Genetic epidemiologic studies on age-related maculopathy

A population-based approach

Caroline C.W. Klaver

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A population-based approach

Genetisch epidemiologische studies over leeftijds-gebonden maculopathie

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Publications and manuscripts based on the studies described in this thesis

Chapter 2

Klaver CCW, Vingerling JR, Hofman A, de Jong PTVM. *Altersabhängige Makuladegeneration.* Kapitel 1. Epidemiologie. Springer-Verlag Berlin Heidelberg 1997: 1-20.

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Chapter 3

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Chapter 4

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Chapter 6

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Contents

Abbreviations

ABCR	ATP-binding cassette transporter gene
AD	Alzheimer's disease
AMD	Age-related macular degeneration
Ape	Attributable proportion in the exposed
APOE	Apolipoprotein E (gene)
App	Attributable proportion in the population
ApoE	Apolipoprotein E (protein)
ARM	Age-related maculopathy
CI	Cumulative incidence
95% CI	95% Confidence interval
CNV	Choroidal neovascularization
IR	Incidence rate
OR	Odds ratio
RPE	Retinal pigment epithelium
RR	Relative risk

Part I

Background

Chapter

1

Aims of this thesis

The western world is aging rapidly. In the Netherlands, the current mean life expectancy for men and women is 74.6 and 80.4 years, respectively, and those over 65 years of age comprise 13.6% of the total population.[1] This proportion of elderly is expected to increase considerably within the coming years, and this will lead to higher frequencies of diseases. Age-related maculopathy (ARM) is one of those frequent geriatric diseases. It is an eye disease ultimately leading to blindness. The prevalence of the clinical end stages of this disorder range from 1% in those aged 60 years of age to 10% in those aged 85 years and older. At least 60000 Dutch subjects[2] are severely affected by these end stages, also called age-related macular degeneration (AMD). AMD has a great impact on visual function and the performance of daily tasks, in particular because there are still no means for long term restoration of vision.

During the last decade there has been steadily increasing research activity investigating the disease etiology. It became better known that the pathogenesis was complex with a variety of risk factors involved. Family reports and twin studies pointed to a genetic background, and epidemiologic studies suggested environmental influences from vascular and dietary factors, sunlight and smoking. However, findings were not unequivocal, and the evidence on most of these relations was insufficient and inconclusive. This called for more extensive research into the causes of ARM.

This thesis aimed to answer the following questions:

Part I: What is the current genetic epidemiologic knowledge on ARM?

Part II: What is the incidence of AMD, what is the natural course of the disease, and what is the relation with visual impairment?

Part III: To what extent is ARM genetically determined, and which genetic factors may be involved?

Part IV: Are environmental factors important in the pathogenesis, and is ARM associated with other disorders?

We used a genetic epidemiologic approach to investigate these issues. All studies were based on the Rotterdam Study, a prospective population-based cohort study of subjects aged 55 years and over taking place in Ommoord, a suburb of Rotterdam. The baseline phase of this study was conducted from 1989 to 1993; the first follow up from 1993 to 1994. A family study originating from the Rotterdam Study was conducted from 1994 to 1996.

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Chapter

2

Epidemiology of age-related maculopathy
A review

INTRODUCTION

The late stage of age-related maculopathy, also referred to as age-related macular degeneration, is the leading cause of permanent visual impairment among the elderly in western countries.[1-4] The loss of vision is a result of degeneration of the photoreceptors in the macular area, which occurs when the retinal pigment epithelium cells with which they are associated deteriorate and die. Useful intervention is limited to only a minority of patients.[5,6] Since the previous reviews concerning the epidemiology of ARM by Ferris in 1983,[7] and by ourselves in 1995,[8] many more investigations have focussed on this disease in an attempt to find etiological clues. This chapter is based on our initial review, but also contains an update of the literature that has appeared since its publication in 1995. We will review the current epidemiological knowledge concerning ARM and discuss diagnosis, frequency, risk factors and prognosis.

DIAGNOSIS

Diagnostic criteria

Age-related maculopathy affects the center of the retina and choroid in the posterior pole of the eye. Generally, it is considered to be present when one or more of the following changes are visible in the macular area:

- large drusen: yellow deposits below the retinal pigment epithelial cells;
- hyper- and hypopigmentary changes of the retinal pigment epithelium;
- atrophic AMD, also known as geographic atrophy: well defined areas of atrophy of the retinal pigment epithelium and choriocapillaris;

- neovascular AMD: serous or hemorrhagic detachment of the pigment epithelium, choroidal neovascularization and subsequent scarring of the macular area.

Although these changes are all manifestations of the disease and associated with increasing age, they show a large range of variety, and for years this has been an obstacle for a uniform definition and classification system. Early epidemiological studies have included decreased central visual acuity as one of the diagnostic criteria. Recently, however, three grading systems have been developed to classify ARM on color photographs of the macula lutea without implication of visual acuity.[9-11] The definitions of these grading systems are summarized in Table 1. In brief, the system of Bressler et al. consists of four categories, and at each step from category one to four, the system leaves out less severe abnormalities. The Wisconsin Age-Related Maculopathy Grading System provides a detailed grading of each abnormality with respect to its size, area and location. It defines early and late stages of ARM.[12] Presently, an international study group has developed a classification system to facilitate comparison of data between the various epidemiological studies. This system defines ARM as all manifestations of this disorder and AMD as the late stages: atrophic or neovascular macular degeneration. For the purpose of this review and this thesis, we will maintain the terminology of the International System.

Differential diagnosis

Drusen must be differentiated from other conditions with white spots in the macula like hard exudates, cotton wool spots and retinal pigment epithelium hypopigmentations such as in fundus flavimaculatus and fundus albipunctatus. Pigmentary changes can also be seen in combination with other abnormal processes in the macular area which are not directly related to ARM, like those accompanying chorioretinal scars due to chorioretinitis, trauma or laser photocoagulation.

Any chorioretinal inflammation or scar may result in the growth of a subretinal neovascular membrane. Therefore, neovascular AMD sometimes resembles similar conditions in myopic macular degeneration, pseudoxanthoma elasticum, Paget's disease, presumed ocular histoplasmosis syndrome, toxoplasmosis, central areolar choroidal sclerosis,[13, 14] laser photocoagulation scars and traumatic, inflammatory, toxic, and congenital processes.[10] In general, these disorders must be excluded before a diagnosis of ARM can be made.

FREQUENCY

Prevalence

Estimation of the occurrence of ARM is not only necessary for assessing the need for ophthalmological care, but comparison of frequency figures from different populations may also suggest etiological clues to the disease. Population-based studies on the prevalence of ARM were conducted in the United States, Europe, Australia, and New Zealand (Table 2). Studies from Framingham,[1] Gisborne,[15] Melton Mowbray,[16] Copenhagen,[17] the National Health and Nutrition Examination Survey (NHANES)[18] and Iceland[19] estimated the prevalence of any type of ARM based on ophthalmoscopical assessment of macular changes with the requirement of central visual loss. As is shown in Figure 1, the prevalence estimates in these studies vary considerably. The studies from Chesapeake Bay,[9] Beaver Dam,[12] and Blue Mountain [21] based their data on photographic grading of macular changes and did not require visual loss, which may explain why their prevalence estimates for any type of ARM are higher (Figure 1). Whatever definition or method of diagnosis, all estimates show a strong rise with age, and a reasonable overall prevalence for any type of ARM in the age-groups 65-74 years and 75-84 years is 20 and 35 percent, respectively.

Separate prevalence estimates of atrophic or neovascular AMD are available from the studies in Framingham, Iceland, Chesapeake Bay, Beaver Dam, Rotterdam,[20] Blue Mountain,[21] Colorado,[22] and Southern Italy[23] (Figure 2). The first two studies based their estimates only on neovascular AMD, while the latter also included atrophic AMD. These prevalence estimates show less variation than with inclusion of drusen and pigmentary changes, and the estimates show an exponential increase after the age of 70 years. A reasonable overall prevalence of neovascular and/or atrophic AMD in the age-groups 65-74 years and 75-84 years is 1 and 5 percent, respectively. Although no other studies showed any prevalence difference in gender, the Blue Mountain Eye Study[21] and the Beaver Dam Eye Study[12] noted that women had a higher prevalence of AMD than men.

Incidence

Recently, two population-based studies reported data on the incidence of ARM. The Chesapeake Bay Waterman Study [24] estimated a 5-year cumulative incidence of neovascular macular degeneration of 2% (one of 50 participants) in men over 70 years of age, while none had developed atrophic macular degeneration during this follow up time. The Beaver Dam Study[25] also reported the 5-year incidence of ARM and macular

degeneration; in subjects aged 65-74 years the 5-year cumulative incidence of atrophic or neovascular AMD was 1.3%, while in subjects over 75 years the incidence of these late stages was 5.4%. As in their prevalence study, they reported that AMD developed more often in women than in men, and the ratio neovascular AMD versus atrophic AMD was 2:1. Other population-based studies are currently performing follow up studies and their incidence data are expected in the coming years.

Table 1. Classification of ARM

Bressler, et al.[9]	Wisconsin Age-related Maculopathy Grading System.[10]	The International Age-Related Maculopathy Study Group.[11]
Grade 1: Presence of grade 4, 3 or 2, or eyes with at least five small drusen within 1,500 μm of the foveal center or at least ten small drusen between 1,500 and 3,000 μm from the foveal center.	Early ARM: Soft indistinct or reticular drusen or any soft or reticular drusen with retinal pigment epithelium degeneration or increased retinal pigment in the macular area and the absence of late ARM.	ARM: All features of the disease excluding hard drusen. Drusen and pigmentary changes are characterized by type, number, size, and area.
Grade 2: Presence of grade 4 or 3, or eyes with ≥ 20 small drusen within 1,500 μm of the foveal center.		
Grade 3: Presence of grade 4, or eyes with large or confluent drusen, or eyes with focal hyperpigmentation of the retinal pigment epithelium.		
Grade 4: Geographic atrophy of the retinal pigment epithelium or exudative changes (eg. choroidal neovascularization, detachment of the retinal pigment epithelium, and disciform scarring).	Late ARM: Signs of exudative AMD or geographic atrophy.	AMD: the end stages of ARM subdivided in atrophic AMD, i.e., geographic atrophy, and neovascular (exudative) AMD.

Table 2. Population-based cohort studies of ARM

Site (reference no.)	Criteria for diagnosis	Age range (yr)	Sample size	Response rate
Framingham (1)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	52 - 84	2675	67
NHANES (18)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	45 - 74	1413	72
Gisborn (15)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	65 +	481	82
Melton Mowbray (16)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	75 +	484	72
Iceland (19)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	43 +	751	81
Copenhagen (17)	Ophthalmoscopy: drusen; pigment disturbances; atrophic or neovascular macular degeneration, visual acuity	60 - 79	1000	71
Chesapeake Bay (9)	Photography: drusen, number, size and distinction of borders; focal hyperpigmentations; non-geographic atrophy; atrophic or neovascular macular degeneration.	30 - 95	777	70
Beaver Dam (12)	Photography: drusen area, number, size and distinction of borders; increased or decreased retinal pigment; atrophic or neovascular macular degeneration.	43 - 84	4926	83
Rotterdam (20)	Photography: drusen number and size; increased or decreased retinal pigment; atrophic or neovascular macular degeneration.	55 +	7583	78
Blue Mountain (21)	Photography: drusen area, number, size and distinction of borders; increased or decreased retinal pigment; atrophic or neovascular macular degeneration.	49 +	3654	82

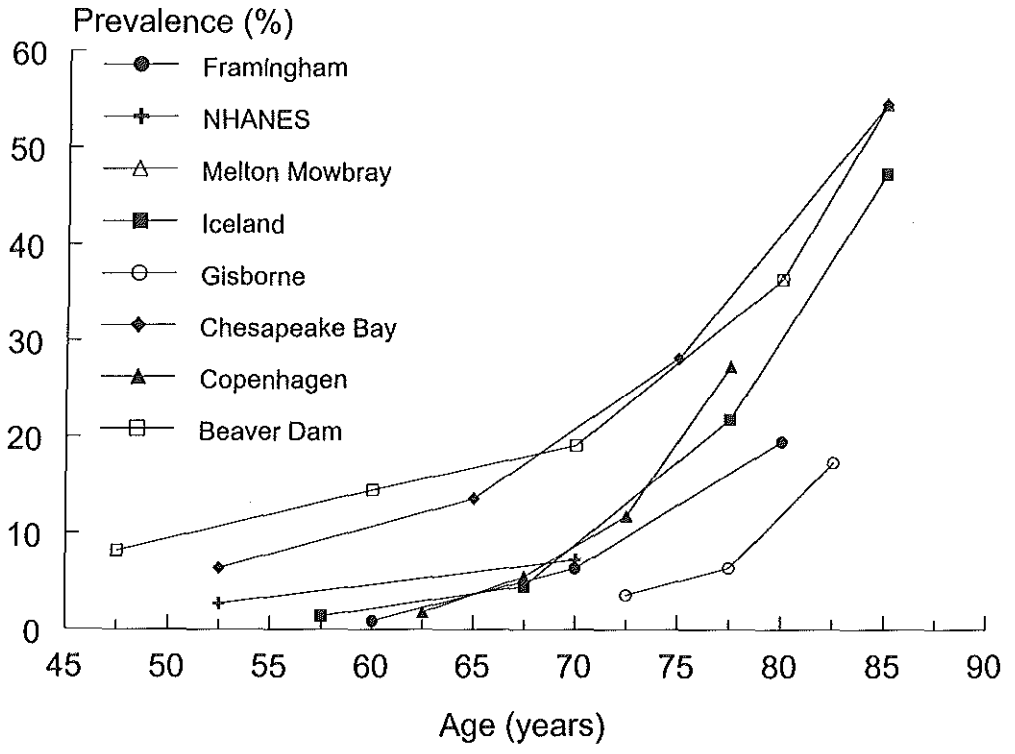

Figure 1. ***Age-specific prevalence of AMD (all types)***

Methodological considerations

The difference in definitions and methodology between studies hampers the comparison of prevalence data. In the studies from Framingham, NHANES, Gisborne, Iceland and Copenhagen, the diagnosis of ARM was only made in patients with central visual loss. This led to lower prevalence rates than the estimates from Chesapeake Bay, Beaver Dam, Rotterdam, and Blue Mountain, which did not use this criterium. In addition, the former studies based the diagnosis on clinical examination, whereas the latter based their grading on fundus photographs. It is known that the frequency of drusen is generally underestimated with clinical ophthalmoscopy, which may be an extra reason for the higher prevalence of ARM in these studies. The Chesapeake Bay Waterman Study was designed to study the relation between sunlight exposure and eye diseases. The study population consisted of a selected group of fishermen and this may have influenced the prevalence rate. Despite these differences, however, there was a similarity in trends: all studies showed a rise of prevalence with increasing age (Figure 1).

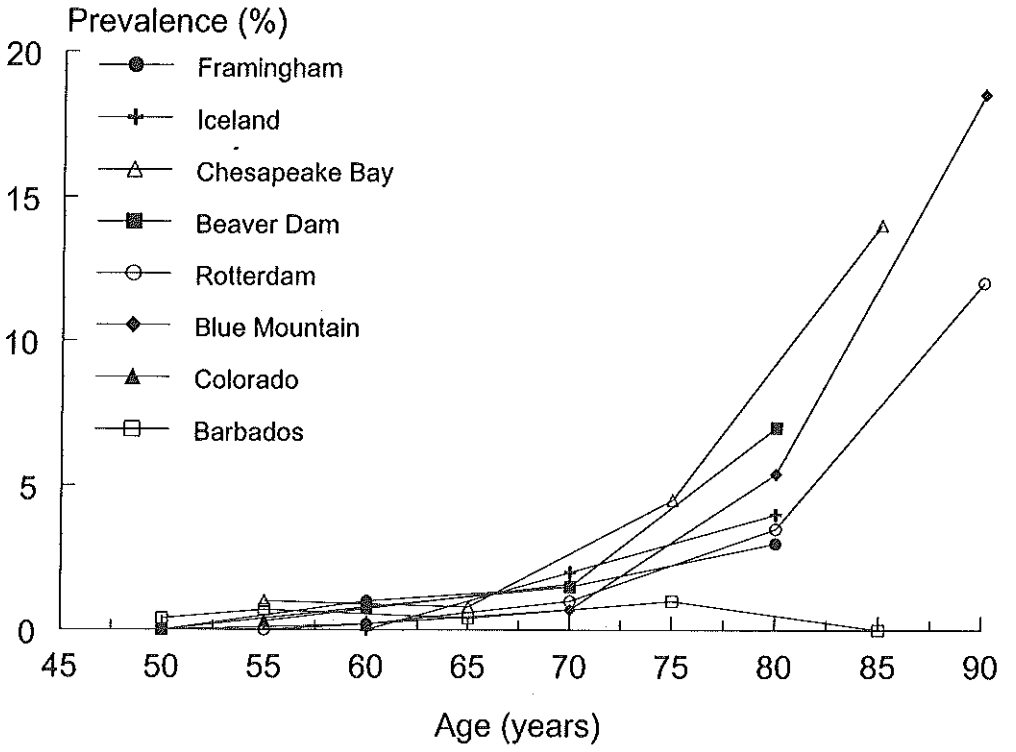

Figure 2. ***Age-specific prevalence of AMD (geographic atrophy or neovascular macular degeneration)***

The comparison of the occurrence of endstages of ARM is also hampered by differences. In the studies from Framingham and Iceland, only the prevalence of neovascular AMD was reported. Atrophic AMD was pooled with drusen and pigmentary changes in these studies. In Chesapeake Bay, Beaver Dam, Rotterdam, and Blue Mountain, atrophic and neovascular AMD were pooled resulting in a higher estimate of prevalence. The differences between prevalences in these studies are therefore likely to be the result of differences in methodology and definition.

RISK FACTORS

A number of case-control and cross-sectional studies has focussed on the etiology of ARM. The main findings are summarized in Tables 2, 3 and 4. A point of

consideration is that most results were based on prevalent cases, having its well-known limitations like selection bias and recall bias which may lead to spurious associations.[26] In addition, the inclusion criteria for cases varied a great deal in the studies. Most studies included early and late stages of ARM. The results of these studies remain, therefore, to be confirmed in follow-up studies based on well-defined incident cases in which the exposure status is measured before onset of disease.

The putative risk factors that will be discussed are family history and genetic factors, ophthalmological characteristics, cardiovascular disease, and environmental exposures.

Genetic factors

Family studies

While Hutchinson and Tay have observed familial occurrence of ARM as early as 1875,[27] the disease has only recently become the subject of extensive genetic investigations. Familial aggregation of drusen has been reported by several studies. Early pedigree reports [13,28-30] have used different terms to describe the familial drusen such as Doyne's honeycomb choroiditis, Tay's central guttate choroiditis, Holthouse-Batten's superficial choroiditis and Mallatia Levantinese. An autosomal dominant trait was suggested for these familial drusen, and they were considered to be different from drusen occurring as a consequence of age.[31] However, Gass postulated in 1973[13] that there was only one entity of drusen, all being manifestations of a heredodegenerative disorder. Evidence for a familial predisposition of drusen was found by a study which compared 53 sibling-pairs and 50 spouse-pairs for concordance of drusen.[32] The investigators found a significantly higher correlation of number and density of drusen between siblings than between spouses.

Family studies incorporating the late stages of ARM have received comparatively less attention. Two studies found a positive family history of macular disease in patients with drusen or pigmentary changes and/or atrophic and neovascular macular degeneration.[32,33] Because the majority of these family data was ascertained by interview alone, they should be interpreted with caution, since anamnestic history of an eye disease is unreliable.[34] Observations in twins led to three case-reports [35-37] of identical twins having atrophic and/or neovascular macular degeneration and a study of nine monozygotic twin pairs[38] having a high concordance in either extensive drusen or AMD. Selection bias or environmental factors may have played a role, but the striking similarity of fundus appearances in each twin pair suggests an increased genetic susceptibility in at least some of the patients. In a more extensive twin study, Meyers et al.[39] compared concordance in presence or absence of any type of ARM in

98 monozygotic and 38 dizygotic twin pairs, who were predominantly ascertained through twin organizations. Of the affected twin pairs, all (25/25) monozygotic twin pairs were concordant, while 42% (5/12) of dizygotic pairs were concordant. The numbers of affected twins in this study was low and skewed towards the monozygotic ones, therefore, the interpretation of these data should be somewhat conservative. More twin studies are currently underway.

Mode of inheritance was investigated by a segregation analysis of data from the population-based Beaver Dam study.[40] This study included 546 sibships with at least two members, and sibling correlations were calculated for age-dependent maculopathy scores. The authors concluded that a single major gene could account for ~55% of the total variability in ARM. A point of consideration in the interpretation of these data is that the genetic models which were compared were three variations of Mendelian dominant inheritance and one more general transmission model. Theoretically, there are numerous possibilities of segregation in complex genetic disorders, and the choice of Mendelian models to test for in such an analysis is arbitrary.

