Age-related maculopathy: its genetic basis

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Age-related maculopathy (ARM) is the present-day name for a disorder that in the past had many names in ophthalmology, among others senile macular degeneration and disciform macular degeneration. Age-related macular degeneration was coined senile macular degeneration in 1885 by Haab,[1] about 100 years after Buzzi from Milan published this paper on the existence of the macula lutea. In 1875 Hutchinson and Tay[2] named probably the same disorder central chorioretinal disease. The international classification system of ARM was the first attempt to systematically classify the ageing changes in the retina, associated with drusen larger than small, hard ones with a diameter ≤ 65 μm, as ARM.[3] This system was based on the presence of various fundus abnormalities associated with ageing in which it was assumed that they form an ascending order of probability to lead to one of the two end-stages of ARM. Indeed from a follow-up study on ARM it seems that there is a more or less constant progression over 2 years in which one-quarter of cases with ARM go on to a higher stage (C.C.W. Klaver, unpublished; see Table 1).

The hallmark of ARM is the presence of drusen, although this may vary from person to person. There may also be racial variations as Japanese subjects seem to have fewer drusen associated with ARM than do Caucasians. Drusen are of course characteristic of ARM but not exclusive to this disorder. In general terms they reflect dysfunction of the retinal pigment epithelium (RPE). This may occur apart from ARM, e.g. above a naevus of the choroid, in several hereditary eye disorders and in kidney diseases. In the kidney glomerulus similar deposits are found (M. Tso, personal communication 2001). Drusen are most probably constituted of basal membrane degradation products and Gass thought that they are only one common end product of RPE failure.[4] Several studies by Hageman have shown in the meantime that a large variety of drusen exists. Clear evidence for the genetic basis of drusen came from one study in which a significantly higher correlation of the number and density of drusen was found in 53 sib pairs compared with 50 spouse pairs.[5]

The end-stages of ARM are dry (geographic atrophy) and wet (neovascular or disciform) macular degeneration and are called age-related macular degeneration (AMD).[3] Thus ARM and AMD are essentially diagnosed by exclusion of other disorders mimicking drusen, pigmentary changes, geographic atrophy and a disciform reaction.

Only about 100 years after Haab's publication it was seriously considered that there might be a genetic component to AMD. We will give a short historical overview of the evidence for the genetic influence on ARM and next will discuss various approaches to the study of the genetics of ARM, starting from the ophthalmologist's point of view.

Evidence for heredity of ARM

Twin studies

Twin studies often given the first indication of a disorder being genetic, and the first twin report on AMD known to us was in 1985.[6] After two more case reports,[7,8] a study in 9 monozygotic twin pairs showed concordance in AMD type in 8 of them.[9] In a larger twin study about one-quarter had ARM.[10] All 25 homozygotic twin pairs who had ARM were afflicted to a similar degree, while this held for only 42% of the dizygotic pairs.[10] The number of monozygotic twins was about 2.5 times higher than the dizygotic ones. As in all the following studies[11,12] caution is needed in interpreting these results. Apart from selection bias, the use of different grading systems for ARM (especially when no fundus pictures are taken) is often a source of misinterpretation of the results. This holds even more for studies using history data, as we will see in the next paragraph. Nevertheless, twin studies clearly point to a genetic factor in ARM.

Family studies

Familial occurrence of ARM was first described in 1875.[2] About 100 years later several more reports of ARM in families appeared.[4,13–15] In a recent study of a family with 10 affected members a locus for AMD was found on

Table 1. *Stratification of ARM in exclusive stages of ascending risk of developing AMD*

