing the sample size rather than by increasing the number of polymorphisms; 3) Association studies have a low reproducibility unless sample sizes are on the order of 500 individuals (49). The latter point specifically explains unsuccessful attempts to reproduce an association, including those described above.

To test our hypothesis-generating finding that heterozygous ABCR mutations may increase susceptibility to AMD, we designed an expanded collaborative study including 15 centers in Europe and North America (50). Two of the most common AMD-associated variants (42), G1961E and D2177N, were genotyped in 1385 unrelated AMD patients and 1478 reportedly unaffected, unrelated individuals as controls. Together, these two non-conservative amino acid changes were found in one allele of ABCR in 53 patients (~4%) and in 13 controls (~0.9%), a statistically significant difference ($P<0.0001$) (50). The results remained significant ($P<0.0001$) even after exclusion of the data from the previous hypothesis-generating study (42). The risk of AMD is estimated to be increased about three-fold in carriers of D2177N and about seven-fold in carriers of G1961E, and these two variants are detected in about 4% of AMD cases. In the context of common complex disorders this represents an important contribution to the disease load. Since AMD affects millions of people worldwide and the described mutations represent only two out of thirteen reported earlier (42), the number of people at increased risk of developing age-related maculopathy as carriers for variant ABCR alleles is substantial.

Model for ABCR involvement in inherited retinal diseases

Based on the findings of mutant ABCR alleles in STGD, FFM, CORD3, RP19 and AMD, a model of ABCR function has been proposed (46,47,51,52). In this model, the severity of the disease is inversely proportional to the residual ABCR activity (Fig. 1). Severe mutations, resulting in a complete loss of ABCR activity, cause retinitis pigmentosa if they affect both alleles of the gene (40,41). One severe allele and one moderate mutation cause cone-rod dystrophy (41), and severe/mild or moderate/moderate combination result in STDG/FFM phenotype (46,47). Heterozygous individuals for an ABCR mutation may be more susceptible to developing AMD over a prolonged time if other genetic factors are involved or exposure to environmental influences occurs. Compound heterozygous or homozygous individuals for mutant ABCR alleles have never been described among AMD patients, an observation consistent with a dominant susceptibility locus (42). One prediction of this model - that parents and grandparents of STGD patients may be more susceptible to AMD - has been supported recently by several studies (46,52-54). Therefore, genetic variation in ABCR is clearly responsible for a substantial amount of retinal pathology (Fig. 1).

Functional studies support the proposed model, in that the ABCR gene has been linked directly to the visual cycle. First, ABCR was localized to the rims of rod photoreceptor outer segment disks (55). Consequently, a potential substrate of this transporter, all-trans retinal, was shown to stimulate ATP hydrolysis by reconstituted ABCR protein *in vitro*,