Silvestri [41] was the first to study familial aggregation of ARM in a case-control setting, cases being siblings of 36 patients with 'dry' and exudative AMD, and controls being siblings of 36 patients undergoing a cataract operation. In this study, the estimated odds ratio for first degree relatives to develop any type of ARM was estimated to be 19.3. In another clinic-based familial aggregation study, Seddon et al.[42] compared the medical records of first degree relatives of 119 probands with extensive drusen or atrophic or neovascular macular degeneration with the records of first degree relatives of 72 control probands. This study estimated a much lower odds ratio of 2.4 (95% CI 1.2, 4.7) for first degree relatives. Possible explanations for this large difference in familial risk are the high chance of selection bias with hospital-derived probands, the large range of ARM features which were combined, and the use of family history, medical charts, and self-report for diagnosis.

Thusfar, only one study investigated the phenotypic variation in affected families. De la Paz [43] examined the appearance of the macula in eight families with multiple affected individuals, and found a broad spectrum of fundus features within each of these families. This implies that genotype-phenotype correlations in ARM may be rather low.

Table 3. Case-control studies of ARM

Site (reference no.)	Fundus abnormalities		Grading method	Age range (yr)	Sample size (case/control)
	Cases	Controls			
Malzman (68)	Age-related maculopathy, not specified, visual acuity < 20/30	Age and sex matched ophthalmological patients without age-related maculopathy	Ophthalmoscopy	52 - 88	30/30
Delaney (80)	Drusen, pigment clumping, neovascular macular degeneration, visual acuity <20/30	Age and sex matched ophthalmological patients without age-related maculopathy	Ophthalmoscopy	50 +	50/50
Hyman (33)	Drusen and/or more severe macular degeneration with some visual loss	Age and sex matched ophthalmological patients without age-related maculopathy or other neovascular retinal diseases	Photography	< 85	228/237
Blumenkranz (82)	Neovascular macular degeneration	15 partners and 8 age and sex matched others without neovascular macular degeneration or more than 10 macular drusen	Not specified	Not specified	26/23
EDCCSG (65)	Neovascular macular degeneration	Residents of clinic area without neovascular macular degeneration or drusen and 6/6 visual acuity	Photography	55 - 80	421/615

Table 4. Risk factors for ARM

Study (reference no.)	Risk factor *					
	Hyperopia	Blue/light iris color	Cardiovascular disease	Hypertension	Smoking	Sunlight exposure
Framingham (83)			0	1.4 (1.1-1.7)		
Malzman (68)	0		NS	1.3 (NS)	NS	
Delaney (80)	0	2.4 (1.0-5.9)		6.1 (2.1-18.5)		
Hyman (33)	0	3.5 (1.7-6.6)	1.7 (1.1-2.7)		1.2 (0.8-1.9)	
Blumenkranz (82)	2.0 (0.5-6.2)	0.7 (0.2-2.3)	4.0 (0.4-102)	0.6 (0.1-4.7)	1.3 (0.3-4.4)	
Chesapeake Bay (63,128)		1.1 (0.6-2.0)			0.6 (0.4-1.1)	1.4 (1.0-1.9)‡
Copenhagen (17,64)		0.6 (0.3-1.1)	1	0.8 (NS)	2.4 ($p<0.01$)	
Beaver Dam (85,116,129)	0.2 (0.1-0.8)	0.6 (0.3-1.3)	0.7 (0.3-1.4)	0.8 (0.1-5.5)	2.5 (1.0-6.2)§	2.2 (1.1-4.3)‖
EDCCSG (65)	1.7 (1.1-2.6)	1.1 (0.7-1.7)	1.1 (0.8-1.5)	NS	2.8 (1.8-4.2)	1.1 (0.7-1.7)‖
Rotterdam (86,103,117)	1.9 (0.5-5.7)	0.6 (0.3-1.1)	2.5 (1.4-4.5)¶	0.9 (0.6-1.4)	2.6 (1.5-4.8)	
Blue Mountain (70,118,130)	0.5 (0.2-1.5)	1.7 (1.0-2.9)	1.4 (0.7-2.9)	1.1 (0.6-1.8)	5.4 (2.4-12.4)	

* Odds ratio's with 95% confidence intervals in parentheses.

NS: Not Significant, but point estimate and confidence intervals unpublished.

‡ Blue light.

§ Women.

‖ Leisure time in summer.

¶ Atherosclerotic plaques in common carotid artery.

Racial variation

Difference in genetic susceptibility explains part of the racial disparities in the frequency of a disease. For years, the clinical impression has been that ARM is rare in blacks. Only few studies have focussed on this issue. Gregor et al.[44] compared frequency of ARM between 1000 blacks in South Africa and 380 caucasians in England. All were 'consecutive' hospital outpatients. Blacks had a significantly lower frequency of the late stages of ARM (0.1% compared to 3.5% among the caucasians). Later, Taylor[45] reported that ARM was a rare cause of blindness in elderly Australian Aborigines. A recent study carried out in Barbados among 3444 blacks found a prevalence of AMD of 0.6%.[46] Although prevalence was much lower in these blacks than in caucasians from Western countries, these studies did not examine a considerable number of caucasians from the same area. Therefore, environmental differences could have affected the results. Data from the third NHANES survey[47], which represents the various populations living in the U.S., showed that racial differences vary by age. Compared to blacks, caucasians more often developed ARM in the age-category over 60 years, but less often in the younger age-categories, giving the impression that blacks have an earlier onset. On the other hand, the Baltimore Eye Survey[48] compared causes of blindness among 2395 blacks and 2913 caucasians and found that blindness due to AMD only occurred in Caucasians. The Colorado-Wisconsin Study[22] also found the late stages to be extremely rare among Hispanic Americans, and the prevalence difference with Caucasians did not change after controlling for known risk factors as smoking and cardiovascular disease. All studies suggest a racial variation of the frequency of age-related maculopathy, but especially the latter study favours a racial difference in genetic susceptibility.

Genes and loci

ARM research has only recently focussed on molecular genetics with the aim to map this disease, to identify genetic forms of the disease, and to create etiologic insights. Until now, there have been very few positive results. The first important candidate gene that was investigated is the tissue inhibitor of metalloproteinases-3 gene (TIMP-3) on chromosome 22, which was identified in autosomal dominant Sorsby's fundus dystrophy, a dystrophy that phenotypically resembles AMD.[49] De la Paz[50] studied TIMP-3 in 38 multiplex families with extensive intermediate drusen, large drusen, geographic atrophy or evidence of exudative AMD, and excluded any linkage with ARM within an area of 10 cM around TIMP-3. Another candidate gene was the photoreceptor cell-specific ATP-binding transporter gene (ABCR), located on chromosome 1p21, which is involved in Stargardt disease (STGD).[51] Allikmets et al.

suggested that this gene was implicated in ARM [52] after observing that certain variations of ABCR were more frequent in 167 patients than in 220 controls. The findings appeared to be controversial: there were critical comments regarding the classification of ARM, the selection of controls, and the statistical methodology.[53, 54] Stone et al.[55] did find allelic variation of ABCR in Stargardt disease, but he could not confirm an association with ARM. To fully comprehend the relevance of the ABCR gene for the etiology of ARM, larger studies are needed in well-defined patients and controls.

Recently, Klein et al.[56] identified a large family in which ARM was segregating as an autosomal dominant trait. In the 10 affected family members ARM was expressed by the presence of large, soft, confluent drusen accompanied by varying degrees of retinal pigment epithelial degeneration and/or geographic atrophy. In this family, the disease locus was mapped to chromosome 1q25-q31.

Ocular risk factors

Iris color

A protective effect of a dark pigmented iris on age-related maculopathy has been suggested after a low prevalence of the disease was reported in black Africans.[44] Cumulative light exposure may have a harmful effect on the photoreceptors and retinal pigment epithelium.[57-61] A dark iris is possibly protecting the retina better against light exposure than a light iris. Initially, two case-control studies reported a protective effect of a dark iris for ARM,[33, 62] but this was not confirmed in later studies.[63-65] Holz et al.[66] reported no association with light iris color, but mentioned that self-reported decrease of iris pigmentation during life was associated with ARM. The inconsistency of data and the absence of an association in the population-based studies suggests a small effect, if any. Sandberg et al.[67] reported that light iris pigmentation is associated with a more extensive disease in patients with neovascular macular degeneration. Because referrals are generally related to severity of symptoms, this may explain some of the inconsistent findings of the clinic-based studies on this matter.

Refractive error

A possible association of hyperopia and ARM was first suggested by Maltzman et al.[68] Later, four case-control studies confirmed this finding.[33,62,65,69] Hyman pointed out that selection bias could have influenced that observation,[33] because the control group may have overrepresented myopic subjects. The control group in another study consisted of non-neovascular AMD cases.[69] The authors suggested that this control group may have comprised a larger proportion of cataract patients, which can result in myopia due

to lens swelling and therefore may have led to a spurious association with hyperopia. A recent report from the population-based Blue Mountain Eye Study found a weak relation between hyperopia and early ARM, but not with the late stages.[70] The reference group consisted of population-based emmetropic subjects, so selection bias is not an explanation for these data. A supportive theory of the association between hyperopia and ARM may be that hyperopics have a thicker sclera than emmetropics, which may influence choroidal vascular flow and the supply of nutrients to the retina. However, considering the inconclusive associations, it is still doubtful whether an association between hyperopia and ARM really exists.

Cataract and cataract extraction

The data regarding the association between cataract and ARM have been inconsistent.[71-73] In Beaver Dam, there was an association between presence of nuclear cataract and prevalent early ARM at baseline, but this finding could not be replicated with incident early or late ARM at 5-year follow up.[74] An increased risk of ARM after cataract extraction was first suggested by a histopathological study.[75] This finding was confirmed later by Pollack et al.[76] in a clinic-based setting. He studied 47 patients with bilateral early ARM after cataract extraction on one eye, and found that the operated eye was much more at risk of subretinal neovascularization than the unoperated eye. The 5-year incidence data of Beaver Dam[74] added to the evidence for this relation. In this study, the odds ratio of incident late ARM for operated eyes was 2.8 (95% CI 1.03, 7.63). The finding is of great clinical importance, for it suggests a conservative policy towards subjects with early ARM and cataract. Possible explanations for the association, apart from detection bias, are photic injury during operation, traumatic ruptures in Bruch's membrane, or inflammatory changes after surgery.

Vascular risk factors

One hypothesis for the pathogenesis of ARM is that vascular disease affects the choriocapillaris. This may result in decreased flow or passage of nutrients.[77-79] The issue was examined in various ways, either by investigating cardiovascular history data or by direct measurements.

History of cardiovascular disease

Conflicting reports have been published about the association between ARM and a history of cardiovascular disease: several case-control studies found a positive association,[33,80,81] whereas others did not.[65,68,82] Self reported history of cardiovascular disease, however, is potentially biased by misclassification, making it more difficult

to detect an association.

Hypertension

Sperduto et al. reported a small and consistent association between ARM and hypertension as determined 25 years prior to the diagnosis maculopathy in the Framingham Heart and Eye Study.[83] The association was stronger with increased duration of systemic hypertension. Vinding, however, did not find an association between blood pressure levels and ARM in a four-year follow-up in the Copenhagen Heart Study.[84] Other studies used blood pressure levels taken at the time of eye examination, and reported a positive association with increased systolic blood pressure.[18,65] No associations with blood pressure, hypertension and prevalent ARM were found in Beaver Dam,[85] Rotterdam,[86] Blue Mountains,[87] Colorado,[22] London,[88] or Oulu.[89] Beaver Dam[90] did find an association between uncontrolled hypertension and incident neovascular ARM (OR 2.1, 95% CI 0.5, 8.1). In summary, the association with blood pressure is inconsistent, and as yet unsettled.

Atherosclerosis

In the Rotterdam Study, Vingerling et al.[86] found that plaques in the carotid bifurcation (OR 4.7, 95% CI 1.8, 22.2) and plaques in the common artery (OR 2.5, 95% CI 1.4, 4.5) were associated with AMD in subjects younger than 85 years. Lower extremity disease was associated with an OR 2.5 (95% CI 1.4, 4.5). The Beaver Dam Study[90] found that high pulse pressure, a presumed indicator of atherosclerosis, was associated with a 30% (95% CI 1.02, 1.65 per 10 mmHg) increased 5-year incidence of neovascular ARM. The Atherosclerosis Risk in Communities Study reported that carotid artery plaques and focal retinal arteriolar narrowing were associated with RPE-depigmentation, but not with early or late ARM.[205] More studies are needed to explore this association in detail.

Hyperglycemia and diabetes

Hyperglycemia has been reported to affect the choroidal circulation, Bruch's membrane, and the RPE.[91-96] A relation between hyperglycemia and ARM has therefore been proposed. A number of case-control[33,65,68,81,82] and two population-based studies[97,80] focussed on this hypothesis. Only one study[81] found a positive association of serum glucose levels and the mean area of drusen in females without diabetes. Klein et al.[98] reported no relation between glycosylated hemoglobin and ARM. Only in men with diabetes aged 75 years or over, a higher frequency of neovascular macular degeneration was found. In the 5-year follow up study of Beaver Dam, no association

with incident early ARM was found, and there were not enough cases to study the relation with late ARM.[90] The effect of hyperglycemia, if any, is likely to be small.

Other vascular factors

Histopathological studies have shown that increased neutral fat deposition in Bruch's membrane may be related to increased serum fatty acids and triglycerids.[99] Many studies have investigated a relation between serum lipids and ARM: some find a positive relation with total cholesterol and LDL,[65] some find a positive relation with HDL,[100] but most find no relation.[22,86,87,89,90,101] Furthermore, postmenopausal estrogens were shown to have a dose-related protective effect against neovascular macular degeneration.[65] The role of estrogen could not be confirmed in Beaver Dam; [102]the power to detect an effect was low, however. The issue was also addressed in a nested case-control study in Rotterdam, which suggested a higher risk of AMD in women who had an early menopause by oophorectomy.[103] These results may be explained by the protective effect of estrogens against cardiovascular disease and atherosclerosis.[104-106] Unfortunately, the possibility of selective survival cannot be ruled out in these cross-sectional studies.

Environmental factors

Smoking

An increased risk of ARM in smokers was first suggested by Paetkau et al.[107] The mechanism of the association is still unclear, but several mechanisms could play a role. It is plausible that, by reducing serum antioxidants,[108-111] smoking decreases retinal antioxidants. These are present in the retina to protect it against oxygen radicals formed during light exposure.[112,113] Several other pathways could be involved in the association, including alteration of the choroidal blood flow.[114,115] The first reports that found a positive association were based on prevalent cases, [33,65,84,117,118] and not all findings were convincing.[63,97] The association was particularly present in neovascular AMD,[116] but seemed to be restricted to relatively young cases.[117] Interestingly, two studies found that the greater the non-smoking period after cessation, the lower the risk for former smokers.[117,119] Recent reports based on incident cases confirmed the strong associations between smoking and ARM, supporting a causal relation. Seddon et al.[120] evaluated the relation between cigarette smoking and any signs of incident ARM in the Nurses' Health study among middle-aged women with 556338 person-years of follow up, and found a relative risk of 2.4 (95% CI 1.4, 4.0) for those smoking over 25 cigarettes/day. Christen et al.[121] evaluated this relation in the Physician's Health Study

among men with 258115 persons-years of follow up, and found a relative risk of 2.5 (95% CI 1.6, 3.9) for those smoking over 20 cigarettes/day. In the 5-year follow up study of Beaver Dam, Klein et al.[122] found an increased risk of incident early features of ARM: for men aged 43-86 years with ≥ 35 pack-years of smoking, the relative risk of large drusen was 2.9 (95% CI 1.2, 7.0), and of increased pigment 2.3 (95% CI 1.2, 4.4). The association is important since smoking is still very common and amenable to prevention, and it suggests that it may be wise to advise patients with early signs of ARM to stop smoking.

Alcohol

At baseline in Beaver Dam,[123] after controlling for confounding factors, consumption of beer was related with increased pigment (OR 1.1, 95% CI 1.0, 1.25 per 105 grams of ethanol). In the 5-year follow up in Beaver Dam,[124] beer drinking was associated with soft indistinct drusen, drusen confluence and increased drusen area. The lack of confirmation of this relation in other studies [125,126] suggests that alcohol is not likely to be a very strong risk factor for ARM.

Light exposure

The damaging effect of light exposure on the photoreceptors and retinal pigment epithelium has been reported in several experimental studies.[59,60] Possibly, long-term exposure to light is a factor in the pathogenesis of ARM.[57,58] In a case-control study by Hyman et al. no significant association was reported between exposure to sunlight and ARM.[33] In a study among fishermen,[63] the ocular exposure was extensively measured.[127] No association between UV-A or UV-B exposure and ARM was observed. In an additional analysis based on a small number of cases, a positive association was observed between blue light exposure and neovascular macular degeneration.[128] Unfortunately, the number of cases suffering from neovascular macular degeneration that could be included in the analysis was very small. Cruickshanks et al.[129] reported a positive association between self-reported time spent outdoors in summer and the presence of drusen or pigmentary changes, as well as an inverse association with the use of hats or sunglasses in men. Furthermore, they observed a positive association between leisure time outdoors in summer and neovascular macular degeneration. The Eye Disease Case-Control Study Group could not confirm an association between history of light exposure and neovascular macular degeneration.[65] The issue was also investigated in two recent Australian studies. In the Blue Mountain Eye Study,[130] neither history of sunburns nor physical signs of excessive sunexposure were related to ARM. In the case-control study described by

Darzins et al, sunexposure was even greater in controls than in ARM cases.[131] One has to keep in mind that the measurement of ocular dose of light exposure is very complex and susceptible to misclassification, especially with history data. A lack of an association could be caused by the dilution of the effect of light exposure. Moreover, the risk period of the exposure may be long before the development of the disease. Long-term follow up studies with objective measures of sun exposure at baseline are needed to unravel this difficult issue.

Antioxidants and diet

Potentially damaging effect of cumulative light exposure on the retinal layers as described above, raised the question whether higher blood levels of antioxidants might protect against ARM.[61,132] Evidence for a protective effect of antioxidant nutrients emerged from basic research.[133-139] A study based on the NHANES data revealed that a low intake of vitamin A was associated with a higher risk of ARM.[101] Newsome et al. suggested a beneficial effect of oral zinc on the natural course ARM.[140] The Beaver Dam study found a decreased risk of RPE pigmentation (OR 0.4, 95% CI 0.2, 0.9) and RPE-degeneration (OR 0.4, 95% CI 0.1, 1.0) for subjects with a history of zinc supplements 10 years earlier,[141] and repeated the evidence for an association with hyperpigmentation in the 5-year incident study.[142] Zinc has a high concentration in ocular tissues, serves as a cofactor for metalloenzymes such as retinol dehydrogenase, and may be important for the RPE by its role in protein metabolism. However, no protective effect of zinc was found in the Eye Disease Case-Control Study [65] or in a small prospective trial of zinc supplements in subjects at risk of neovascular AMD in the fellow eye.[143] In the Eye Disease Case Control Study, a decreased risk of neovascular AMD was found for subjects with higher levels of serum cartenoids (OR 0.4, 95% CI 0.2, 0.6) [145] or for those eating cartenoid rich foods as spinach and collard green (OR 0.6, 95% CI 0.4, 0.9).[144] The Beaver Dam Eye Study [145] confirmed a relation only with lycopene, not with the cartenoids that compose macular pigment (lutein, zeaxanthin). One study reported that higher serum levels of α-tocopherol (vitamin E) were associated with a decreased risk of neovascular macular degeneration,[146] but that association could not be confirmed in a nested case-control study of the Beaver Dam,[147] nor in the Blue Mountain.[148] In the Beaver Dam Eye Study [147] as well as in the Baltimore Longitudinal study of Aging [146] there was some evidence for a protective effect of vitamin C, but findings were inconsistent. The French POLA study recently showed that enzymatic antioxidant processes may also be involved. In a cross-sectional analysis among 2584 participants aged 60 years and older, this study found an increased risk of late ARM for subjects with high levels of plasma glutathione

peroxidase.[149]

The Beaver Dam Eye Study evaluated dietary fats.[147] Subjects in the highest quintile of saturated fat and cholesterol intake had 80% (95% CI 1.2, 2.7) and 60% (95% CI 1.1, 2.4) increased odds of prevalent early ARM. There are no other reports on fat intake.