Stage of ARM	Stage of ARM	Criteria
No ARM		No ARM features or only drusen $\leq$ 63 μm
Early ARM	Stage 1 (a)	Soft distinct drusen only
	(b)	Pigmentary irregularities only
	Stage 2 (a)	Soft indistinct drusen or reticular drusen
	(b)	Soft distinct drusen with pigmentary irregularities
	Stage 3	Soft indistinct or reticular drusen with pigmentary irregularities
Late ARM, equal to AMD	Stage 4	Dry (= atrophic = geographic atrophy) or wet (= neovascular = disciform) AMD

chromosome 1q25-31.[16] This seems to be at present one of the best pieces of evidence for the heredity of AMD. Several more families with ARM were described in which a wide spectrum of fundus features of ARM was found within a single family.[17] Recently we identified a large family with 9 members with ARM and 13 with AMD in a genetically isolated population.[18] Thus family studies also provide evidence for a genetic component in ARM.

Clinical association studies

The first study to compare data on the history of AMD in relatives of affected subjects and those of a control population was published in 1983.[19] A positive family history was found in 21.6% of affected subjects versus 8.6% of controls. The value of this study was that it was the first of its kind; later, however, the feeling that history data are less reliable was substantiated.[20] Recently another study found an increased risk of ARM related to a positive family history.[21] The flaw in using family history data without checking them is probably also the reason why in two clinic-based studies the familial risk of ARM varied from 19.3[22] to 2.4.[23] The first study[22] compared first degree relatives of 36 patients with AMD with those of 36 patients having a cataract extraction. In the second study[23] records of first degree relatives of 119 cases with early or late ARM were compared with those of 72 control subjects. The discrepancy in odds ratios between these two studies may be explained by differences in the examination and classification methods, by the use of non-verified data on family history, self-reported diagnoses and data from non-standardized medical charts, as well as by possible bias due to the fact that the studies were clinic-based rather than population-based.

Population-based case–control study

One learns from those leading the way and thus we designed a study to overcome some of the pitfalls mentioned in the previous section.[24] All first degree relatives of 87 participating (response rate 86.1%) patients with AMD from the population-based Rotterdam Study were compared with all first degree relatives of 135 participating (response rate 87.7%) control subjects from the same population. In the sibs and children of these people the participation rates varied between 80% and 88%.[24] All participating subjects were examined using the same standardized

examination protocol of the Rotterdam Study,[24] including grading on fundus colour transparencies according to the International Classification System.[3] Thus the odds ratio for early ARM (Table 1) in first degree family members of ARM cases was found to be 4.8 (95% confidence interval (CI) 1.8–12.2) and for AMD 19.8 (95% CI 3.1–126). The life-time risk for AMD was estimated to be 50% (95% CI 26–73) for relatives of cases versus 12% (95% CI 2–16) for those of controls, a risk ratio of 4.2. The population risk attributable to genetic factors was calculated to be 23%.[24] Together with the risk factors that became known in the meantime from epidemiological studies, smoking being a major and consistent one, this means that ARM is most probably a multifactorial disorder. In a later study this overall risk for relatives of AMD cases was differentiated and found to vary remarkably among families of subjects having AMD.[25] About 70% of those families had no increased risk and at the other end of the distribution a few per cent of the families had odds ratios for developing AMD of up to 30, with quite wide confidence intervals.[25] At present we are trying to fine-tune these findings by adjusting for the size of the families.

Racial studies

Most early reports on racial differences in the prevalence of ARM indicated a higher prevalence in Caucasians.[26,27] As those studies did not examine a large number of Caucasians at the same time in the same geographic area, one never can be sure whether the differences really were racial or environmental. Similarly, the notion that the prevalence of ARM is rising steeply in Japan cannot be substantiated as long as there are no good baseline data available. However, of late several new studies[28–31] that overcame these problems showed that there are racial differences in ARM, if only in terms of varying expression in different age strata. Thus blacks seem to have early ARM at an earlier age than Caucasians[30] but a lower prevalence of AMD.[28]

There are also racial differences in refraction: for example, myopia is more common in Asian than in Caucasian populations. Myopia is in itself a multifactorial and complex anomaly of refraction. Nevertheless it is intriguing why hypermetropia seems to be associated with ARM,[32] although this did not reach significance in a few studies[33,34] or even was protective in others.[35] Also from our clinical impression ARM, and especially early ARM with many or confluent drusen, is rarer in myopic eyes. We might hypothesise that this is

due to a thinner retinal pigment epithelium, choriocapillaris, choroid, sclera (or all four of them) in myopic eyes compared with hypermetropic eyes. Less disciform AMD in myopic eyes might partly be due to confounding because one would be inclined not to classify these as AMD according to the International Classification System[3] but as a Fuchs' spot or disciform reaction due to myopia.