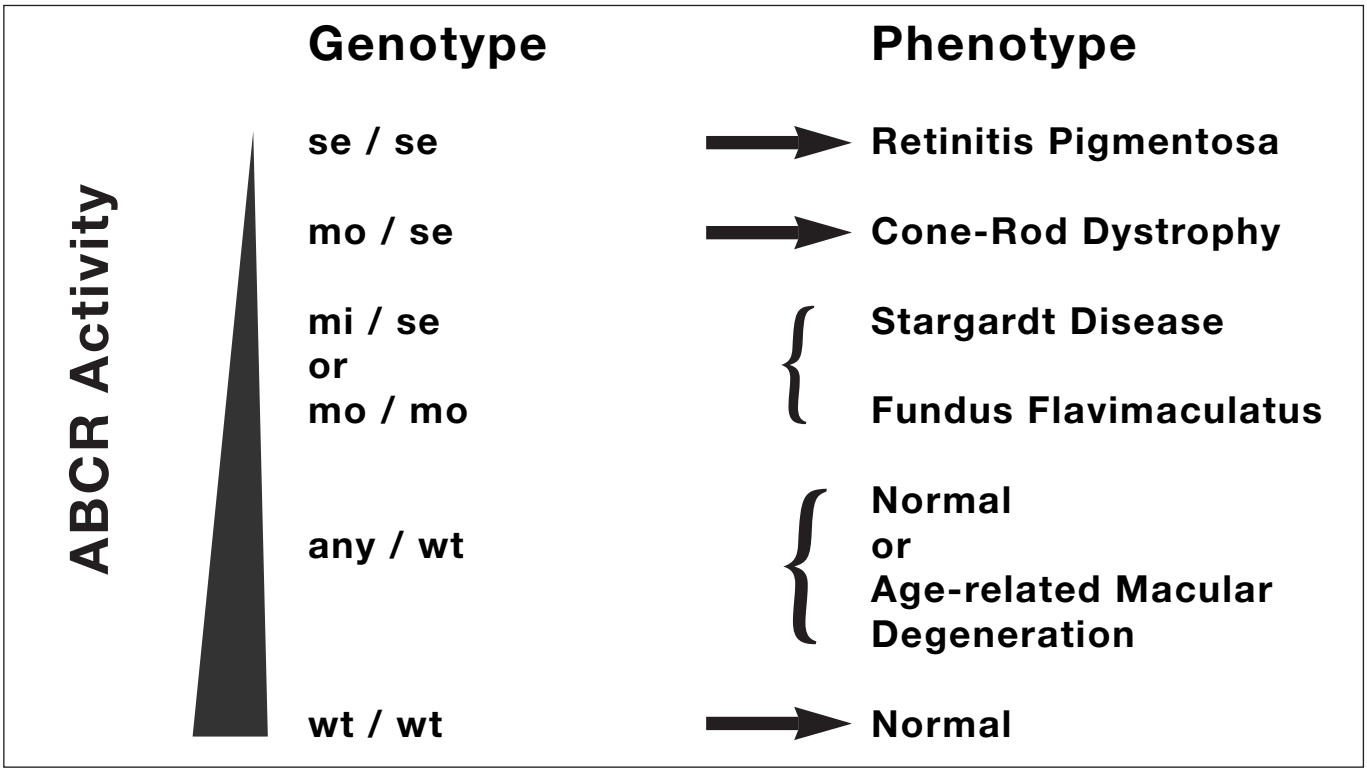

Fig. 1 - *Model for ABCR in inherited retinal diseases.*
Correlation of disease phenotypes with ABCR alleles and the residual ABCR activity is demonstrated. The model is derived from those published earlier (47,52). ABCR activity is shown at left by the filled triangle, decreasing towards the top. Both alleles for each genotype are depicted. wt, "wild type" or normal allele; se, severe mutation leading to a complete absence of a protein product (null allele); mo, moderate mutation; mi, mild mutation; any, any mutation of the three possible subtypes. Combinations of severe (null) mutations result in a form of RP; combinations of severe and moderate mutations result in CRD; combinations of two moderate mutations or a severe mutation with a mild mutation result in STGD/FFM; and some heterozygous ABCR mutations increase susceptibility to AMD.

suggesting that retinal could also be the *in vivo* substrate for ABCR (56). Studies of mice lacking the ABCR gene demonstrated delayed dark adaptation, increased all-trans retinal following light exposure, elevated phosphatidylethanolamine (PE) in rod outer segments, accumulation of the protonated Schiff base complex of all-trans-RAL and PE (N-retinylidene-PE), and striking deposition of a major lipofuscin fluorophore (A2-E) in retinal pigment epithelium (RPE) (57). Based on these findings, Travis et al proposed that the ABCR-mediated retinal degeneration may result from "poisoning" of the RPE due to A2-E accumulation, with secondary photoreceptor degeneration due to loss of the RPE support role (57). A2-E, a pyridinium bis-retinoid, which is derived from two molecules of vitamin A aldehyde and one molecule of ethanolamine, has been characterized as one of the major components of retinal pigment epithelial lipofuscin. Accumulation of lipofuscin in the macular region of RPE is characteristic to aging eyes and is the hallmark of both STGD1 and AMD. Together, these data define ABCR as the "rate-keeper" in the cycling of retinal. ABCR is apparently not absolutely essential for this process, since individuals lacking the protein (RP19 patients) maintain their eyesight for many years. Over time, however, even mild dysfunction of ABCR affects the vision irreparably.

CONCLUSIONS

In summary, our current knowledge of the genetic predisposition of AMD allows us to be very optimistic when looking into the future of scientific research in this field, and for several reasons.

First, tremendous progress has been achieved in the field of genetic studies of monogenic inherited eye diseases in the last few years. Rapidly increasing knowledge of the genetic causes of single-gene diseases will aid in defining which alleles of these genes, and in what combinations, contribute to AMD (Tab. IB). As an example, one would expect that proteins interacting with ABCR or those involved in the same functional or genetic pathway(s) would be reasonable candidates for investigation. The most recent data suggests that a gene on the long arm of human chromosome 6, implicated in the dominant Stargardt-like disease, could be in the same pathway with ABCR (58). The highly anticipated completion of the human genome sequence in the near future will add a myriad of genes that may be suitable candidates for association studies.

Second, all components are in place for immediate advancement in AMD research. Many laboratories have established vast collections of DNA samples from affected individuals and matched controls. Methods of mutation detection and genotyping technology are improving rapidly, both qualitatively and quantitatively. Interest in this research has been growing steadily, translating into ample funding by government and private agencies. New knowledge of methods in population genetics enables researchers to avoid costly and time-consuming mistakes in future research. It is obvious that collaborative, multicenter studies as the one described above would be instrumental in obtaining information important for deciphering the genetic determinants of AMD. International consortiums would: 1) increase the number of analyzed samples substantially; 2) achieve consensus on diagnostic criteria; 3) submit the obtained information to the most rigorous and critical analysis.

Third, any information on the function of the genes associated with AMD can be used for evaluation of possible therapeutic targets or pathways for disease intervention. For example, in *in vitro* experiments the ATPase activity of the ABCR protein has been synergistically stimulated by several compounds, including amiodarone, a widely used drug for cardiac arrhytmias (56). That raises a reasonable optimism that finding a way to enhance the transport activity of the protein may delay some of the symptoms associated with its defects. At the same time, studies of mice lacking ABCR (*abcr*$^{-/-}$) have suggested some preventive measures including avoiding bright sunlight as a potential treatment to delay the onset of symptoms stemming from ABCR defect(s) (57).

Defining the role of ABCR in pathogenesis of AMD is the first, small step towards deciphering the genetic cause of AMD. The ultimate prize for the successful completion of this difficult task will be improved quality of life and, eventually, restored eyesight to millions of people. This said, there is no reason to doubt that we are on the right track.

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