PROGNOSIS

Visual loss

The risk of loss of visual acuity and the central visual field is the primary reason for concern about ARM. Several studies have shown that the disease usually affects both eyes of patients.[13,150-153] Generally, severe visual loss is caused in these patients by a choroidal neovascular membrane and in a smaller amount of cases by atrophy of the retinal pigment epithelium involving the fovea.[13,33,154] The risk of visual loss in cases with bilateral drusen was reported in two follow-up studies. In the first study, Gass reported that nine of 49 cases developed severe visual loss in one eye during an average follow-up period of 4.9 years.[13] In the other study of 71 patients by Smiddy et al.[155] severe visual loss due to neovascular disease occurred in seven eyes of six patients. Using life-table analysis, the five-year cumulative risk of visual loss was 12.7 percent. The interpretation of the results remains difficult, because both studies were based on prevalent cases with different duration of disease.[156] Furthermore, the cases were obtained from specialized clinics. This could have resulted in the selection of more severe cases, and extrapolation of the results to a general population may, therefore, be misleading.

With both eyes affected, one has a severe visual handicap. The prognosis of the second eye in cases with unilateral neovascular AMD is, therefore, a matter of great concern. The issue was studied in several case-series.[13,151,152,156-160] Roy et al. summarized the risk of second eye involvement to be somewhere between 4 and 12 percent annually for the first three years following the diagnosis of ARM in the first eye.[157] More accurate risk estimates are to be expected from cohort studies.

Prevention of visual loss

The need for effective treatment of AMD to prevent blindness is evident. The development of treatment techniques has mainly focussed on suppression of subretinal neovascular membranes. Effective treatment is as yet not available for drusen,

pigmentary changes and atrophic AMD. For neovascular AMD, laser photocoagulation has been shown to effectively occlude subretinal neovascular membranes.[161] Laser treatment of a subretinal membrane leads to an immediate irreversible decline of visual acuity when performed close to the fovea due to the destruction of the overlying photoreceptors, but results after two years in a smaller scar and scotoma than no treatment.[162] Estimations of the proportion of patients with neovascular AMD that may be treated for this indication vary between 13 and 57 percent.[5,6,163,164] Unfortunately, more than half of the treated patients suffer from recurrences of choroidal neovascularization within five years,[165] possibly by recanulation of the occluded vessels or by incomplete treatment. It is as yet inconclusive whether the new technique of digital indocyanine green videoangiography will be able to increase the chances of a favourable visual outcome by better detection of well-demarcated neovascular membranes.[166,167]

Treatments under investigation

Interferon

Systemic interferon alfa-2 has been used to treat vascular tumors.[168,169] It inhibits the growth of iris neovascularization in monkeys and even induces its regression.[170] In vitro, interferon alfa inhibits vascular endothelial cell proliferation.[171] First results from case series[172-177] and from one small randomized trial [178] suggested that interferon may be effective as a treatment for ARM with a slower growth of the choroidal membrane in neovascular AMD. However, later results from a large prospective randomized placebo-controlled clinical trial showed no benefit for interferon alfa-2a as a treatment for choroidal neovascularization.[179] On the contrary, when given at a dose of 6 MIU, those on treatment had a poorer visual outcome after one year than those on placebo.

Radiotherapy

Low doses of ionizing radiation lead to regression of ocular hemangiomas [180] and of new vessel formation in wound healing.[181-182] The effect of radiotherapy on subretinal neovascularisation was first investigated by Chakravarthy [183] who reported higher visual acuity and smaller choroidal neovascular membranes in the treatment group after one year follow-up. After this study, phase I/II trials were performed which all [184-188] but two [189,190] found a benificial effect for radiotherapy. Bergink et al.[186] was the first to perform a prospective randomized clinical trial that consisted of 36 subjects undergoing treatment and 32 subjects who did not. At 12 months follow up, 32% of the treatment group lost 3 or more lines of visual acuity versus 53% of the observation group (P=0.03). Larger randomized controlled clinical trials that study long-term

effects, methods and dose of irradiation, and possible side effects are currently underway. These results should be awaited before this treatment can be applied at large.

Photodynamic therapy

A large drawback of conventional laser therapy is that, due to heat conduction, the entire retina, RPE, and choroid surrounding the coagulation spot are irreversibly damaged. Photodynamic therapy is a relatively new treatment which combines laser with a non-thermal localized chemo-toxic reaction. The intention is to selectively occlude the neovascular vessels by inducing a localized photochemical process with low energy laser light in an area with a high concentration of photosensititizing dye. This dye is injected intravenously and has the property to preferentially accumulate in pathologic vessel proliferations. The concept of this treatment appeared promising in experimental treatments and animal models.[191,192] A recent phase I/II clinical trial [193] reported the results of 61 patients with classic CNV undergoing photodynamic therapy. All subjects showed complete cessation of leakage after 1 week, but leakage reappeared in 75% after 12 weeks, although markedly less than before treatment. Retreatment was effective for another short period,[206] and no adverse affects occurred below a light dose of 150 J/cm^2.[207] A large phase III trial has now started to evaluate the long-term prognosis.

Antioxidants

If one assumes that the cumulative damaging effect of radiant energy, such as sunlight, on the retinal layers is caused by the formation of free radicals, a benificial effect of anti-oxidants may be expected. The effect of anti-oxidant therapy is likely to be small but of clinical relevance.[195] Treatments with megadose vitamins E, C, ß-carotene, and zinc are now being investigated in the Age Related Eye Disease Study, a multicenter randomized trial in the USA. By design, the conclusions of this study will be limited to the benefits of megadose therapy in a population with an already sufficient intake of micronutrients. A nutricious diet seems a good recommendation, but there is not enough evidence at this time to advise zinc and/or antioxidant supplements.[196] Aside from all the potential benefits, more extensive study of the possible harmful effects is needed.

Surgical intervention

Several studies have examined the effect of surgical removal of subretinal hemorrhages or neovascular membranes.[197-202] This treatment aimed at minimizing the size of the

scar. The indications for these techniques, however, are still a matter of debate. Most studies report a beneficial effect on anatomical scar size but the results fail to improve the functional status of the macula. Recurrence rates are likely to be as frequent as after laser treatment. The viability of the RPE seems to play an important role as results are better in younger subjects. It seems reasonable to reserve these techniques for cases with large subretinal hemorrhages.

Lasertherapy of early lesions
Several studies are investigating whether the disappearance of drusen after laser photocoagulation has a beneficial effect on visual prognosis. The first results are disappointing. Preliminary findings from the Choroidal Neovascularization Prevention Trial[203,204] show that significant exudative manifestations of AMD associated with the region of treatment may develop after photocoagulation of soft drusen.

Other potential future treatments
Experimental treatments that are currently under investigation are neuroprotective and antiangiogenic drugs, gene therapy, noninvasive laser targeted drug delivery, retinal translocation, and RPE transplantation.

CONCLUSIONS

ARM is a major cause of severe visual impairment in the elderly of western countries. The visual handicap has major consequences for the quality of life of patients and their relatives. In this paper we reviewed the epidemiologic findings concerning frequency, risk factors, and prognosis of the disease. The classification of ARM has been a matter of debate, but recently international agreement has been reached on the classification of each of the separate disease features for epidemiological studies. Although they established a diagnosis of the late stages, researchers still have to reach agreement on the classification of early stages.

The frequency of AMD rises with age; the prevalence increases from 1% in subjects aged 65-74 to 10% in those over 85 years in the Netherlands. The incidence of AMD, expressed as 5-year cumulative incidence in the Beaver Dam Eye Study, is 1.3% in subjects under 75 years, and 5.4% for those aged 75 years or older. There is a clear need for more incidence studies of ARM, with the particular aim to confirm known risk factors, to specify their magnitude, and to investigate potential new factors. These

studies are currently taking place in North America, Europe, and Australia.

Most known risk factors for ARM have been studied in case-control studies and cross-sectional population-based studies. Apart from age, genetic predisposition and smoking are the most definite risk factors that have been found. More extensive research is needed to evaluate the magnitude of the genetic component and mode of inheritance. Genes need to be identified, since that will lead to a better understanding of the underlying causes of ARM, and identification of family members at risk will provide the basis for future therapeutic and preventative interventions. The origin of the association with smoking is not fully understood yet, but the association is interesting since smoking habits are potentially modifiable. Other risk factors that have been suggested in the etiology of ARM are light exposure, atherosclerosis, and oxidative stress. The role of light exposure remains unclear; as yet, the possible harmful effect of light seems to be small. A vascular basis to the disease seems plausible and may provide leads for intervention.

Patients with atrophic AMD may retain useful vision for years until the atrophy reaches the fovea. The prognosis in patients with neovascular AMD is generally worse. Further studies are needed to provide a better estimate of the prognosis. Currently, there is no proven treatment for the disease except for a selected group of patients with neovascular AMD, in which treatment with laser photocoagulation has some benefits. New interventions as anti-oxidant suppletion, radiotherapy, and photodynamic therapy are currently being investigated, and these may have beneficial consequences for patients who are not eligible for conventional laser treatment. Investigations to date certainly render encouraging perspectives, but future epidemiological studies will be needed to provide a better insight in course, determinants and prevention of ARM in the elderly.

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Part II

Disease frequency and impact

Chapter

3

Incidence and progression of age-related maculopathy
The Rotterdam Study

Abstract

Objective: To describe the incidence of late stages of ARM and the progression of earlier stages, and to study the hierarchy of fundus features that determine progression.

Design: Population-based prospective cohort study.

Participants: A population of 4948 subjects aged 55 years and older living in Rotterdam, the Netherlands, was studied to determine the incidence of neovascular and atrophic AMD. A subgroup of 1244 subjects was studied for progression of early stages of ARM.

Methods: At baseline and at 2-year follow-up, fundus transparencies were graded for features of age-related maculopathy using the International Classification System. ARM was stratified in four exclusive stages according to type of drusen and presence of pigmentary irregularities.

Main Outcome Measures: AMD, ARM.

Results: The overall 2-year cumulative incidence of AMD was 0.2%, increasing to 1.2% in subjects of 85 years and older. Of the early stages, 22% showed progression to a more severe stage. Most important predictors for progression were more than 10% of macular area covered by drusen (OR 5.7, 95% CI 2.5, 13.1), presence of depigmentation (OR 5.0, 95% CI 3.2, 7.8), and hyperpigmentation (OR 3.1, 95% CI 2.0, 4.7).

Conclusions: The incidence of AMD appears to be lower in the Netherlands than in the United States. Progression of early stages occurs in a distinct pattern at a stable rate with a large area of drusen and pigmentary changes as most important predictors.

INTRODUCTION

A large number of studies have shown that ARM is a frequent eye disorder in the elderly,[1-3] and that its end stages are the most important cause of irreversible blindness in the Western world.[4-6] The design of most of the epidemiologic studies has been cross-sectional, and they have provided information on disease prevalence and prevalence associations. In etiologic research, however, incidence is commonly preferred over prevalence. Incidence represents the actual disease occurrence, and risk analyses based on incident cases are more suggestive of a causal relation, since exposures are measured before the onset of disease. Incidence data of ARM would improve the knowledge on the etiology, early development and progression of this disease. At present, these data are still scarce.[7,8]

The purpose of this study was to describe the incidence and progression of ARM in the population-based Rotterdam Study in the Netherlands. We studied the incidence of the late stages of ARM in the entire cohort, and investigated progression of early features in specific subgroups. Furthermore, we aimed to assess the prognostic value of the various fundus features that are associated with ARM.

SUBJECTS AND METHODS

Population

The Rotterdam Study is a population-based prospective cohort study conducted in a suburb of Rotterdam, the Netherlands, in which chronic ophthalmologic, neurologic, cardiovascular, and locomotor disorders are investigated. Methods used to identify and describe the population have appeared in previous reports.[2,9] Baseline interview and screening examinations took place from 1990 to mid 1993, follow up examinations from mid 1993 to the end of 1994.

Of 10,275 eligible subjects aged 55 years and older living in Rotterdam, 7983 (78%) agreed to participate in the baseline phase of the study. Gradable fundus transparencies were available on 6411 subjects, of whom 104 (1.6%) subjects were diagnosed with the late stages of ARM, i.e., atrophic or neovascular AMD. This resulted in a cohort of 6307 subjects at risk for incident AMD.

Procedures and definitions

The screening for presence of ARM followed the same protocol at baseline and at

follow up. Procedures have been described in detail elsewhere.[2,9] In brief, during the screening eye examination color transparencies centered on the macula were taken with a monoscopic (35°field; Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) and stereoscopic camera (20°field; Topcon TRV-SS2 fundus camera, Topcon Optical Company, Tokyo, Japan). The diagnosis of ARM features was based on grading of fundus transparencies according to the International Classification System,[10] in which all features of age-related maculopathy are called ARM and the two late stages are called AMD. At baseline, fundus transparencies of the entire cohort were graded in a detailed manner to identify all features of ARM present in the macular grid area (radius, 3000μm). At follow up, all fundus transparencies of the entire cohort were graded for presence of atrophic or neovascular AMD. Inter- and intra-rater agreement on all fundus features was checked every 1000 subjects, and consensus training was performed when weighted kappa values fel below 0.6.

Table 1. Stratification of ARM in exclusive stages of severity

Stage of ARM	Criteria	No. at baseline	No. at follow-up	No. selected for analysis
No ARM	No ARM features or only drusen ≤ 63µm	4025	3234	327
Stage 1 (*i*)	Soft distinct drusen	1465	1144	331
(*ii*)	Pigmentary irregularities without soft drusen	332	248	248
Stage 2 (*i*)	Soft indistinct drusen or reticular drusen	180	121	121
(*ii*)	Soft distinct drusen with pigmentary irregularities	222	170	170
Stage 3	Soft indistinct or reticular drusen with pigmentary irregularities	83	47	47
Stage 4	Atrophic or neovascular macular degeneration (AMD)	104	54	0

To assess the incidence and progression of early ARM features, ARM at baseline was stratified in four exclusive stages of disease (Table 1). On the basis of previous findings,[7,8,11,12] we assumed more clinical severity and a higher risk of development of AMD with each successive stage. The stage classification of a subject was based on

the eye with the most severe stage of ARM. ARM stages 1 (*i*) and (*ii*) were considered one stage of clinical severity, as were stages 2 (*i*) and (*ii*). For reasons of feasibility and efficiency, only a randomly selected subset of subjects with no ARM or ARM stage 1 (*i*) at baseline underwent detailed grading of early ARM features at follow up. Of all other stages, the entire group of subjects with gradable fundus transparencies underwent detailed grading at follow up.

Incidence of an ARM lesion was defined as absence of this particular lesion within the grid area of either eye at baseline and presence of this lesion in at least one eye at follow up. Progression of ARM was defined as an increase in one or more stages of ARM; no progression was defined as no change or a decrease in stage.

Statistical methods

Subjects with AMD at baseline were excluded from the incidence and progression analyses. The age-specific incidence of AMD was obtained per 10-year age-categories by dividing the number of incident cases by the number of person-years per age-category. The latter was calculated by summing each participant's contribution of follow up time per age-category. Confidence intervals of incidence rates were calculated with the exact method. Age at follow up was regarded as age at onset of incident AMD. Cumulative incidences were calculated from the incidence rates with the formula

$$CI\,(t) = 1 - e^{-IR*t},$$

where CI is the cumulative incidence over a period of t years, IR is the incidence rate, and e is the constant 2.71828, the base of the natural logarithm.

Progression of early ARM stages was studied by logistic regression analysis with age, gender, baseline stage of ARM and duration of the follow up period fixed in the model. In an initial 'univariate' analysis with these fixed factors the predictive power of drusen size and location, proportion of macular grid area covered by drusen, most frequent drusen size, largest drusen size, drusen confluence, presence and area of hyperpigmentation, and presence and area of depigmentation was evaluated. Statistical interaction between macular area of drusen and hyper- or depigmentation, between hyper- and depigmentation, and between area of drusen and drusen confluence was studied by entering the product term of these factors in the model. Fundus features or product terms with a significant odds ratio entered a subsequent 'multivariate' analysis to determine the independence and magnitude of prognostic factors.

RESULTS

Incidence of AMD

Of the 6307 subjects at risk for incident AMD, 5442 (86%) participated in the 2-year follow up phase of the Rotterdam Study. Of the non-participants, 326 subjects had died before follow up, and 539 (10%) subjects refused to participate in any follow up examination. Of subjects that consented to follow up, 5097 participated in the re-screening eye examination. Gradable fundus transparencies of at least one eye were present in 4948 subjects, 78% (4948/6307) of the total number of subjects at risk for incident AMD, and these subjects were included in the incidence analyses. They significantly differed from other eligibles by age, but, after adjustment for age, not by stage of ARM at baseline (table 2).

Table 2. Baseline characteristics of subjects at risk of incident AMD

	Eligible subjects (n=6307)		
Characteristics	**In analysis[†] (n=4948)**	**Not in analysis[‡] (n=1359)**	***P***
Age at baseline (%)			
55 - 64 y	43.2	24.3	<0.001
65 - 74 y	38.1	30.2	
75 - 84 y	16.2	33.0	
85 + y	2.5	12.5	
Gender (% women)[§]	58.5	61.3	0.07
Institutionalized (%)[§]	4.4	10.2	<0.001
ARM at baseline (%)[§]			
Stage 1	29.1	28.4	0.15
Stage 2	6.5	5.9	
Stage 3	1.1	2.1	

[†] subjects with gradable fundus transparencies
[‡] deceased, non-participants, and subjects with ungradable fundus transparencies
[§] adjusted for age

After an average follow up period of 2.0 (SD 0.6) years, 11 cases were identified with incident AMD. Of those, 5 cases were identified with atrophic AMD and 6 with neovascular AMD. Given a total of 9833 person-years, the overall incidence of AMD was 1.1 per 1000 person-years (2-yr cumulative incidence 0.22%). The incidence increased with age (table 3). Figure 1 shows the estimated 5-year cumulative incidence risk in the Rotterdam Study in comparison with the two other population-based incidence studies that report 5-year incidences.

Table 3. Age-specific incidence rates per 1000 person-years and 2-year cumulative incidences (%) of AMD in the Rotterdam Study

Age-group	Person-years	N	Rate	95% CI	2-yr incidence
55-64	3530	0	0	(0, 0.001)	0
65-74	4009	1	0.2	(0.01, 1.4)	0.05
75-84	1955	8	4.1	(1.8, 8.1)	0.82
85+	338	2	5.9	(0.7, 21.4)	1.18
Total	9833	11	1.1	(0.6, 2.0)	0.22

Overall, there were no statistically significant gender differences in incidence of AMD. Among the 2078 men, the overall incidence was 1.00 per 1000 person-years (2-yr cumulative incidence 0.2%), while among the 2870 women, the overall incidence was 1.20 per 1000 person-years (2-yr incidence 0.2%, $P = 0.24$, adjusted for age and follow up time).

Incident AMD was strongly associated with stage of ARM at baseline. Neither ARM stage 0 nor stage 1 progressed to incident AMD. ARM stage 2 gave rise to 3 subjects with incident atrophic AMD and 4 with incident neovascular AMD. For this stage, the overall incidence rate of AMD was 12.1 per 1000 person-years (2-year cumulative incidence 2.4%), ranging from 4.0 per 1000 person years (2-yr incidence 0.8%) in subjects under 65 years to 35.4 per 1000 person-years (2-yr incidence 6.8%) in subjects aged 85 years and older. Stage 3 at baseline gave rise to 2 subjects with incident atrophic AMD and 2 with incident neovascular AMD. For stage 3, the total incidence rate of AMD was 38.6 per 1000 person-years (2-yr incidence 7.4%), and the age-category in which this occurred was 74-85 years .

Of the 31 subjects with AMD in only one eye at baseline, 3 subjects with atrophic

AMD and 4 subjects with neovascular AMD developed incident AMD in the second eye at 2-yr follow up. This resulted in an incidence rate of 109.9 per 1000 person-years (2-yr cumulative incidence 19.7%) for involvement of the second eye. The 3 subjects with unilateral atrophic AMD at baseline developed the same type of AMD in the second eye. Of the 4 subjects with neovascular AMD, 2 developed neovascular AMD and 2 developed atrophic AMD in the other eye. The baseline ARM stages of the second eye were stage 2 (3 subjects) and stage 3 (4 subjects).