Genetic analytical methods for studying ARM

Apart from the type of studies mentioned above, to which we will refer in the following sections, we would like to mention a few different approaches to genetic studies depending on what kind of patient material one has. A clinician studying genetics of ARM may have at her or his disposal isolated patients with ARM, single sib pairs, extended sib pairs with three to five sibs, monozygotic or dizygotic twins, multigeneration families, clinic-based groups of isolated cases and controls, and similar cases and controls derived from general or genetically isolated populations. Depending on the available patient material there are often several options for studying the genetics of ARM. In each approach one often also has to make assumptions about the mode of inheritance or about ARM being a monogenic, oligogenic or complex disorder. Usually one has to carry out multiple analyses for different hypotheses about the mode of inheritance. If this mode is unknown we will see below that one can perform a segregation analysis to see what models best fit the available data. Given the late expression of ARM it will be hard to find large multigeneration families with this disorder and thus most studies have looked at unrelated cases and controls, mostly from clinics. In such a case–control setting association studies using a candidate gene approach are indicated. If one nevertheless has at one's disposal one or more multigeneration families segregating ARM, linkage analysis is indicated followed by positional cloning of the disease gene. One should be aware, however, that ARM seems to be only partly genetically determined, multifactorial, and not a monogenic disorder. This will complicate linkage analysis. In the following sections we will mention some of the studies on ARM performed so far.

Segregation analysis

'Segregation' in general terms means separation, but in genetics it is used in a special way: segregation analysis statistically tests genetic models on available data. In essence one examines various models for the best fit. In the first segregation analysis on AMD in the Beaver Dam Eye Study four models – three of dominant Mendelian inheritance and one general model – were used.[36] The authors came to the conclusion that their data on 546 sibships best fitted an autosomal dominant inheritance model in which a single major gene could explain between 59% and 62% of the variability in ARM. A major random environmental factor was improbable. The

number of genes involved could not be predicted.[36] We think at present, given the locus found on chromosome 1q,[16] the small number of ABCR mutations found in ARM[37] and our own data, that it is hardly probable this model[36] still holds.

Association studies

In genetic association studies one compares unrelated cases with a disorder with unrelated controls, from either a clinic or a general population, in order to test the contribution of genetic factors in known candidate genes to a disease. General population means here also not derived from a genetic isolate. One assumes that the variables in the population leading to the associations are dependent on each other, leading to a so-called non-parametric test. Association studies are often performed in multifactorial disorders. In order to perform an association study one mostly needs large numbers of well-defined cases and controls. Because AMD is a diagnosis by exclusion of other disorders, such as disciform macular degeneration due to myopia, trauma or inflammation, there is always a danger of confounders such as mixing similar phenotypes with different genotypes. This could be one of the reasons for the controversy that arose around the role in AMD of mutations in the ABCR gene, known to be involved in Stargardt's disease.[14,37–41] It is beyond the scope of this review to go into much detail here. In essence the general feeling at present seems to be that, due to the classification of ARM in the cases, the method of selecting controls, selective pooling of alleles and the statistics used, the role of ABCR in AMD is much smaller than originally assumed.[37] In this last publication, finally, only two ABCR mutations were tested. It is to be expected that in the near future more clarity will be obtained in this matter.