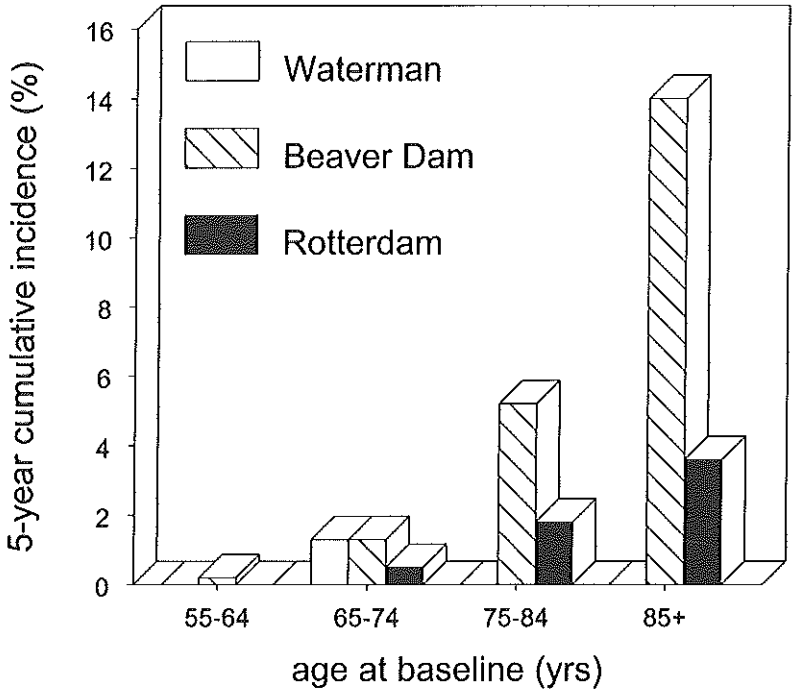

Figure 1. ***Comparison of the estimated age-specific 5-year cumulative incidence of AMD in the Rotterdam Study with the 5-year cumulative incidences in the Waterman Study[7] and in the Beaver Dam Eye Study[8]***

Progression of early stages

Of the 1244 subjects who were selected for the early ARM progression analyses, 315 subjects progressed to a more severe stage of ARM. For the total cohort, this implied a 2-year cumulative progression rate of 21.5%. Table 4 shows the incidence rates of the various stages of ARM at follow up. Age was associated with progression: adjusted for gender, follow up time and baseline stage of ARM, the odds ratio of progression

for age per year was 1.02 (95% CI 1.00, 1.03). Gender was not associated with progression: the odds ratio for women versus men was 0.98 (95% CI 0.75, 1.27; adjusted for age, follow up time and baseline stage of ARM).

Table 4. Incidence rates of the various stages of ARM per 1000 person-years based on 2-year follow-up (2-yr cumulative incidences,%)

Stage of ARM	*Follow-up Stage 1*	*Follow-up Stage 2*	*Follow-up Stage 3*	*Follow-up Stage 4*
Baseline Stage 0	112.8 (20%)	4.5 (1%)	0	0
Baseline Stage 1		112.5 (20%)	15.8 (3%)	0
Baseline Stage 2			119.9 (21%)	11.7 (2%)
Baseline Stage 3				37.6 (7%)

In the 'univariate' analysis of prognostic factors, macular area covered by drusen, presence and area of hyperpigmentation, presence and area of depigmentation, number of small drusen ($\leq 63\mu m$), number of large drusen ($\geq 125\mu m$), and drusen confluence were significantly associated with progression (data not shown). In the 'multivariate' analysis with these significant factors in the model, all factors except number of large drusen ($\geq 125\mu m$) remained statistically significant. A large area of drusen was the most important predictor of ARM progression; the odds ratio for >10% of macular area covered by drusen was 5.8 (95% CI 2.5, 13.3) (table 5). The other important independent predictors were presence of depigmentation, hyperpigmentation, 10 or more small drusen, and at least 10% drusen confluence. Area of depigmentation with a total diameter larger than 500 μm had a higher odds ratio than did smaller area's (odds ratio for area >500 μm versus area <175 μm 4.61 (95% CI 2.48, 8.56), indicating that larger area's of depigmentation were more prognostic than smaller area's. Area's of hyperpigmentation larger than 125 μm did not have higher odds ratio's than area's of 125 μm or smaller, indicating that larger area's of hyperpigmentation were not of additional prognostic value. We found no evidence for statistical interaction between area of drusen and pigmentary irregularities, between hyper- and depigmentation, or between area and confluence of drusen (data not shown).

Table 5. Fundus features prognostic for progression of ARM

Fundus feature	OR (95% CI)*
Total drusen area ≥10% of grid	5.78 (2.52, 13.30)
Presence of depigmentation	4.95 (3.15, 7.79)
Presence of hyperpigmentation	3.09 (2.02, 4.72)
≥10 small drusen (≤63μm)	3.01 (1.90, 5.11)
≥10% drusen confluence	2.77 (1.81, 4.24)

* based on a model which included these factors, plus age, baseline stage of ARM, and duration of follow-up period

DISCUSSION

In the Rotterdam Study, the incidence of ARM's late stage, AMD, was 1.12 per 1000 persons per year for subjects aged 55 years and over. The incidence of AMD showed a strong relation with age and increased to 5.9 per 1000 persons per year for those aged 85 years and older, which appeared to be lower than in the United States. The incidence of AMD in the contralateral eye of subjects already affected by unilateral AMD was 109.9 per 1000 persons per year. The most predictive stage for development of incident AMD was ARM stage 3, which comprises the presence of either soft indistinct or reticular drusen, or soft drusen with pigmentary irregularities. Progression of early stages of ARM occurred in a very distinct pattern at a rate of 22% in two years. Most important predictors for progression were more than 10% of macular area covered by drusen, presence of depigmentation, and presence of hyperpigmentation.

A good estimate of the incidence of AMD requires the follow up of many subjects over a long period of time, because the occurrence of this clinical end stage is relatively infrequent. A large study population with a significant number of elderly is one of the strengths of the Rotterdam Study. However, the length of the follow up period was limited, and the number of subjects who developed incident AMD was low. Therefore, our estimated incidence rate of AMD is rather imprecise. On the other hand, the short follow up period was a benefit for the study of the progression of early ARM stages. This enabled us to register small changes and to determine a pattern of

progression, which may add to the understanding of the natural course of this disease.

Lost to follow up is a concern in this study, as it is in all cohort studies. Non-participation at the second round was mainly due to mortality and non-response to the entire study, not to the eye examination itself. Comparison of subjects in the analysis with subjects that were not, showed that the latter group was older, but the groups did not significantly differ in stage of ARM at baseline. Thus, the effect of lost to follow up on our findings may be limited.

The age-specific incidences of AMD appeared to be lower in the Rotterdam Study than in the Waterman Study or the Beaver Dam Eye Study (figure 1).[7,8] The American studies took place in different parts of the United States, but show incidences within the same range. We estimated a five-year cumulative incidence by extrapolation of our data in order to allow for a meaningful comparison, and in coming years we will be able to evaluate whether this estimation is correct. Nevertheless, the difference seems considerable, consistent over the age-groups, and in agreement with earlier reports indicating global differences in the occurrence of AMD. Comparison of prevalence data from the Beaver Dam Eye Study, the Blue Mountain Eye Study, and the Rotterdam Study showed that the prevalence of AMD was highest in the United States and lowest in the Netherlands.[1-3] The three studies used very similar methods of diagnosis based on fundus photography, which makes it less likely that the differences were a result of observation bias. Known risk factors such as smoking and cardiovascular disease did not explain the differences ("Smith et al, submitted"), and it remains a key point of interest to identify the environmental and genetic factors that are accountable.

The 2-year incidence of AMD in the fellow eye in subjects with unilateral AMD was 20%, and the type of AMD was not necessarily concordant with the first eye. The Beaver Dam Eye Study found a 5-year incidence of 22% for the second eye,[8] considerably lower than the Rotterdam Study. The lower age-range in Beaver Dam may well account for this difference. Our data are in line with clinic-based studies reporting the rate of fellow eye involvement. The majority of these studies focussed on patients with neovascular AMD, and estimates for annual second eye incidence mostly ranged from 4 to 10%,[11,13-17] although annual incidences up to 15% have been published.[18] Comparison of rates is generally hampered by differences in age, duration of disease, and diagnosis, and long-time follow up of large, well-defined study groups will be needed to provide valid and precise estimates.

An important objective of the study was to describe the progression of early features of ARM. For long it has been known that soft drusen and pigmentary changes are precursor lesions that increase the risk of geographic atrophy and neovascular

AMD.[7,8,11-20] After appearing, drusen and pigmentary changes may regress and disappear, but generally this is a result of appearance of more severe lesions.[7,8] In the Rotterdam Study, we did not focus on individual fundus lesions. To enhance clinical relevance, we preferred to study progression of ARM in exclusive stages of disease. We stratified early features of ARM in three stages based on type of drusen and presence of pigmentary changes, the factors which have been shown to be strong predictors for the development of AMD.[7,8,11,12] The ranking of the stages was in accordance with clinical severity: the risk of AMD increased from virtually no risk for stages 0 and 1, to a 2-year risk of 2.4% and 7.4% for stages 2 and 3, respectively. An interesting finding was that progression predominantly occurred to only one more advanced stage at a rate of approximately 20% in 2 years for the earliest stages (table 4). Progression from stage 3 to 4 was slower and occurred at a rate of 7% in 2 years. Some subjects progressed fast and skipped one stage, but no subjects skipped more than one stage in the 2 years of follow up. Although future studies are awaited to confirm these data, our findings add to the view that development of ARM is not a random occurrence of events, but rather seems to follow a well-defined pattern at a stable rate.

In accordance with others,[8] we found that a large area of the macula covered by drusen and pigmentary irregularities were important and independent predictors of ARM progression. Other predictors were number of small drusen, and drusen confluence. The number of intermediate (64-124μm) and large drusen (≥ 125μm) did not have additional predictive power, neither did location of drusen. Although small drusen (≤ 63μm) are not considered an ARM feature in the International Classification System, our data indicate that more than 10 small drusen are predictive of ARM progression independent of other features. This is consistent with findings from the Waterman Study[7] and the Beaver Dam Eye Study,[8] which both reported that many small drusen increased the risk of large and soft indistinct drusen, but not of AMD. From our results and those of others we conclude that progression of early ARM appears to follow a distinct pattern. A large number of small hard drusen or isolated pigmentary changes may indicate the very early start of ARM. Then soft drusen emerge. Subsequently, at a stable rate, multiple drusen of various sizes appear and become confluent, the total area increases and some of the drusen become soft indistinct. The appearance of pigmentary changes at this stage, especially large areas of depigmentation, then further increases the risk of AMD. Subretinal neovascularization or development of geographic atrophy denote the etiologic end stage of ARM.

In conclusion, the 2-year incidence of AMD in the Rotterdam Study was 2.2 per

1000 subjects. Our data provide further evidence that ARM is a progressive disease with a distinct temporal sequence of events ultimately ending in AMD.

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Chapter

4

Age-specific prevalence and causes of blindness and visual impairment in an older population The Rotterdam Study

Abstract

Objectives: To study the prevalence and causes of blindness and visual impairment in various age categories of a large population-based study.

Methods: For the study, 6775 subjects aged 55 years and older underwent an extensive ophthalmological screening examination, including measurements of visual acuity and the visual field, and fundus photography. The causes of blindness or visual impairment were determined using all screening information and medical records.

Results: The prevalence of blindness, according to WHO criteria, ranged from 0.1% in subjects aged 55-64 years to 3.9% in subjects aged 85 or older; the prevalence of visual impairment ranged from 0.1% to 11.8%. For persons younger than 75 years, myopic degeneration and optic neuropathy were the most important causes of impaired vision. For persons aged 75 years or older, AMD was the major cause of the increased prevalence of blindness, whereas age-related cataract predominantly caused the increased prevalence of visual impairment.

Conclusions: The hierarchy of causes of blindness and visual impairment is highly determined by age. As yet, little can be done to reduce the exponential increase of blindness; however, adequate implementation of surgery to treat cataract could reduce visual impairment by one third. Underuse of ophthalmological care is a prominent cause of the high frequency of untreated cataracts among the elderly.

INTRODUCTION

Blindness is the functional end stage of many eye disorders. The occurrence and course of these disorders differ markedly throughout the world, and this is reflected by differences in the prevalence of blindness and visual impairment.[1] International comparison of these data may help to provide insight in the risk factors associated with blinding eye disorders, and facilitate evaluation of therapeutic modalities and prevention programs.

Various population-based studies have provided precise estimates on prevalence and incidence of blindness and visual impairment in Western countries.[2-10] All show a clinically significant increase in the prevalence of impaired vision with increasing age. To fully understand this increase, to make a meaningful comparison between countries, and to develop efficacious strategies for eye care for a wide spectrum of ages, accurate data on the age-specific causes of impaired vision are essential. Few such data exist, however.

The Rotterdam Study is a population-based study of the occurrence and determinants of various disorders in a middle-aged and elderly population. The age range is wide in this study, and the proportion of 80- and 90-year-old subjects substantial. In the present study, we analyzed data from the Rotterdam Study to describe the age-specific prevalence and causes of blindness and visual impairment in an older, predominantly white Western population.

SUBJECTS AND METHODS

Subjects

The rationale and design of the Rotterdam Study have been described elsewhere.[11] In brief, this population-based prospective follow-up study focuses on chronic ophthalmologic, neurologic, cardiovascular and locomotor diseases among subjects aged 55 years or older living in Ommoord, a city district of Rotterdam. Baseline data were collected between 1990 and 1993. Eligibles were identified by drawing names and addresses from the municipal register. During an initial home interview, demographic characteristics, medical and ophthalmological history, the use of eye care, attained level of education, the level of ability in daily activities, and a variety of other variables were evaluated. Subsequently, participants underwent a physical examination at the screening center. Subjects living in the six nursing homes of the target area were examined at their homes.

Procedures and definitions

The ophthalmological examination included measurements of visual acuity, ocular refraction, visual fields, and intraocular pressure; slitlamp examination; and direct and indirect ophthalmoscopy. The examination was performed by two ophthalmologically trained physicians who determined the presence of cornea and lens opacities, and vitreous and fundus changes using a standardized grading protocol. In addition, 20° stereoscopic fundus color transparencies were taken of the optic disc (Topcon TRC-SS2 stereoscopic fundus camera, Topcon Optical Company, Tokyo, Japan), and 35° color transparencies were taken of the macular area (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan). Visual acuity was measured at a 3 m distance using the Lighthouse Distance Visual Acuity Test, a modified Earley Treatment Diabetic Retinopathy Study chart.[12] To evaluate best-corrected visual acuity, optimal refraction was obtained subjectively after objective autorefraction. Screening of visual fields was performed using a modified 76-point supra-threshold perimetry test (Humprey Visual Field Analyzer, Zeiss, Oberkochen, Germany); visual field defects were subsequently confirmed by using Goldmann perimetry.

The various population-based studies evaluating blindness have used different criteria for blindness and visual impairment. We used two sets of criteria for blindness and visual impairment to enable comparison of our prevalence data with others. The first set of criteria was established by the World Health Organization and used in the International Classification of Diseases; blindness is defined as a best-corrected visual acuity of less than 0.05 (Snellen, 20/400) in the better eye or a visual field no greater than 10° around central fixation; and visual impairment, as a best-corrected visual acuity of less than 0.3 (20/60) but no less than 0.05 (20/400) in the better eye.[13] The second set of criteria is used most commonly in the United States; blindness is defined as a best-corrected visual acuity of 0.1 (20/200) or less in the better eye, and visual impairment as a best-corrected visual acuity less than 0.5 (20/40) but better than 0.1 (20/200) in the better eye. The cause of visual loss was determined for blindness and visual impairment according to the WHO criteria. Two clinical investigators (C.C.W.K. and P.T.V.M.J.) reached consensus on the final determination of the cause of visual loss after reviewing all screening information, fundus transparencies, and, when necessary, information provided by ophthalmologists. Standard procedures and standard clinical criteria were applied. In most cases, the cause of visual loss was a single disorder. When multiple disorders were present, we attempted to identify the disorder causing the greatest limitation of vision. In a few subjects, no primary cause of the visual loss could be identified, and visual loss was considered due to a combination of mechanisms.

To evaluate the presence of diabetes mellitus, a non-fasting oral glucose tolerance test was performed for all subjects not using antidiabetic medication. Diabetes mellitus was defined as the use of antidiabetic medication or a random or postload glucose level greater than 11 mmol/L (198mg/dL). A screening test for cognitive function comprised the Mini-Mental State Examination; a low score indicates poor cognitive function.[14] The attained level of education was evaluated according to the standard classification of education,[15] which is comparable to the international standard classification of education (UNESCO, Paris, France, 1976). Four levels of education were included, the lowest was primary education and the highest university or higher vocational education. Ability in daily activities was measured in eight components (ie, dressing, rising, reach, hygiene, eating, walking, grip, and activity) as described previously.[16,17] Moderate disability was present when subjects had difficulties in four out of eight components.

Data analysis

The prevalences of blindness and visual impairment were calculated as percentages of the total study population and stratified by age and gender. The prevalences of causes of blindness and visual impairment were calculated as percentages of affected eyes in three age-categories. The proportions of categorical variables and the differences in the categorical variables between groups were calculated by using multiple logistic regression analysis with adjustment for age and gender; means and differences between continuous variables were adjusted by using analysis of covariance. The sum of the age-specific prevalences of blindness and visual impairment was calculated to represent the total prevalence of poor vision (i.e., blindness and visual impairment).

RESULTS

A total of 10 275 eligible subjects were identified during recruitment, and 7983 (77.7%) consented to an initial home interview. Of these subjects, 6775 participated in the ophthalmologic examination. On the basis of other available data, differences in general characteristics between subjects who underwent the ophthalmologic examination and subjects who did not could be evaluated. Compared with participants in the ophthalmologic examination, nonparticipants were significantly older, were more often women and were more often institutionalized; nonparticipants had lower scores on the Mini Mental State Examination, and were more likely to have visual and

other health problems. The use of anti-diabetic medication was not higher among nonparticipants.

By using the WHO-criteria, we identified 32 subjects who were blind and 96 subjects who were visually impaired in both eyes. Table 1 gives the more specific distribution of characteristics among these subjects compared with subjects with better vision. Compared with subjects with better vision, subjects who were blind or visually impaired were significantly older (Student *t* test; $P<0.001$). After adjustment for age, they were still more likely to be institutionalized (21% vs 9%; $P<0.001$), showed more disability in daily activities (41% vs 33% $P<0.001$),and had slightly lower scores on the Mini Mental State Examination (25.0 vs 27.2; $P<0.001$). There were no significant differences in presence of diabetes mellitus (12% vs 11%; $P=0.1$).

Table 1. General and clinical characteristics*

		Blind subjects (n=32)	Visually impaired subjects (n=96)	Subjects with visual acuity ≥ 0.3 (n=6647)
Age		83‡	84‡	69
Women (%)		75	71†	59
Institutionalized (%)		45‡	28‡	7
Homebound (%)		30‡	21‡	6
Moderately disabled (%)		52†	47‡	30
Mini-Mental State Examination score		25.1‡	26.1‡	27.3
Level of education (%)	Lowest	36	42	38
	Highest	10	8	9
Diabetes mellitus (%)		12	6	11
Visited ophthalmologist for other reason than to obtain glasses (%)		77‡	59†	41

* Values are means, adjusted for age and gender, unless otherwise indicated.
† P<0.05 for the difference with subjects with visual acuity ≥0.3 (Snellen, 20/60)
‡ P<0.001 for the difference with subjects with visual acuity ≥0.3 (Snellen, 20/60)

The prevalence of blindness and visual impairment stratified by age and gender is given in Table 2. Whatever criteria were used, blindness and visual impairment showed a significant increase in prevalence in subjects aged 75 years or older. Women had

slightly higher prevalences of blindness or visual impairment in most age strata, although the differences were not statistically significant after additional adjustment for age within the age strata. Figure 1 shows a comparison of our data with prevalence data from other studies based on white populations. Compared with the results of other studies, the prevalence of blindness and visual impairment in the Rotterdam Study was low for all groups older than 55 years, although the Rotterdam Study did not have the lowest prevalence at every age point.

Table 2. Prevalence of blindness and visual impairment according to WHO and US criteria, stratified by age and gender*

		Blindness		Visual impairment	
Age (yrs)	Total no.	WHO	US	WHO	US
Men		% Percentage of study subjects (n)			
55-64	1097	0.1 (1)	0.1 (1)	0.1 (1)	0.3 (3)
65-74	1054	0.2 (2)	0.2 (2)	0.1 (1)	0.7 (7)
75-84	504	0.4 (2)	1.0 (5)	2.2 (11)	6.0 (30)
≥ 85	89	1.1 (1)	3.4 (3)	9.0 (8)	28.1 (25)
Total	2744	0.2 (6)	0.4 (11)	0.8 (21)	2.4 (65)
Women					
55-64	1464	0.1 (2)	0.2 (3)	0.1 (2)	0.5 (7)
65-74	1354	0.1 (2)	0.1 (2)	0.6 (8)	1.0 (13)
75-84	894	0.8 (7)	1.6 (14)	2.8 (25)	8.5 (76)
≥ 85	319	4.7 (15)	6.6 (21)	12.5 (40)	30.4 (97)
Total	4031	0.6 (26)	1.0 (40)	1.9 (75)	4.8 (193)

* WHO indicates World Health Organization. Data are given as percentage (number) of study subjects.

In most subjects, the cause of visual loss was the same for both eyes. However, in 3 (9%) of the 32 blind subjects and in 18 (19%) of the 96 visually impaired subjects, the two eyes had different causes of visual loss. For this reason, the prevalences of the various causes of visual loss are most clearly presented as percentages of eyes rather than percentages of subjects. Table 3 gives the causes of blindness for three age categories. Optic neuropathy was the most frequent cause of blindness for subjects

aged 55 to 74 years. In subjects aged 75 years or older, age-related macular degeneration became the most important cause of blindness and was most apparent in the oldest age category. Primary open-angle glaucoma and cataract were second and third most important causes of blindness, respectively. In the two cases of combined mechanisms, we could not determine which disorder limited vision the most, myopic macular degeneration or primary open-angle glaucoma. The rare causes included pigment dispersion syndrome with secondary glaucoma, congenital syphilis, and atrophy of the eyeballs as a complication of surgery to correct a retinal detachment

Table 3. Causes of blindness in 64 eyes of 32 blind subjects, stratified by age*

Cause	55-74 yrs (14 eyes)	75-84 yrs (18 eyes)	≥ 85 yrs (32 eyes)	All (64 eyes)
Age-related macular degeneration	14 (2)	56 (10)	78 (25)	58 (37)
Cataract	0 (0)	11 (2)	6 (2)	6 (4)
Primary open angle glaucoma	14 (2)	0 (0)	9 (3)	8 (5)
Myopic degeneration	14 (2)	0 (0)	6 (2)	6 (4)
Optic neuropathy	29 (4)	0 (0)	0 (0)	6 (4)
Retinitis pigmentosa	14 (2)	0 (0)	0 (0)	3 (2)
Rare causes	14 (2)	22 (4)	0 (0)	9 (6)
Combined mechanisms	0 (0)	11 (2)	0 (0)	3 (2)

* Blindness according to WHO-classification: best-corrected visual acuity < 0.05 (Snellen, 20/40) in the better eye. Data are given as percentage (number) of eyes.

The causes of visual impairment according to WHO criteria are listed in Table 4. Myopic macular degeneration was the predominant cause of visual impairment in subjects younger than 75 years. For subjects aged 75 years or older, cataract, as a single cause or in combination with other disorders, became the leading contributor to visual impairment. In 62 (65%) of the 96 visually impaired subjects aged 85 years or older, cataract contributed at least partially to the visual impairment. The disorder most frequently accompanying cataract as a cause of impaired vision was age-related macular degeneration, followed by primary open-angle glaucoma. The combined mechanisms included corneal dystrophy with macular hole, myopic macular degeneration with optic neuropathy, and age-related macular degeneration with

primary open-angle glaucoma. The rare causes comprised hereditary macular degeneration, neuroretinitis, enucleation of the eyeball after complications of combined surgery to treat glaucoma and cataract, retinopathy without a known cause, and venous branch occlusion.

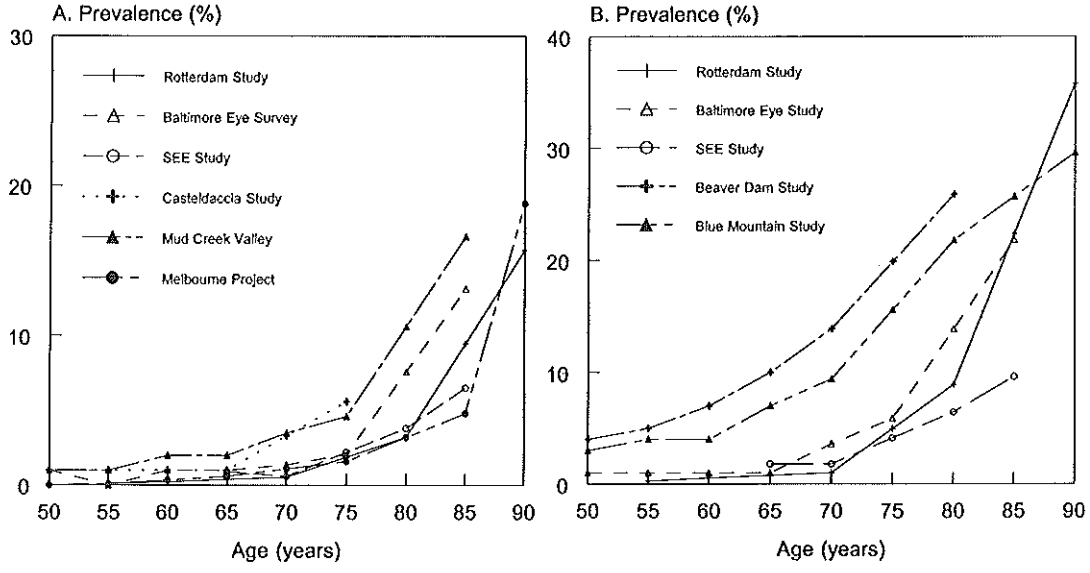

Figure 1. ***Total prevalence of poor vision (blindness and visual impairment) as a function of age, according to (A) World Health Organisation criteria and to (B) criteria used in the United States***

Figure 2 shows, by age, the proportions of poor vision (blindness and visual impairment, WHO-criteria) caused by age-related macular degeneration, age-related cataract and primary open-angle glaucoma. As single causes, these 3 disorders comprised the largest part of the increase in the prevalence of poor vision; combinations of these disorders and other single causes increased the prevalence of impaired vision only moderately with age.

Of the three major causes, age-related cataract is the only cause for which treatment may be sufficiently successful to restore vision. Cataract-extraction was a common surgical procedure in our study population of 6775 subject; its overall prevalence was 5.5% (371 subjects), ranging from 1.4% (35 subjects) in persons aged 55 to 65 years to 21.3% (87 subjects) in persons aged 85 years or older. Cataract extraction had prevented or treated possible bilateral blindness and visual impairment (as bilateral

cataract extraction or unilateral extraction with blindness or visual impairment in the other eye) in 3.6% (234 subjects) of the total study population and up to 15.2% (62 subjects) of persons aged 85 years or older. Of interest is that more than half (53%) of the subjects who were blind or visually impaired owning to untreated cataract indicated that they had never visited an ophthalmologist. To identify possible reasons for not seeking appropriate care, we compared the variables listed in Table 1 between subjects who were bilaterally blind or visually impaired due solely by cataract (n=34) and subjects who had undergone surgery to treat cataract (n=371). Compared with subjects who had undergone surgery to treat cataract, blindness or visual impairment due to untreated cataract was associated with a higher proportion of subjects over 85 years (21% vs 58%, respectively, P<0.001), being homebound owing to health reasons (17% vs 33%, respectively, age-adjusted, P=0.03), and a higher proportion of low scores (≤20) on the Mini Mental State Examination (6% vs 20%, respectively, age-adjusted, P=0.004). Differences in gender and level of education were not statistically significant; however, none of the subjects with untreated cataract had attained university or higher vocational education.

Table 4. Causes of visual impairment in 192 eyes of 96 visually impaired subjects, stratified by age*

Cause	55-74 yr	75-84 yr	≥ 85 yr	All
Age-related macular degeneration	5 (1)	28 (21)	27 (26)	25 (48)
Cataract	18 (4)	35 (26)	42 (40)	36 (70)
Cataract in combination with another cause	18 (4)	12 (9)	23 (22)	18 (35)
Primary open angle glaucoma	0 (0)	3 (2)	1 (1)	2 (3)
Myopic macular degeneration	23 (5)	4 (3)	3 (3)	6 (11)
Optic neuropathy	9 (2)	0 (0)	0 (0)	1 (2)
Corneal dystrophy	9 (2)	1 (1)	0 (0)	2 (3)
Diabetic retinopathy	9 (2)	0 (0)	0 (0)	1 (2)
Rare causes	9 (2)	11 (8)	3 (3)	6 (13)
Combined mechanisms	0 (0)	5 (4)	1 (1)	3 (5)

* Visual impairment according to WHO-classification; best-corrected visual acuity ≥0.05 (Snellen, 20/40) and <0.3 (Snellen, 20/60) in the better eye. Data are given as percentage (number) of eyes.

DISCUSSION

We have presented age-specific prevalences and causes of blindness and visual impairment in a population ranging in age from 55 to 106 years. Our data indicate that AMD is the main contributor to the exponential increase in the prevalence of blindness in persons aged 75 years or older and that age-related cataract causes the major increase of visual impairment. Myopic macular degeneration, optic neuropathy, and various other less frequent disorders have important contributions to the poor vision occurring before the age of 75 years.

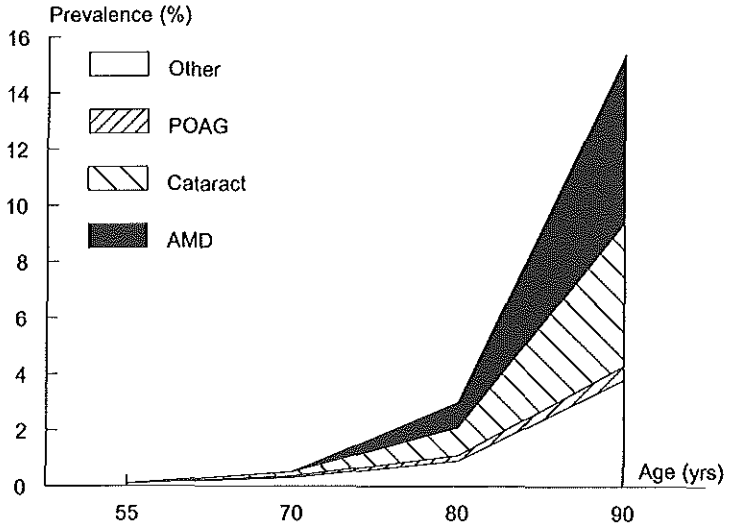

Figure 2. ***Total prevalence of poor vision (blindness and visual impairment according to WHO-criteria) as a function of age, specified by cause. AMD indicates age-related macular degeneration; POAG, primary open-angle glaucoma***

All population-based studies during the 1990s on the prevalence of blindness and visual impairment show an exponential increase with age.[2-10] However, the age-specific prevalences vary considerably among studies (figure 1). Although the variations may be due to study design, population sampling, or differences in measuring techniques, they may indicate real geographic variation in prevalence and

course of vision-impairing disorders. This points out a need for detailed information on the age-specific causes of impaired vision.

The size of our study enables relatively precise estimates of the prevalence of blindness and visual impairment and facilitates an accurate determination of the proportions of causes. Our population was predominantly white, and because black populations are known to have higher prevalence of poor vision,[18] we limited a comparison of prevalences to white populations. Our age-specific prevalences were similar to the SEE Study[9] and the Melbourne Visual Impairment Project,[10] but lower compared with all other studies. The Beaver Dam and Blue Mountain Eye Studies showed substantially higher prevalences.[7,8] This may be due in part to differences in definition because those two studies included a visual acuity of 0.5 (20/40) in the definition of visual impairment. When comparing only legal blindness, the prevalences were still higher, but closer to our data (data not shown).

A major concern in prevalence studies is non-participation. The Rotterdam Study had a reasonable response, but evaluation of differences between participants and nonparticipants indicated that nonresponse was selective and may have produced an underestimate of the prevalences of blindness and visual impairment. A higher proportion of nonparticipants among the oldest subjects is a general problem in studies of elderly populations, as is the higher nonresponse among subjects with poor physical or mental health. Our study consisted of more subjects aged 85 years or older than the other studies, and especially for this age-group, the true prevalence must be even higher. We consider it unlikely that non-response influenced the proportions of causes of blindness and visual impairment.

Knowledge of the age-specific causes of blindness elucidates the increase in prevalence of impaired vision and may facilitate adequate management. In this study, most of the disorders responsible for blindness and visual impairment were age-related, mostly of unknown cause, and, as yet, unpreventable. We confirm the observation that AMD is the leading cause of blindness among white populations,[8, 18, 19] but this was true only for subjects aged 75 years or older. Then it became the main contributor to the steep increase in the prevalence of blindness, leading to bilateral blindness as a single cause in 12 (3%) of all 408 subjects aged 85 years or older in the present study. In common with other studies, age-related cataract was the most important cause of bilateral visual impairment,[5,20] and the second most frequent cause of blindness. The visual impairing effect of cataract was highly associated with age, causing a larger proportion of visual impairment with increasing age. Successful treatment for this disorder is readily available; cataract extraction is one of the most frequent surgical procedures in the Netherlands (Netherlands Foundation of

Information Systems for Health Care[SIG-Zorginformatie], National Medical Registration, Utrecht, the Netherlands).[21] If adequate facilities and personnel are not a logistic constraint to treatment, why does cataract still impair vision to such a great extent in the elderly? Before we enlarge on this issue, we emphasize that 62 (15%) of 408 subjects aged 85 years or older were "saved" from bilaterally blindness or visual impairment by cataract-extraction, a much greater proportion than the 32 (8%) that were blind or visually impaired by cataract. Most of the subjects in the latter category received no eye care. Our study provides limited information on possible barriers, but old age, unawareness of treatment possibilities and comorbidity (with other disabling disorders) seem to hamper access to appropriate care. Policies to implement referrals on a more uniform basis are needed, for even in the very old or disabled, restoration of visual function may improve the quality of life and reduce the nursing care required.

Diabetes mellitus was a frequent disorder in our population, but diabetic retinopathy rarely led to poor vision. Although findings from studies on subjects with diabetes suggest a larger influence of diabetes on visual loss,[21,22] diabetic retinopathy was not a major cause of blindness in any of the other population studies of older white populations.[18-20] As described by Stolk et al,[22] active proliferative retinopathy was not observed in the Rotterdam Study. In addition, a low frequency of laser photocoagulation scars indicated that the absence of active proliferative retinopathy did not directly result from ocular treatment. Selective nonresponse of subjects with diabetes with known complications may have occurred, although the frequencies of the use of antidiabetic medication between participants and nonparticipants were similar. Possible explanations for the small impact of diabetes on vision in this relatively old population are selective mortality of persons with diabetes with severe systemic complications, the uncommon progression from background retinopathy to proliferative retinopathy in the elderly,[22,24] and the intensified control of hyperglycemia in persons with diabetes.[25,26]

Our data indicate that age must be specified when determining the frequency of causes of visual loss. Appropriate medical care to further reduce the prevalence of blindness is not available, but improving accessibility to surgery for the treatment of cataract among the old and disabled will help diminish the number of untreated cataracts that still leads to visual impairment.

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Part III

Genetic risk of age-related maculopathy

Chapter

5

Genetic risk of age-related maculopathy
A population-based familial aggregation study

Abstract

Objective: To investigate to what extent ARM is genetically determined.

Design and Setting: Familial aggregation study based on probands derived from the population-based Rotterdam Study.

Participants: First degree relatives of 87 cases with late ARM, i.e., atrophic or neovascular macular degeneration, were compared with first degree relatives of 135 controls without ARM.

Main Outcome Measures: Presence and stage of ARM as diagnosed on fundus transparencies; odds ratio; lifetime risk; risk ratio; population attributable risk.

Results: Independent of other risk factors, the prevalence of early (OR 4.8; 95% CI 1.8-12.2) and late (OR 19.8; 95% CI 3.1-126)ARM was significantly higher in relatives of cases with late ARM. The lifetime risk estimate of late ARM was 50% (95% CI 26%-73%) for relatives of cases versus 12% (95% CI 2%-16%) for relatives of controls ($P<0.001$), yielding a risk ratio of 4.2 (95% CI 2.6-6.8). Relatives of cases expressed the various features of ARM at a younger age. The population attributable risk related to genetic factors was 23%.

Conclusions: First degree relatives of cases with late ARM developed ARM at an increased rate at a relatively young age. Our findings indicate that approximately one fourth of all late ARM is genetically determined and suggests that genetic susceptibility may play an important role in determining the onset of disease.

INTRODUCTION

ARM is by far the leading cause of blindness in the elderly of developed countries.[1] The prevalence and severity of ARM increase substantially with age. By the age of 80 years, approximately 10% of patients have developed one of the two late stages of ARM, atrophic or neovascular macular degeneration.[2] Treatment such as laser photocoagulation is available for only a minority of patients, and even then improvement of visual function is limited.[3] The growing population of elderly and increased life expectancy necessitate research into the causes and risk factors of this disease.

The etiology of ARM is largely unknown, but environmental factors and genetic factors have been implicated in the disease. Environmental factors that have been associated are cardiovascular risk factors such as smoking,[4-6] atherosclerosis,[7] and estrogens.[8,9] A role for genetic factors has been supported by various twin studies,[9,10] and by a population-based segregation study.[12] Recent findings from a molecular study suggest that the Stargardt ABCR gene may be associated with ARM.[13]

The presence of a genetic component may be widely acknowledged, the magnitude of its causative role is controversial. Results of two clinic-based studies show familial aggregation of ARM and estimate a familial risk of 19.3 and 2.4, respectively.[14,15] Possible explanations for this large difference are the high chance of selection bias with hospital-derived probands, the large range of ARM features that were combined, and the use of family history and self-reported diagnoses.

The purpose of this study was to investigate to what extent ARM is genetically determined, on the basis of a collection of population-derived probands ascertained without regard to family history, and selected without regard to any known risk factors. We determined the diagnosis of ARM in first-degree relatives by actual examination using a standardized protocol, defined the risk of ARM for these relatives, and identified those factors associated with increased risk. Furthermore, we aimed to quantify the attributable risk of genetic factors to the overall occurrence of late ARM.

SUBJECTS AND METHODS

Collection of families

All probands were derived from the Rotterdam Study, a population-based prospective follow-up study in the Netherlands of subjects aged 55 years and over. The rationale and design of the Rotterdam Study have been described elsewhere.[3,16] In brief, 6775

participants were ophthalmologically examined; the diagnosis of ARM was based on grading of fundus transparencies according to an internationally accepted classification system.[17] For the present study, we identified all subjects with atrophic or neovascular macular degeneration as case probands (n=101). As control probands (n=154), we randomly selected a sample of study participants who did not have any features of ARM, i.e., no soft drusen of intermediate (63-124μm) or large (≥125μm) size and no late ARM, i.e., no atrophic or neovascular macular degeneration. Probands differed in age (mean age of cases vs controls 81.9 vs 76.7 years; P<0.001), but not in gender (cases vs controls, 63% vs 56% women; age-adjusted P=0.64). Eligible relatives were siblings and offspring of cases and controls living in the Netherlands or Belgium who could be contacted by letter and telephone. Relatives were invited for an extensive screening examination at the research center of the Rotterdam Study, located in Ommoord; those who were homebound were examined at their home. The study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, the Netherlands, and written informed consent was obtained from all participants.

Diagnosis

The ophthalmological examination included measurements of best-corrected visual acuity, ophthalmoscopy, and fundus photography. After mydriasis, 20° stereoscopic fundus color transparencies (Topcon TRC-SS2 stereoscopic fundus camera, Topcon Optical Company, Tokyo, Japan) and 35° color transparencies (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan) were taken of the macular area. Participants who were examined at their home were photographed with a portable camera (35° fields, Kowa RC-2 fundus camera, Kowa Corporation LTD, Tokyo, Japan). Fundus transparencies were graded for presence of ARM in a masked fashion according to the International Classification System,[17] identical to the protocol that was used for probands. In accordance with this system, drusen larger than 63 μm, increased pigmentation, RPE-degeneration, atrophic macular degeneration (geographic atrophy) or neovascular macular degeneration present in the macular grid area (radius, 3000 μm) were considered outcomes of ARM. These lesions were subsequently stratified into four exclusive stages increasing clinical severity: no ARM was defined as the absence of any type of soft drusen (>63μm), atrophic or neovascular macular degeneration;[17] preliminary or very early ARM as the presence of soft distinct drusen without pigmentary irregularities; early ARM as either the presence of soft indistinct or reticular drusen or the presence of both soft distinct drusen and pigmentary irregularities;[18,19] and late ARM as the presence of atrophic or neovascular macular degeneration.[17]

Environmental risk factors

To investigate whether familial aggregation of ARM could be caused by clustering of environmental risk factors, we considered smoking, atherosclerosis, and estrogen deficiency as potential correlates of ARM.

Presence of atherosclerosis was non-invasively assessed using ultrasound as described earlier.[20, 21] Peripheral arterial disease was judged to be present when the ankle-brachial systolic blood pressure ratio was less than 0.90. Participants were questioned about current and former smoking by interview, and women were asked about age and type of menopause, use of contraceptives and post-menopausal estrogen therapy.

Statistical analyses

Prevalence of ARM lesions, adjusted for age and gender, was compared between siblings of cases and siblings of controls, and between offspring of both groups. The prevalence odds ratio was estimated for siblings and offspring of cases using multiple logistic regression analysis, with siblings and offspring of controls as reference categories, and early and late ARM as outcomes. Odds ratios were adjusted for age and gender, and in additional analyses, for smoking, peripheral arterial disease, early menopause and exogenous estrogen use. Interaction between genetic factors and smoking was studied by performing a stratified analysis and by performing the analysis on the full data set, including the product term for smoking and proband status (case or control).