As apolipoprotein E (apoE) polymorphism plays a role in Alzheimer's disease, the ε4 allele leading to an increased risk, we hypothesised that it would also lead to increased ARM. In an associated study between 88 AMD cases and 901 controls from the population of the Rotterdam Study, we found an inverse association. Subjects with the apo ε4 allele had a decreased risk of AMD with an odds ratio of 0.43 (95% CI 0.21–0.88) and the risk in those with the ε2 allele was increased (OR 1.5; 95% CI 0.8–2.8).[42]

Allele sharing studies

Allele sharing methods are mostly used in related cases, i.e. sib pairs or large families with a multifactorial disease, or in genetically isolated populations. In these populations mostly a combination of linkage analysis and association study is used. So far no ARM study on a large number of sib pairs has appeared. This is probably due partly to the limited number of sib pairs one may find in such an age-related disorder and to the difficulty of the sib pair approach in a multifactorial disorder. Thus

for a successful sib pair study we would estimate that one needs 500 to 1000 sib pairs that, of course, would have to be well documented.

Studies using linkage analysis

Linkage analysis is mostly used in families with a clear inheritance pattern, although this latter may be also only assumed. Finding large families with more than 10–15 affected members is hampered by the late onset of the disease, leading to a paucity of surviving parents and uncertainty about the genetic status of the children. One large family published so far led to a locus on chromosome 1q25-31.[16] In another family no mutation in the *TIMP-3* gene (responsible for Sorsby's fundus dystrophy)[43] was detected. Nor could mutations in the *ABCR* gene be found in 38 ARM families.[15] In the family we have studied, so far no known mutations of candidate genes have been encountered, also suggesting that ARM is a multifactorial disorder.[18]

Candidate gene approach

Mention should be made of the candidate gene approach and new methods of identifying retina (ARM)-specific genes.

We have seen that candidate gene approaches have been widely used in association with positional cloning studies. So far this approach has been of limited value in finding genes for ARM, perhaps because most candidate genes have been related to monogenic disorders and ARM most likely is a multifactorial disease. One can find nearly every month new candidate genes on several databases on the internet,[44] among others on http://www.sph.uth.tmc.edu/retnet, and in addition more and more genes from other species become available. Given the speed with which new data on the human genome are becoming available, it is to be hoped that the candidate gene approach may contribute more to the ARM genetic solution.

There are several new methods of finding retina-specific genes, such as differential hybridisation, cDNA library subtraction and clone sequencing, and computer-assisted collection of expressed sequence tags (ESTs). By checking ESTs preferentially expressed in the human retina one can try to find new candidate genes for AMD.[45] The majority of the genes involved in single-gene retinal disorders are expressed only in the eye. There is a redundancy of ESTs and by testing the *in vitro* expression profile of ESTs from eye cDNA libraries 39 genes with specific transcription in the human retina were found.[45] This seems to be a very promising tool for finding new genes that might also be involved with ARM. Finally, multi-array systems for specific analysis of up- or down-regulated genes in the ageing retina and in the retina affected by ARM become more and more suitable for research in ARM, so that we expect that in the coming years genetic research in ARM will advance at a dazzling speed compared with the past 200 years.

Once genes for ARM have been found, functional studies on them will have to prove the genetic basis for ARM. It might be a problem, however, to find a suitable animal to study the phenotype. So far it is uncertain whether one could elicit ARM, or even more AMD, in, for example, mice, which have no fovea. The future will have to tell us whether the zebra fish will be a good model for ARM.

Conclusions

There is sufficient evidence that ARM is a partly genetically determined, multifactorial disorder. The best pieces of evidence so far are the locus on chromosome 1q and the two *ABCR* gene mutations described. The candidate gene approach to ARM is hampered by ARM being a multifactorial disorder. However, the availability of the human genome sequence will enhance the possible use of candidate genes. It is further to be expected that complementary techniques such as the use of micro-arrays, comparative gene mapping in different species, and *in silico* cloning (obtaining computer data from large gene bases) will speed up the search for ARM genes.

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