The cumulative risk estimating the lifetime absolute risk of ARM for relatives of cases and controls was calculated using Kaplan-Meier product-limit survival analysis with early and late ARM as outcomes. Participants above 85 years were pooled to maintain unbiased estimates.[22] Cumulative risks were compared between groups using the logrank test.

The attributable proportion of genetic factors to the occurrence of late ARM in the exposed and general population was estimated using the formulas presented by Miettinen.[23] The attributable proportion for genetically exposed (Ape) was calculated with the formula

$$Ape = \frac{RR - 1}{RR},$$

where RR is the relative risk. The attributable proportion for the total population (App) was calculated with

$$App = Ape * Pe,$$

where Pe is the proportion genetically exposed in the cases.

Table 1. General characteristics*

		Siblings		Offspring	
		of cases (n=73)	of controls (n=142)	of cases (n=113)	of controls (n=201)
Age in years (SD)		76 (9)	75 (9)	54 (10)‡	49 (9)
Age range (years)		59-96	45-96	30-74	23-73
Gender (% women)		55	58	43	46
Smoking (%)	Current	18	9	32	28
	Former	46	49	41	42
	Never	36	42	27	30
Peripheral atherosclerosis (%)		12	16	5	2
Menopause < 45 years (%)†		13	13	0	0
Ever used oral contraceptives (%)†		20	15	76	86
Ever used postmenopausal estrogens		16	17	16	21

* Values are given as means (SD) or proportions, adjusted for age and gender.
† Percentage of women
‡ P < 0.001 for the difference with relatives of controls

RESULTS

Family description

The overall response of eligible subjects was 83.6%. Of cases, 87 (86.1%) gave permission to contact their families; 34 cases had been diagnosed with atrophic macular degeneration and 53 as having neovascular macular degeneration. Of controls, 135 (87.7%) consented. Of relatives of case probands, 73 (85%) of 86 siblings as well as 113 (86.2%) of 131 children agreed to participate, of relatives of controls, 142 (79.8%) of 178 siblings and 201 (81%) of 248 children participated. The frequency of home visits was equally distributed among the participating relatives of cases and controls (16.7% and 17.5%,respectively; P=0.90).

Table 2. Prevalence of ARM-characteristics*

		Siblings		Offspring	
		of cases (n=73)	of controls (n=142)	of cases (n=113)	of controls (n=201)
Drusen					
Largest size, total number					
63-124 μm	1-9	33	31	32	23
	≥ 10	8	4	1	1
≥125 μm	1-9	19†	7	5	2
	≥ 10	7	3	2	0
Most severe type	soft distinct	39	35	34	25
	soft indistinct	17†	6	4	1
	reticular	0	1	0	0
Grid area occupied	< 10%	89†	97	96†	100
by drusen ‡	10-24%	7†	1	2	0
	≥ 25%	4	2	2	0
Pigmentary irregularities					
Increased pigment		14†	4	4	1
RPE-degeneration		11†	4	6†	1
Late ARM					
Atrophic macular degeneration		10†	1	0	0
Neovascular macular degeneration		4	2	1	0

* Values are given as proportions in percentages adjusted for age and gender. Features may occur concurrently and do not add up to 100%.
† $P < 0.05$ for the difference with relatives of controls
‡ Circular grid with radius 3000 μm centered on the fovea

Table 1 shows the distribution of age, gender, and risk factors among relatives. There were no significant differences in these characteristics between groups, except for the age distribution among offspring. Smoking appeared to be more frequent among relatives of cases but did not differ significantly from relatives of controls.

The prevalences of the various features of ARM are given in Table 2. Although siblings had higher frequencies of almost all ARM characteristics than offspring, in siblings and offspring these lesions were significantly more frequent among relatives of cases than among relatives of controls. Features given in Table 2 may overlap; hence, we subsequently calculated the prevalence of ARM by exclusive stages of disease. For siblings: the prevalence of no ARM was 35.5% for siblings of cases vs 57.8% for siblings of controls (P=0.001, age and sex adjusted); the prevalence of very early signs of ARM was 41.6% vs 37.1% (P=0.52, age and sex adjusted); the prevalence of early ARM was 9.5% vs 2.9% (P=0.04, age and sex adjusted); and the prevalence of late ARM was 13.4% vs 2.2%, (P=0.001; age and sex adjusted) respectively. For offspring: the prevalence of no ARM was 57.4% for offspring of cases vs 72.4% for offspring of controls (P=0.02, age and sex adjusted); the prevalence of very early signs of ARM was 34.9% vs 25.7% (P=0.09, age and sex adjusted); the prevalence of early ARM was 6.3% vs 1.9% (P=0.05, age and sex adjusted); and late ARM was present only in 1.4% offspring of cases (P=0.20, age and sex adjusted).

In the nuclear families of cases, 10 siblings and 2 children were identified with late ARM. To investigate whether there was an association with subtype of macular degeneration, we determined the concordance of subtype in the 12 relative-proband pairs with late ARM. The concordance was low as only three pairs had the same type of late ARM (neovascular macular degeneration).

Table 3. Odds ratio of early ARM for first degree relatives of cases*

	Early	No ARM	OR† (95% CI)	OR‡ (95% CI)
Siblings of cases	15	22	4.5 (1.8-11.3)	4.8 (1.8-12.2)
Siblings of controls	12	77		
Offspring of cases	8	60	4.9 (1.2-20.6)	6.6 (1.4, 31.8)
Offspring of controls	3	150		

* Early ARM indicates age-related maculopathy, defined as either the presence of soft indistinct or reticular drusen or the presence of both soft distinct drusen and pigmentary irregularities; OR, odds ratio; and CI, confidence interval.

†Adjusted for age and gender

‡Adjusted for age, gender, smoking, and atherosclerosis

Genetic risk estimates

Relatives of cases had an increased risk of ARM when compared with relatives of controls (Tables 3 and 4). For siblings, the point estimate of the odds ratio increased

with greater severity of ARM (odds ratio point estimate of early ARM 4.5; of late ARM 14.3). However, confidence intervals were wide. For offspring, the odds ratio estimate of early ARM (4.9) was similar to the estimate for siblings. The strength of the associations did not diminish after adjustment for smoking and atherosclerosis, or after additional adjustment for early menopause and exogenous estrogen use in women (latter data not shown), indicating that the associations were not confounded by familial clustering of these risk factors. Furthermore, we found no statistical evidence for interaction between familial risk and smoking (data not shown).

Kaplan-Meier product-limit estimates indicated that the lifetime absolute risk of developing early ARM by the age of 85 years (Figure 1a) was 48% (95% CI=31%-65%) for relatives of cases, whereas this risk was 23% (95% CI=10%-37%) for relatives of controls (P=0.001), yielding a risk ratio of 2.1 (95% CI=1.4, 3.1) and a risk difference of 25%. The lifetime absolute risk of developing late ARM by age 85 years (Figure 1b) was 50% (95%CI=26%-73%) for relatives of cases vs 12% (95% CI=2%-16%) for relatives of controls (P<0.001), yielding a risk ratio of 4.2 (95% CI=2.6-6.8) and a risk difference of 38%. Although the pattern was most pronounced for late ARM, both cumulative risk curves showed similar patterns, with an earlier rise in risk for relatives of cases. When relatives were stratified by proband gender, no significant evidence for difference in risk of early or late ARM was obtained. When relatives were stratified by proband subtype of ARM, i.e., atrophic or neovascular macular degeneration, there was no significant difference in cumulative risk of early ARM. This showed that genetic risk was not confined to gender or subtype of late ARM.

Table 4. Odds ratio of late ARM for first degree relatives of cases*

	Late ARM	No ARM	OR† (95% CI)	OR‡ (95% CI)
Siblings of cases	10	22	14.3 (3.0-67.8)	19.8 (3.1-126)
Siblings of controls	3	77		
Offspring of cases	2	60	-	-
Offspring of controls	0	150		

*Late ARM indicates age-related maculopathy, defined as the presence of atrophic or neovascular AMD; OR, odds ratio; CI, confidence interval.

†Adjusted for age and gender

‡Adjusted for age, gender, smoking, and atherosclerosis

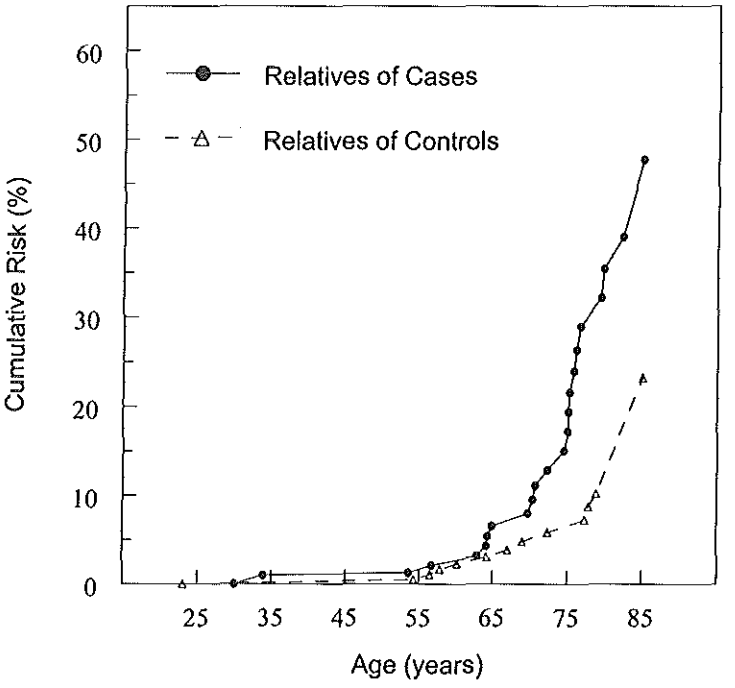

Figure 1a. ***Comparison of cumulative risk of early ARM, i.e., either soft indistinct or reticular drusen or both soft distinct drusen and pigmentary irregularities, between relatives of cases and relatives of controls***

Attributable risk

We restricted the calculation of the attributable risk related to genetic factors to late ARM because this is the clinically most relevant stage and the diagnosis on which cases had been selected. The attributable proportion, or excess case load, was calculated for the genetically exposed participants and for the total population using the ratio of the cumulative risks of late ARM in relatives as the best approximation of the true relative risk for genetic factors (RR=4.2) in the *Ape* and *App* formulas. The attributable proportion among the genetically exposed (Ape) was 76%, i.e., in 76% of the participants with a familial occurrence the disease may be attributed to a genetic component. We estimated the proportion of exposed cases (Pe) as the ratio of case probands with affected relatives divided by all case probands with relatives who were at least 68 years old, which was the minimum age of late ARM onset in our study. This proportion was 12 of 39, and subsequently we calculated that the proportion of late ARM in the total population that may be attributed to a genetic component (App) was 23%.

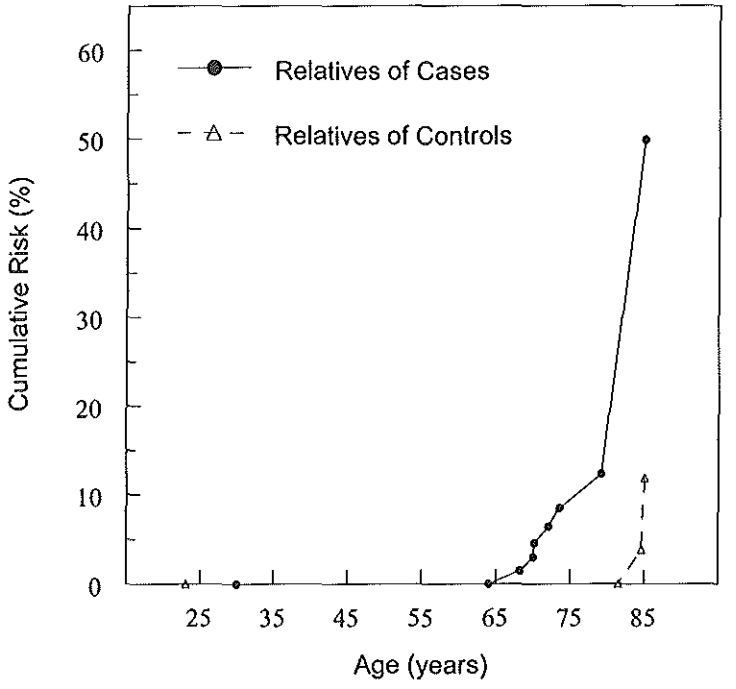

Figure 1b. ***Comparison of cumulative risk of late ARM, i.e., atrophic or neovascular macular degeneration, between relatives of cases and relatives of controls***

DISCUSSION

We demonstrated that ARM aggregates in families of a general white population, which we cannot attribute to clustering of known risk factors. Independent of smoking, atherosclerosis and early menopause, first degree relatives of cases with late ARM had a substantial excess risk of developing ARM. Their life time absolute risk to be likewise affected by late ARM was 50%. Results of our study suggests that almost one fourth of all late ARM in the general population may be caused by a genetic component.

The design of our study has several benefits. First, we took advantage of the database from the Rotterdam Study, which included detailed information on ARM in a population-based setting. This enabled us to ascertain probands without knowledge of family history and without regard to any known risk factors. Previous studies have used hospital registries for sampling probands, where differential referral of patients according to family history or other correlates of disease may have distorted results.

Second, in contrast to others, we did not rely on family history but actually examined all first-degree relatives. Third, because ARM is clinically heterogeneous with a large range of variance in its manifestations and an age-dependent penetrance, enforcement of standard clinical criteria is important. We based the diagnosis of ARM on a masked grading of fundus transparencies using internationally accepted criteria. For probands we used rigorous criteria and selected cases and controls who were at either end of the clinical spectrum to improve classification of truly affected and unaffected participants. By contrast, we registered all characteristics of ARM in relatives and stratified them according to stage of disease to study aggregation of the entire spectrum of ARM in the families.

There are also several limitations to our study. The size of the study was relatively small, which resulted in imprecise risk estimates, and low statistical power to detect interaction with environmental factors. Only larger studies can overcome this problem. Another issue is the age-distribution of the study participants. Although siblings of both groups were similar in age, offspring of cases were significantly older than offspring of controls. This may have distorted the prevalence odds ratios for offspring estimated with logistic regression analysis. Distortion of other risk estimates is less likely, because they were based on the Kaplan-Meier analysis which carefully accounts for age at examination. The last point is the limited study of potential confounding variables. The environmental factors that we considered were those risk factors identified in the Rotterdam Study.[4,5,7,8] Other environmental factors such as diet[24,25] and cholesterol level[26] have been suggested, but the risk associations are inconclusive and could not be replicated in the Rotterdam Study (Vingerling and Klaver, unpublished data). Simple clustering of unknown risk factors may partly account for our findings, but it has been shown that genetic factors are the most likely explanation for strong familial aggregation.[27]

The notion has long existed that genetic susceptibility is one of the strongest risk factors for ARM apart from advanced age. However, it has remained unclear to what extent ARM is genetically determined. We based the relative risk for a genetic component on the proportions affected in relatives of affected and unaffected subjects, and estimated the odds ratio and the ratio of cumulative lifetime risks. The latter was estimated with Kaplan Meier product-limit analysis, which censored participants who had not developed the disease at the time of examination and thereby accomplished an adjustment for age-dependent expression. Given this benefit, the 4.2 ratio of cumulative lifetime risks is the better estimate of the true lifetime relative risk of late ARM for first degree relatives. We based the attributable risk on this ratio and on the proportion exposed cases, considering those having an affected relative to be exposed.

We limited our analysis to cases with relatives aged 68 years or older, but our proportion of 23% may be an underestimation of the true attributable risk if exposed cases have relatives who have not developed the disease .

Although both cumulative risk curves of ARM demonstrated an exponential rise in risk, the curve for relatives of cases shifted to the left, suggesting that a strong effect of familial predisposition is an earlier onset of disease. This contention is supported by the observation that the frequencies of ARM features were remarkably similar between offspring of cases and siblings of controls, whereas they differed approximately 20 years in age (Table 2). Unfortunately, our study had no information on age of disease onset, and we, therefore, could not make a distinction in familial risk for probands with early vs late onset of ARM. A higher risk for probands with an early onset would have added to the evidence of an association between age at onset and familial risk. Nevertheless, it is an interesting observation that needs further exploration, for knowledge of this relation will direct genetic research to focus on subjects with a high familial risk of disease.

Whether differences in phenotypic manifestations of ARM reflect differences in genetic background has been subject for debate. Various reports describe familial occurrence of drusen only,[28, 29] whereas Klein et al[10] report a striking similarity of late ARM features in monozygotic twins. On the other hand, a recent publication[30] reporting eight families with a high prevalence of ARM describes a large variance of ARM features among relatives. We compared families of subjects with late stages of ARM with families of participants without any manifestations of disease. All early and late manifestations of ARM occurred more frequently among relatives of cases. Concordance of ARM features between family members was low apart from stage of disease, and there was no difference in familial risk between probands with atrophic vs neovascular macular degeneration. Hence, genetic susceptibility to late ARM increased expression of all manifestations of ARM, with the highest risk for either type of late ARM.

In conclusion, we showed that all manifestations of ARM occur at a higher frequency and at an earlier age in relatives of cases with late ARM. The high relative and attributable risks demonstrate that genetic factors play a major role in the cause and overall occurrence of ARM. Further studies are needed to reveal whether this genetic contribution is mainly caused by a major gene, the result of several genes, or involves interaction with other risk factors. This will improve understanding of the molecular basis of this disease and may eventually lead to strategies for prevention and treatment.

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Chapter

6

Heterogeneity of the genetic risk in age-related maculopathy

Abstract

Earlier studies demonstrated an increased risk of age-related maculopathy (ARM) for first degree relatives of affected subjects. We aimed to assess whether the genetic risk of ARM shows heterogeneity among families. Case (n=64) and control (n=100) probands were selected from the population-based Rotterdam Study, and first degree relatives were examined for diagnosis of ARM by fundus photography. The family score method was used to estimate a risk for each family taking into account the risk of disease expected on the basis of age. Families of cases were at a higher risk of ARM than families of controls. Familial heterogeneity of risk was suggested within the case families, with 17% at low familial risk of ARM, 13% at intermediate risk, and 3% at high familial risk of ARM. Subjects with an intermediate or high familial risk were 30 times (OR 29.9, 95% CI 3.4, 262) more likely to develop ARM than subjects with no excess risk. Our results show that the risk of ARM varies among families, and suggest that only a small fraction of all ARM is due to a strong genetic component.

INTRODUCTION

Age-related maculopathy (ARM) is becoming a frequent eye disease as the population ages, and it is currently the leading cause of blindness in developed countries.[1] It has been well recognized that genetic factors are implicated in the etiology of the disease,[2,3] but estimates of the strength of this component vary significantly. The relative risks reported for first degree relatives of cases ranges from 2.4 to 19.3.[4,5] One study suggested that ~56% of the total ARM variability was compatible with a single major gene.[6] In a previous familial aggregation study based on probands from the Rotterdam Study, we estimated that the life time relative risk of end stage ARM was 4.2 (95% CI 2.6, 6.8) for first degree relatives of cases, and calculated that genetic factors attributed ~23% to all end stage ARM in the population.[7]

Although the ABCR and APOE genes have been associated with ARM, the knowledge of the disease-causing genes is limited.[8-11] Most former study designs regarded ARM families as a genetically homogeneous population. In a complex disease such as ARM, that is rather unlikely. Presumably, there are families with a strong genetic susceptibility as well as families with only a mild or no genetic risk, and the relative frequencies of these families will determine the overall magnitude. In addition, knowledge of the familial risks is particularly relevant for clinical counseling.

In this report, we further explored the data of our previous familial aggregation study with the aim to detect variation in risk among ARM families. For each family, we calculated a risk score based on observed and expected number of affected relatives using demographic and epidemiologic data.[12] This methodology allowed us to discriminate between high and low risk families, to investigate their frequencies, and to assess their risk differences.

SUBJECTS AND METHODS

Collection of families

Design of the familial aggregation study and methods of data collection have been described previously.[7,13] In brief, all probands were identified from the baseline phase of the Rotterdam Study; case probands (n=101) were all subjects with atrophic or neovascular macular degeneration, and control probands (n=154) were a

randomly selected sample of study subjects who did not have any soft drusen (≥63μm), nor any atrophic or neovascular macular degeneration. Probands differed in age (cases mean age 81.9 years vs controls 76.7 years, $P<0.001$), but not in gender (cases 63% women vs controls 56% women, age-adjusted $P = 0.64$). Genealogical data of the last five generations were obtained from probands; no probands were genealogically linked. First degree relatives were subsequently invited for a screening examination at a research center or at home.

Diagnosis of ARM

The ophthalmologic examination included fundus photography of a 20° and 35° macular field with a stereoscopic (Topcon TRC-SS2 stereoscopic fundus camera, Topcon Optical Company, Tokyo, Japan) and monoscopic camera (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan). Subjects who were examined at home were photographed with a portable camera (35° field, Kowa RC-2 fundus camera, Kowa Corporation LTD, Tokyo, Japan). Fundus transparencies were graded for presence of ARM features in a masked fashion according to the International Classification System, identical to the protocol that was used for probands.[14] ARM was stratified in two stages of disease. Early ARM was defined as the presence of either soft distinct drusen with pigmentary changes or the presence of soft indistinct or reticular drusen. Late ARM was defined as the presence of atrophic (geographic atrophy) or neovascular AMD.

Table 1. Age-specific prevalence (%) of ARM in the baseline phase of the Rotterdam Study, 1989-1993

Age (years)	Early ARM	Late ARM	Total ARM
55 - 59	2.2	0.2	2.4
60 - 64	3.2	0.1	3.3
65 - 69	5.1	0.5	5.6
70 - 74	10.6	0.8	11.4
75 - 79	12.6	1.7	14.3
80 - 84	14.8	6.5	21.3
85 - 89	18.5	9.2	27.7
90+	21.2	17.7	38.9

Statistical analyses

Age-specific prevalences of ARM were determined from the baseline phase of the Rotterdam Study,[12] and they served as the expected outcome of ARM for each relative (Table 1). For each family, the expected number (E_i) of affected relatives for the ith family was compared to the observed number (O_i) to give a family score (FS) for this family as

$$FS = O_i - E_i.$$

This is a modified version of the method originally described by Houwing-Duistermaat et al.[15,16]

Family scores were subsequently stratified in four risk groups: no increased risk ($FS < 0.5$); low risk ($0.5 \leq FS < 1$); intermediate risk ($0.5 \leq FS < 1$); and high risk ($FS \geq 2$).[15-17] The relative frequencies of these strata were calculated. The risk of ARM was estimated for each stratum using logistic regression analysis, adjusting for the possible confounding effect of age, sex, smoking, and atherosclerosis.

Table 2. General characteristics of the study population

	Case families (n = 64)	Control families (n=100)
Total no. of relatives	186	343
No. of siblings	73	142
Mean age of siblings, yrs, ± SD	76.0 ± 8.7	75.4 ± 9.4
% women among siblings	55	59
No. of offspring	113	201
mean age of offspring, yrs, ± SD	53.7 ± 10.4*	48.8 ± 8.9
% women among offspring	43	46

* P < .001 for the difference with relatives of controls

RESULTS

Of case probands, 87 (86%) subjects consented to participation in the familial aggregation study; of control probands, 135 (88%) responded. Of the relatives of cases, 73 (85%) siblings and 113(86%) children responded. Of relatives of controls, these responses were 142 (80%) and 201(81%), respectively. This resulted in 64 case families and 100 control families available for the family score analyses. Table 2 shows the distribution of age, gender, and composition of the families; table 3 shows the number of affected and unaffected relatives among case and control families.

Table 3. Frequency of ARM among first degree relatives per family*

No. of relatives affected	Case families† (n = 64)	Control families (n = 100)
0	41 (64)	84 (84)
1	14 (22)	15 (15)
2	7 (11)	0 (0)
3+	2 (3)	1 (1)

* Frequencies in numbers (percentages)

† The difference in distribution of number of affected relatives between cases and controls was statistically significant, P=0.002

The individual family scores ranged from -0.9 to 3.3 in the entire study group. Table 4 shows the distribution of family scores among cases and controls. The family scores in the case families varied from -0.7 to 2.7. Eight families had a family score between 1 and 2, and 2 families had a score above 2. No significant differences were found in distribution of low, medium, and high family scores between probands with atrophic and neovascular AMD (data not shown). The family scores in the control families ranged from -0.9 and 0.8 with an outlier of one family with a family score of 3.3. This family consisted of 12 relatives of whom four were affected. Two affecteds were relatively young and largely determined this high family score. The control proband in question was still unaffected at the time of this analysis.

Table 4. Distribution of *Family Scores* in risk categories among case and control families*

Family score		Case families† (n=64)	Control families (n=100)
$FS < 0.5$	no risk	43 (67)	90 (90)
$0.5 \leq FS < 1$	low risk	11 (17)	9 (9)
$1 \leq FS < 2$	intermediate risk	8 (13)	0 (0)
$FS \geq 2$	high risk	2 (3)	1 (1)

* Frequencies in numbers (percentages)
† The difference in distribution of *FS* between cases and controls was statistically significant, P=0.0004

In Figure 1A and 1B, the relative frequencies of family scores are plotted. Among cases, there was a peak around zero with a skewed tail composed of families with higher than expected rates of ARM. Among controls, the distribution of the family scores was, apart from the outlier, centered around 0.

Table 5 shows the odds ratio of ARM for each risk stratum adjusted for age and gender, and smoking and atherosclerosis. Intermediate and high risk families were pooled since no control families contributed to the intermediate risk group. The risk of ARM increased with higher family scores; these risks further increased after additional adjustment for smoking and atherosclerosis.

Table 5. Odds ratio's of ARM for low, intermediate, and high family scores

Family score		ARM	no ARM	OR (95%CI)*	OR (95%CI)†
$FS < 0.5$	no risk	43	90	reference	reference
$0.5 \leq FS < 1$	low risk	11	9	2.8 (1.0, 7.8)	6.6 (1.8, 23.7)
$FS \geq 1$	intermediate and high risk	10	1	29.9 (3.4, 262)	47.1 (4.6, 484)

*Adjusted for age and gender of proband
†Adjusted for age, gender, smoking, and atherosclerosis

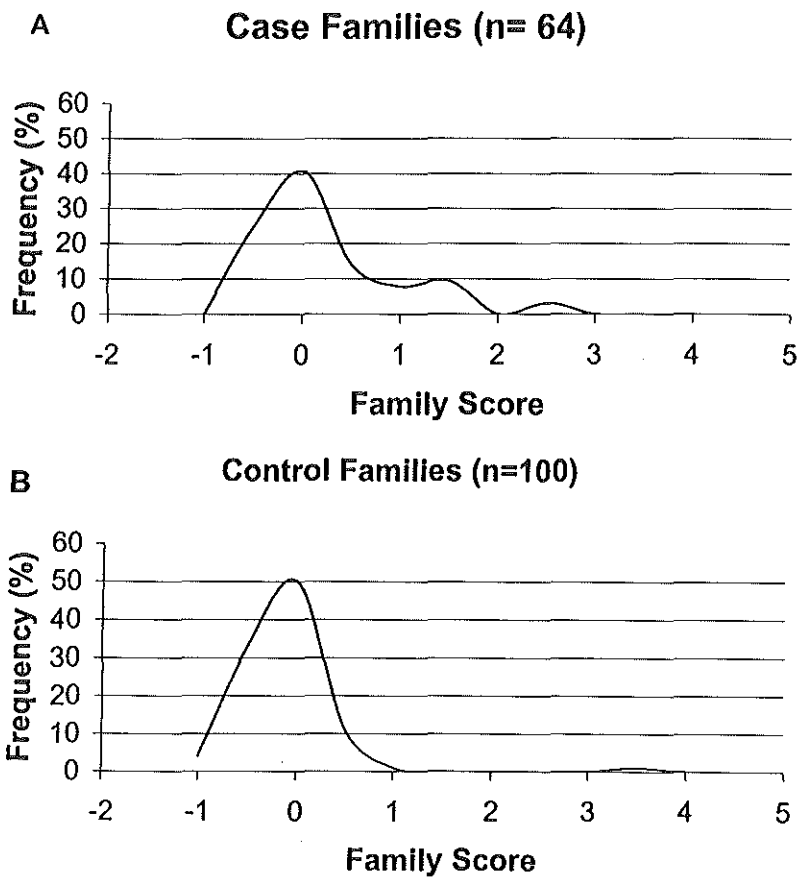

Figure 1. *Frequency (%) of family scores in case (A) and control (B) families*

DISCUSSION

In this analysis, we demonstrated familial heterogeneity of the ARM risk using a family score method.[15-17] The majority of ARM families did not appear to have higher frequencies of disease than expected on the basis of their age distribution. However, 17% of families had a low increased risk, 13% an intermediate risk, and 3% a high of disease, much higher than expected by the age distribution of the family members. Subjects with an intermediate or high familial risk were at least 30 times more likely to be affected than subjects with no excess familial risk. This increased risk could not be explained by the known risk factors smoking and

atherosclerosis; on the contrary, the risk accrued when the effect of these factors was taken into account.

There are several advantages of the design and method of analysis in this study. In contrast to former familial risk analyses of ARM which pooled relatives from different families, we used a family score method which regarded individual families as the unit of analysis. This strategy was developed earlier for modeling family history in logistic regression models.[15,16] Per family, the observed number of affected relatives was compared with the expected number, resulting in a risk estimate for each family. An important benefit is that this allowed for discrimination of risk in families from the same proband group. Other strengths of the study include the calculation of the expected ARM risk from the age-specific prevalences in the Rotterdam Study, the same source population as where the case and control probands originated from. Moreover, relatives were actually examined and photographed, and the diagnostic criteria for observed and expected number of affected relatives, as well as for cases and controls, were identical.

Among the limitations of our approach is, that consideration of the family as the unit of analysis created loss of statistical power. Due to the relatively small number of families, and the small number of relatives per family, statistical significance between familial risk strata could not be achieved. For the total number of families that were studied, we were limited by the frequency of ARM in the Rotterdam Study. Increasing the number of families and expanding the study population with second degree relatives would improve precision of the risk estimates. Another issue is that our study population consisted of prevalent rather than incident cases, leading to misclassification of family scores due to an unknown age of onset. On the other hand, this will be the situation encountered in clinical practice. By using age-adjusted prevalences for the expected number of relatives, the excess familial risk was adjusted for age at examination. Finally, control probands were on average younger than case probands, and may have harbored 'subclinical' familial cases of ARM. The effect of this potential misclassification appeared to be small, for observed family scores were close to the expected score among the control families. The exception was one family with four relatives affected, of whom the proband was still unaffected at the age of 87 years.

Given these considerations, what can be learned from this study? Our data emphasize that ARM is a genetically complex disorder, which can not readily be explained by one single major gene.[6] On the contrary, the large range in familial risk indicates that the genetic contribution to disease differs considerably among families. In the high risk families, the disease is probably caused by a strong genetic factor with a Mendelian inheritance. However, this proportion of families

factor with a Mendelian inheritance. However, this proportion of families appears to be small. In the majority of ARM families, multiple etiologies are involved: some genetic, some environmental, and most likely a combination of these.

In summary, the results of this analysis complement our earlier findings.[7] We confirmed that families of cases are at an increased risk of ARM, but now demonstrated that the variation in familial risk is large. The classification of ARM families into high, intermediate, and low risk families may allow molecular genetic studies to focus on the appropriate risk groups in the search for disease-causing genes.

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Chapter

7

Genetic association of apolipoprotein E with age-related macular degeneration

Abstract

Age-related macular degeneration (AMD) is the most common geriatric eye disorder leading to blindness and is characterized by degeneration of the neuro-epithelium in the macular area of the eye. Apolipoprotein E (apoE), the major apolipoprotein of the central nervous system and an important regulator of cholesterol and lipid transport, appears to be associated with neurodegeneration. The apoE gene (APOE) polymorphism is a strong risk factor for various neurodegenerative diseases, and the apoE protein has been demonstrated in disease-associated lesions of these disorders. Hypothesizing that variants of APOE act as a potential risk factor for AMD, we performed a genetic association study among 88 AMD cases and 901 controls derived from the population-based Rotterdam Study in the Netherlands. The APOE polymorphism showed a significant association with the risk for AMD; the APOE $\epsilon 4$ allele was associated with a decreased risk (odds ratio 0.43 [95% CI 0.21-0.88]), and the $\epsilon 2$ allele with a slightly increased risk of AMD (odds ratio 1.5, [95% CI 0.8-2.82]). To investigate whether apoE is directly involved in the pathogenesis of AMD, we studied apoE immunoreactivity in 15 AMD and 10 control maculae and found that apoE staining was consistently present in the disease-associated deposits of AMD-maculae, that is, drusen and basal laminar deposit. Our results suggest that APOE is a susceptibility gene for AMD.

Chapter 7

INTRODUCTION

AMD is the most common cause of blindness in the elderly in developed countries,[1-4] severely affecting over 10% of octo- and nonagenarians.[5] Histopathologically, the hallmark of early AMD is accumulation of extracellular drusen and basal laminar deposit,[6-8] the end stage is characterized by a complete degeneration of the neurosensory retina and of the underlying retinal pigment epithelium in the macular area.[9] The etiology of AMD is largely unknown, but the current understanding is that AMD is a genetically complex eye disorder[10-12] possibly caused by a variety of molecular defects. Less frequent macular disorders have been linked to a significant number of genomic loci,[13-18] whereas mutations in the TIMP3[19] and peripherin/RDS[20-25] genes have been identified in specific earlier-onset retinal dystrophies. Despite close clinical similarities with these disorders, neither the TIMP3 gene nor the peripherin/RDS gene have been associated with AMD. A recent publication reports that the Stargardt disease gene shows a consistent variation of the ABCR gene in 4.2% of AMD patients, significantly different from the 0.45% in population controls.[26] This variation may account for approximately 4% of the total occurrence of AMD, and, presumably, more genes are involved.

Apolipoprotein E (apoE) is unique among apolipoproteins in its special relevance to nervous tissue. It mobilizes and redistributes lipids, in maintenance and repair of neuronal cell membranes 1988;[27-29] thereby playing a pivotal role in the reinnervation process following peripheral injury[30] and central nervous system injury.[31] The gene for apoE (APOE), located on chromosome 19q13.2,[32] is polymorphic, with the occurrence of three common alleles: $\epsilon 2$, $\epsilon 3$, $\epsilon 4$. The $\epsilon 3$ allele is considered to be the ancestral allele; and $\epsilon 2$ and $\epsilon 4$ are considered as variants by single point mutations.[28] APOE's polymorphism is of particular interest within the framework of neurodegeneration, for it is strongly associated with the risk of Alzheimer disease [34,35] and may be associated with various other neurodegenerative disorders.[36,37] Moreover, apoE is expressed in lesions that characterize Alzheimer disease, Down syndrome, and prion diseases.[38,39]

Expanding these data to a neurodegenerative eye disorder, we investigated the possible role of APOE in AMD in a genetic association study. We have used a case-control design implemented within a population-based study, to assess whether the APOE alleles are associated with the risk of AMD. In a subsequent immunohistochemical procedure, we studied apoE expression in human maculae with and without AMD.

SUBJECTS AND METHODS

We studied APOE genotype and allele frequencies in AMD-cases and controls derived from the Rotterdam Study, a population-based study, in the Netherlands, of subjects aged 55 years and over. The rationale and design of the Rotterdam Study are described elsewhere.[5,40] A total of 6775 participants in that study had undergone an extensive ophthalmological examination, including fundus photography. Diagnosis of AMD was based on grading of fundus transparencies according to an internationally accepted classification system.[41] Cases were all subjects with end stages of AMD of whom data on APOE genotype were available (n=88). The end stages comprised atrophic macular degeneration, that is, geographic areas of atrophy of the retinal pigment epithelium and choriocapillaris, and neovascular macular degeneration, that is, serous or haemorrhagic detachment of the pigment epithelium or choroidal neovascularization. Controls were a randomly selected sample of study subjects without atrophic or neovascular AMD (n=901). There were no significant differences in baseline characteristics between cases and controls apart from the known risk factors age and atherosclerosis. (Mean age [SD]: 81 [8] vs 69 [9] years, $P< 0.001$; frequency of lower-extremity arterial disease [an indicator of atherosclerosis] 37% vs 16%, $P<0.001$ [age-adjusted prevalence data]).

Genomic DNA was extracted from peripheral blood leucocytes, and the subsequent analysis of APOE genotypes was performed as described elsewhere.[42,43] Genotype and allele distributions between cases and controls were calculated by use $\chi 2$ statistics. With multiple logistic-regression analysis, we estimated the odds ratio (OR), as a measure of relative risk, for the various genotypes, using the ancestral E3E3 genotype as a reference. ORs were adjusted for age and gender and, in a separate analysis, for presence of lower extremity arterial disease, to investigate the possible confounding effect of atherosclerosis.

For the immunohistochemical study, maculae were obtained from 25 human eye-bank eyes from 25 subjects. The times from death to processing of the maculae ranged from 1-10 hours, with a mean of 7 hours. Tissues were fixed in 4% formaldehyde, embedded in paraffin, and sectioned into 5 μm thickness. Sections were stained with hematoxylin-eosin, the periodic-acid-Schiff reaction, and Mallory staining and subsequently were classified histologically according to quantification of drusen and basal laminar deposit, as described elsewhere.[6,8] Accordingly, maculae with no or only solitary patches of basal laminar deposit and with no more than three drusen were classified as controls (n=10); maculae with a continuous layer of basal laminar deposit

and/or with many or confluent drusen were classified as cases (n=15). Cases were, although non-significantly, older than controls (mean age [SD]: 82 [10] vs 72 [14] years, P = 0.08). After deparaffinization and rehydration, sections were incubated with 5.5 mU/ml pronase E (Sigma), to reveal antigenic epitopes of APOE, and were placed in a Sequenza Immunostaining Workstation (Life Sciences International). Sections were successively incubated with a mouse-monoclonal antibody directed against apoE (clone 3D12, dilution of 1:25, Monosan), biotinylated-secondary antibodies (Multilink, dilution of 1:75, Biogenex), and alkaline-phosphatase-conjugated streptavidin (dilution of 1:50, Biogenex). Between these incubations, sections were washed thoroughly with phosphate-buffered saline. After final rinsing with 0.2 M Tris-HCl pH 8.0, the presence of apoE was visualized with 0.3% New Fuchsin/Tris-HCl (Sigma).

Table 1. Distribution of APOE genotypes and allele frequency

APOE characteristics	Frequency in	
	AMD cases (n=88)	controls (n=901)
Genotype		
E2E2	0.000	0.010
E2E3	0.227	0.144
E2E4	0.023	0.017
E3E3	0.636	0.555
E3E4	0.114[a]	0.252
E4E4	0.000	0.022
Allele frequency		
ϵ2	0.125	0.090
ϵ3	0.806	0.753
ϵ4	0.068[b]	0.156

[a] P=0.02 versus controls;
[b] P=0.004 versus controls; Hardy-Weinberg equilibrium: cases χ^2 2.27, P=0.26; controls χ^2 4.24, P=0.11

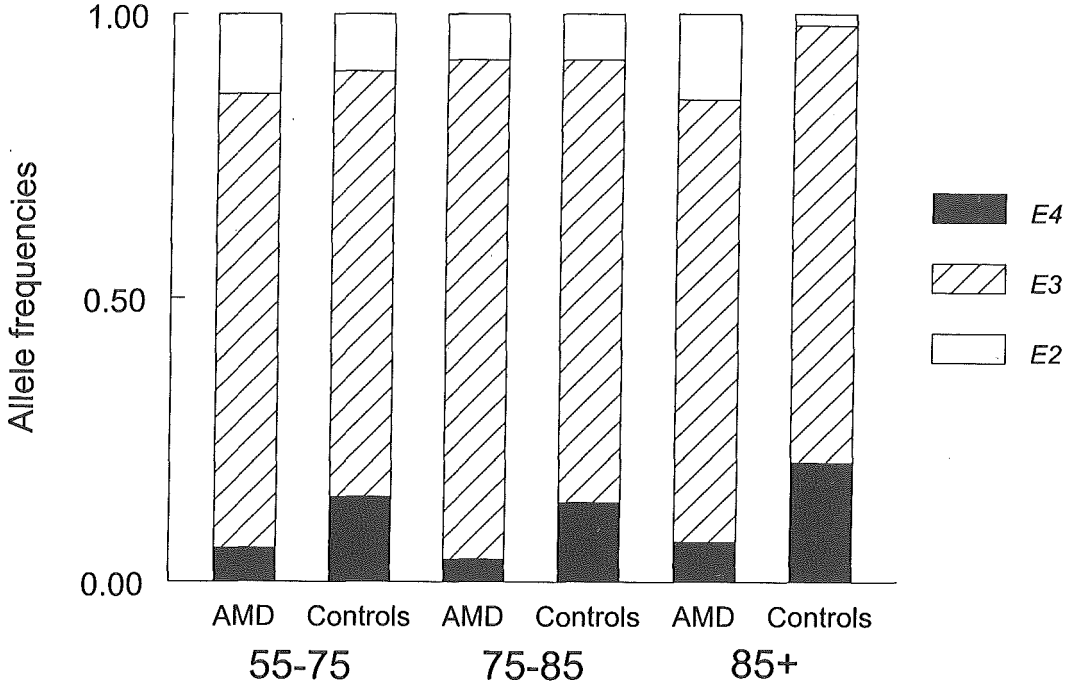

Figure 1. ***APOE allele frequencies in age-categories 55-75 years, 75-85 years and ≥85 years. The number of cases and the number of controls, respectively, in the successive categories are 17 and 687, 40 and 188, and 26 and 31. The proportional areas (sub-bars) indicate the relative allele frequencies of the ϵ2, ϵ3, and ϵ4 alleles in AMD-cases and in controls***

RESULTS

APOE genotype and allele distributions differed significantly between cases and controls (Table 1). Compared with controls, the frequency of the APOE ϵ4 allele was significantly lower among cases (0.07 in cases vs 0.16 in controls, P=0.002), whereas the frequency of the ϵ2 allele was, although not-significantly, higher (0.13 vs 0.09, P=0.17). Because the ϵ4 allele may adversely affect longevity given its association with Alzheimer disease and coronary heart disease,[44] we investigated the prevalence of the APOE alleles as a function of age (Figure 1). There were no significant differences in allele frequencies in the three age groups, indicating that our findings cannot be explained by the age-distribution difference between cases and controls.

Table 2 shows the relative risks of AMD for the different APOE alleles. When adjusting for age and sex, subjects with the ϵ4 allele were more than two times less

likely to develop AMD than were subjects with the E3E3 genotype. Subjects with the ϵ2 allele were at a slightly, but non-significantly, increased risk of AMD. Additional adjustment, for lower extremity arterial disease, did not significantly alter the risk estimates (data not shown), suggesting that APOE and atherosclerosis are independent risk factors for AMD.

Table 2. Risk of AMD[a] for the APOE[b] genotypes

APOE genotype[e]	AMD (n=88)	Controls (n=901)	Crude OR[c] (95% CI)	Adjusted OR[d] (95% CI)
E*2	22	154	1.28 (0.75-2.21)	1.50 (0.80-2.82)
E3E3	56	500	reference	reference
E*4	12	262	0.41 (0.22-0.78)	0.43 (0.21-0.88)

[a]Age-related macular degeneration
[b]Apolipoprotein E
[c]Odds ratio
[d]Adjusted for age and gender
[e]APOE genotypes with the ϵ2 allele are grouped, and genotypes with the ϵ4 allele are grouped. Subjects with the E2E4 genotype (2 of 88 cases, 15 of 901 controls) are present in both the E*2 and the E*4 group.

ApoE immunoreactivity was present in the extracellular deposits that characterized the AMD maculae, that is, basal laminar deposit and soft drusen. Basal laminar deposit stained positive for apoE in 13 of 15 maculae with this type of deposit (Figure 2A), and drusen stained positive in 9 of 11 maculae with drusen (Figure 2B). One eye with atrophic AMD showed both a thick layer of basal laminar deposit and drusen staining positive for apoE (Figure 2C). In both case and control maculae, staining was seen in the outer collagenous zone of Bruch's membrane, in blood vessels, and in Müller cells. Particularly of interest is the finding that solitary, hard hyaline drusen, a type of deposit that is clinically not associated with AMD, did not show any apoE immunoreactivity.

DISCUSSION

Our results show that the APOE gene polymorphism is significantly associated with the risk of AMD and that apoE is expressed in lesions that characterize AMD. A decreased risk of AMD was associated with the ϵ4 allele, whereas an increased risk

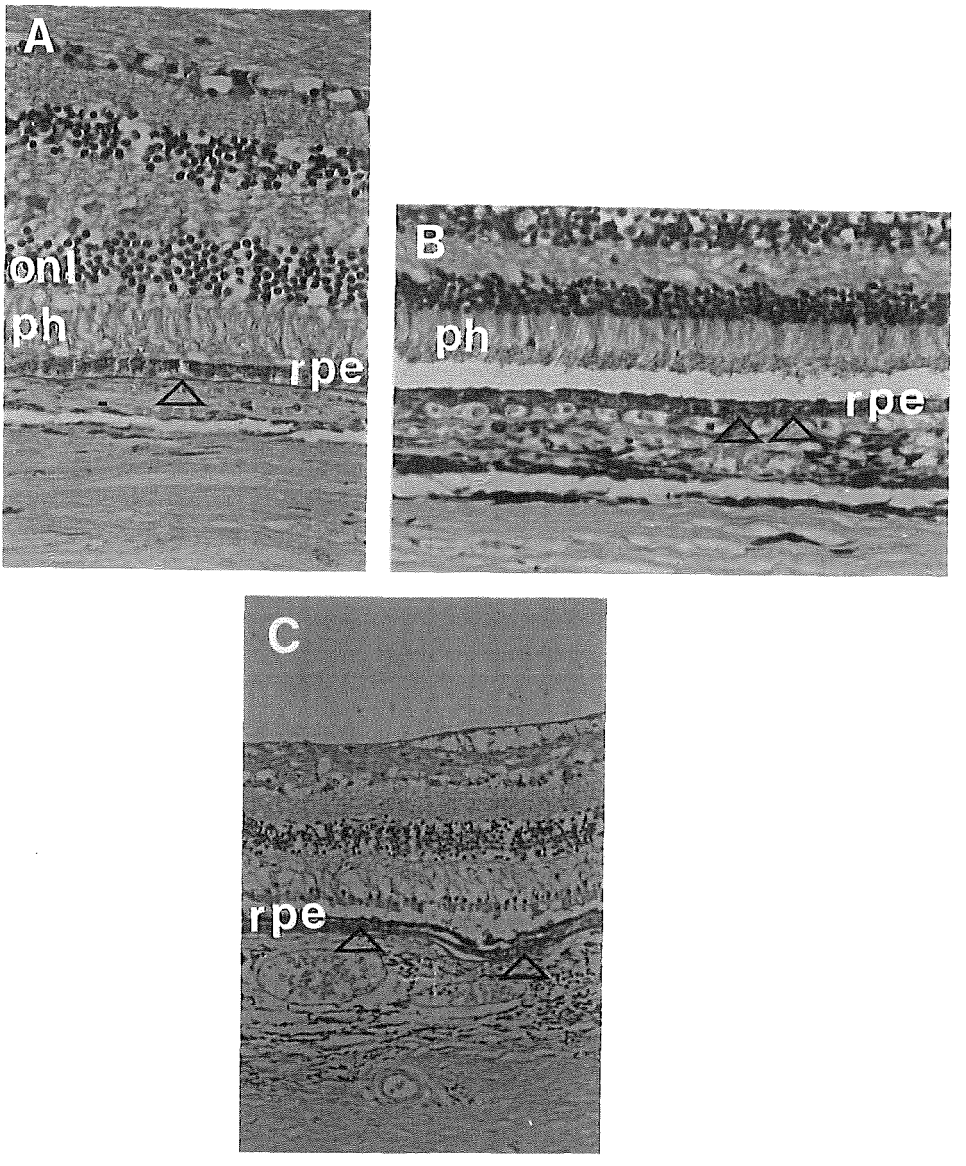

Figure 2. *Immunohistochemistry of apoE in human maculae with AMD (New Fuchsine, x400). Shown is positive staining (in red) of (a) a thin layer of basal laminar deposit located between the retinal pigment epithelium (rpe) and Bruch's membrane (arrow); (b) soft drusen (arrows); and (c) thick layer of diffuse drusen in subject with AMD. (Note disappearance of the photoreceptors and most of the rpe.) ONL = outer nuclear layer; PH = photoreceptors*

was associated with the ϵ2 allele. The consistent immunoreactivity in soft drusen and basal laminar deposit in the AMD maculae suggests importance of apoE in the pathogenesis of AMD.

We carefully avoided selection bias, a frequently encountered problem in association studies. Since cases and controls were both derived from the same homogeneous source population, and the distribution of the APOE genotypes in cases and controls was in Hardy-Weinberg equilibrium, selection on the basis of genotype is unlikely. Moreover, the allele frequencies among the controls were in close agreement with the average allele frequencies estimated for the Dutch[45] and other Caucasian populations.[46] Because our extensive ophthalmologic examination demanded attentiveness from the study subjects, it may have selected against other neurodegenerative diseases - such as Alzheimer disease - which are known to be associated with increased ϵ4 frequency. Nevertheless, this cannot account for differences in allele frequencies between cases and controls, because both groups underwent identical procedures. Finally, we showed that allele frequencies were similar across all age groups (figure 1), indicating that the association cannot be explained on the basis of age.

Given the limited amount of data available, we can only speculate on the possible role of apoE in the neuronal dynamics of the macular area. In the central nervous system in general, a major physiological role for apoE is to mediate the interaction of apoE-containing lipoproteins and lipoprotein receptors, including the low density lipoprotein (LDL) receptor[47] and the LDL receptor-related protein receptor (LRP).[48] After neuronal cell loss, large amounts of lipids are released from degenerating cell membranes and myelin, and, in response, astrocytes synthesize apoE, to bind the free cholesterol and lipids and distribute them for reuse in cell membrane biosynthesis.[31,49] ApoE may have a significant role in retinal membrane renewal. The high turnover of photoreceptor membranes,[50] especially in the macular area, makes cell membrane remodeling of critical importance for maintaining the normal physiology of the retina. Failure of this process may then result in macular degeneration.

In the central nervous system, apoE is primarily synthesized by the major glial cell, the astrocyte. In our series, cell bodies of the Müller cell, the retinal analogue of the astrocyte, showed significant apoE expression, which may indicate a site of apoE production. This assumption is supported by findings from previous reports, which show that these cells are capable of apoE synthesis[51] and which show increased expression in eyes with retinal damage.[52] The distribution of the LDL or LRP receptor in the neuroepithelium of the eye is unknown, and it is therefore unclear which cells are able to take up and process apoE-complexed molecules in this compartment. The

retinal pigment epithelium cell, which has digestion of photoreceptor outer segments as its primary function, may be an appropriate candidate.

Interestingly, we found a reduced risk of AMD for subjects carrying the ϵ4 allele, whereas for most other neurodegenerative disorders the risk is increased for these subjects. Isoform-specific alterations in apoE-lipoprotein metabolism consist of differences in net charge[46] and total serum[53] and brain level of apoE.[54] Recently, it has been shown that the isoforms also differ in cell-specific binding properties.[33] The apoE mediated binding, internalization and degradation of lipids in the central nervous system appear to be different for each apoE isoform, depending on the type of target cell. A possible interpretation of our findings is that apoE isoforms in the macular area may either differ in binding affinity or elicit a response different than that at other sites in the nervous system. Since it is not immediately clear how the APOE alleles may be a source of genetic risk for AMD, it will be intriguing to investigate whether accumulation of deposits in AMD occurs in an isoform-dependent manner.

An alternative explanation to our findings is that the ϵ4 allele is associated with a distinct mutation in a gene in linkage disequilibrium with APOE. This may be the gene that actually determines susceptibility to AMD. According to the August 1997 OMIM (Online version of Mendelian Inheritance in Man), 20-30 genes are located in the immediate vicinity of APOE, and they may be considered in this context; among these genes, we could not find an obvious candidate gene for retinal disease.

To conclude, we have shown a significant association between the APOE gene and AMD in a general population of elderly people, and we have immunohistochemically localized the apoE protein in defining lesions of AMD. Although in need for confirmation, our data further emphasize the role of APOE in neurodegeneration and may indicate that we have identified a susceptibility gene for AMD.

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Part IV

Environmental risk and comorbidity

Chapter

8

Smoking is also associated with age-related macular degeneration in persons aged 85 years and older

The article by Vingerling et al.,[1] published in the October 1996 issue of the ARCHIVES, describes the association between smoking and age-related macular degeneration (AMD). Smoking increased the risk of macular degeneration only in persons aged 55 to 84 years; there was no increased risk for smoking in persons aged 85 years and older. The authors mentioned that the lack of association in this age-group was possibly due to competitive risk factors, and hypothesized that selective survival and a decreased response among the oldest persons might have influenced the results.

To be consistent with the international classification and grading system of AMD,[2] we re-graded the fundus transparencies from the Rotterdam Study. In addition, historical data and information from medical records were obtained to exclude other causes of maculopathy. Re-grading did not alter the overall and age-specific prevalence of AMD in the Rotterdam Study. However, re-grading did affect the association between smoking and AMD in persons aged 85 years and older. In contrast to the former results, smoking is significantly associated with an increased risk of AMD (Table 1).

This is the first observation of an association between smoking and AMD among persons aged 85 years and older in a general population.[3,4] We conclude, that the association between smoking and the increased risk of AMD is irrespective of age.

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Table 1. Smoking status and age-related macular degeneration (AMD) in two age strata, using the International Grading and Classification System for AMD

	55-84 years			85 years and over			All ages		
	AMD		Odds ratio* (95% CI)	AMD		Odds ratio* (95% CI)	AMD		Odds ratio* (95% CI)
	present	absent		present	absent		present	absent	
Total number of	62	5954		36	282		98	6236	
Smoking status:									
Never	17	1986	1.0†	23	184	1.0†	40	2170	1.0†
Former	22	2561	2.1 (1.0-	6	70	0.8 (0.3-2.4)	28	2631	1.5 (0.8-2.8)
Current	23	1407	3.6 (1.6-	7	28	5.2 (1.2-23.1)	30	1435	3.5 (1.8-7.0)

* adjusted for age and gender

† reference

Chapter

9

Is age-related maculopathy associated with Alzheimer's disease ? The Rotterdam Study

Abstract

The authors examined the relationship between age-related maculopathy and Alzheimer's disease in the Rotterdam Study, a prospective population-based study in the Netherlands. From 1990 to mid-1993, subjects aged 75 years or older (n=1438) were screened for presence of age-related maculopathy and Alzheimer's disease, and follow-up examinations were conducted from mid-1993 to the end of 1994. Subjects with advanced age-related maculopathy at baseline showed an increased risk of incident Alzheimer's disease (RR 2.1, 95% CI 1.1, 4.3; adjusted for age and gender), but the risk decreased after additional adjustment for smoking and atherosclerosis (RR 1.5, 95% CI 0.6, 3.5). These findings suggest that the neuronal degeneration occurring in age-related maculopathy and Alzheimer's disease may, to some extent, have a common pathogenesis.

INTRODUCTION

Age-related maculopathy (ARM) and Alzheimer's disease (AD) are both chronic neurodegenerative disorders that affect a substantial proportion of elderly persons, imposing a significant burden on public health and quality of life. In the Netherlands, 8% of those aged 75 years and older are affected by end-stage ARM[1] and 13% of those are diagnosed with AD.[2] Characteristic of these disorders is the irreversible loss of neuronal function, for which there is no cure.

Although the etiology of both is largely unknown, the pathogeneses of ARM and AD show some striking similarities. In ARM, early histopathologic manifestations are the extra-cellular drusen deposits and basal laminar deposit. These lesions contain lipids, glycoproteins, and glycosaminoglycans, which are presumably derived from a degenerating neuroretina.[3-6] Accumulation of these deposits is associated with deterioration of macular function and subsequent loss of photoreceptors.[7-9] In AD, an early pathological hallmark is the presence of extra-cellular senile plaques. These plaques contain β-amyloid, activated microglia, and axons and dendrites from dystrophic neurons.[10,11] Analogous to those in ARM, these deposits are associated with neuronal malfunction and cell loss.[11-13]

The pathogenic parallels between ARM and AD prompted us to study their comorbidity within the population-based Rotterdam Study. This study was designed to investigate the determinants of various chronic geriatric disorders among middle-aged and elderly subjects. In the present analysis, we studied the relation of ARM at baseline to the two-year incidence of AD among subjects aged 75 years or older.

SUBJECTS AND METHODS

Study design and population

The Rotterdam Study is a population-based prospective cohort study conducted in a suburb of Rotterdam, the Netherlands, in which chronic neurologic, ophthalmologic, cardiovascular and locomotor disorders are investigated.[14] The study was approved by the Medical Ethics Committee of the Erasmus University Medical School. Informed consent and permission to retrieve information from physicians was obtained from all participants. Baseline interview and screening examinations took place from 1990 to mid-1993, follow up examinations were conducted from mid 1993 to the end of 1994.

Of 10.275 eligible subjects of the entire Rotterdam Study, 7983 (78%) of those aged 55 years or older agreed to participate in the baseline phase. In the present study, we included only subjects aged 75 years or older (n=2016); of these, 1599 (79%) underwent a complete screening for ARM and AD. A total of 139 subjects in this age group were diagnosed with prevalent Alzheimer's disease in this age group, while 22 subjects had a dementia other than AD. Therefore, 1438 subjects were at risk of incident AD during the follow-up period and were included in the analysis.

Diagnosis

Case-finding procedures for ARM and AD have been described in detail elsewhere.[1,2] In brief, during the ophthalmologic screening examination, 35° color transparencies were taken of the macular area (Topcon TRV-50VT fundus camera, Topcon Optical Company, Tokyo, Japan). The diagnosis of ARM was based on grading of fundus transparencies according to the international classification system.[15] ARM was stratified on the basis of 4 exclusive stages, which increased in clinical severity: no ARM, the absence of any type of soft drusen and atrophic or neovascular macular degeneration; stage 1, the presence of only soft distinct drusen of more than 63 μm in the absence of pigmentary irregularities and atrophic or neovascular macular degeneration; stage 2, the presence of either distinct drusen with pigmentary irregularities or indistinct or reticular drusen; stage 3, the presence of indistinct or reticular drusen with pigmentary irregularities; and stage 4, the presence of either atrophic or neovascular end-stage macular degeneration. Best-corrected visual acuity was measured at a distance of 3 m by using a modified Early Treatment Diabetic Retinopathy chart.

Dementia screening and diagnosis at baseline and at follow-up followed a three-step protocol that included the Minimental-State Examination (MMSE) and the Geriatric Mental State schedule, the Cambridge Mental Disorder Examination (CAMDEX) diagnostic interview, and an examination by a neurologist, as described previously.[2] In addition, the entire cohort was monitored during follow-up to detect cases of dementia by through linking the general practitioner's automated medical record system to the database of the Rotterdam Study. Data on subjects who could not be rescreened at follow up (refusals, deceased) were obtained from informants, medical files, and the regional institute for outpatient mental health care. The final diagnosis of AD was based on all collected information using NINCDS-ADRDA criteria,[16] which in brief imply the presence of dementia not caused by systemic or other brain disorders, deficits in at least two

areas of cognition, gradual progression of disease, and no disturbance of consciousness.

Table 1. Baseline characteristics of subjects at risk of incident Alzheimer's disease, stratified by stage of ARM

Characteristic	No ARM (n=811)	Stage 1 (n=349)	Stage 2 (n=165)	Stage 3 (n=46)	Stage 4 (n=67)
Age at baseline (years) (SD*)	80.5 (4.4)	80.0 (4.0)	81.3 (4.5)	82.2 (5.6)	84.5 (4.8)
Gender (% women)	64.4	67.9	63.6	67.4	68.7
Institutionalized (%)	14.8	16	21.2	34.8	34.3
MMSE* score at baseline (SD)	26.9 (2.2)	26.9 (2.1)	26.8 (2.1)	25.5 (4.8)	26.3 (2.5)
Smoking Former (%)	35.6	29.1	33.5	30.2	28.4
Current (%)	12.1	11.8	13	23.3	19.4
Atherosclerosis (%)	18.3	14.9	22.5	18.6	25

*SD, standard deviation; MMSE, Mini-Mental State Examination

Smoking, atherosclerosis, and apolipoprotein E

Smoking habits were assessed during the baseline home interview; subjects were stratified as non-cigarette smokers, former cigarette smokers, and current cigarette smokers. The presence of generalized atherosclerosis was evaluated by using the ratio of the ankle to brachial systolic blood pressure,[17,18] as described previously.[19] Atherosclerosis was considered present when the left or right ankle-brachial index was less than 0.90. Genomic DNA was used for genotyping of apolipoprotein E. The apolipoprotein E gene was amplified by using the primers and conditions, as described.[20,21] Genotypes E2/E4, E3/E4 and E4/E4 were grouped and defined as the presence of the apolipoprotein $\epsilon 4$ allele.

Statistical methods

The incidence of AD was obtained for the successive stages of ARM. Person-years were calculated per age-category after summation of each participant's contribution of follow-up time to each category. We calculated the relative risks of incident AD for the four stages of ARM as well as for low visual acuity by using Cox proportional hazards regression analysis, adjusting for age and gender. Additional adjustment for smoking, atherosclerosis, and the presence of the apolipoprotein $\epsilon 4$

allele was performed in separate analyses. To increase statistical power of the risk analyses, ARM stages 1 and 2 were combined, as were stages 3 and 4.

RESULTS

Population at baseline

ARM was absent in 56.4% (n=811) of study subjects. Of the remaining 627 subjects, 24.3% (n=349) were diagnosed with stage 1, 11.5% (n=165) with stage 2, 3.2% (n=46) with stage 3, and 4.7% (n=67) with stage 4 ARM. The baseline characteristics of these subjects are given in Table 1. In the more severe stages of ARM, subjects were older and were more likely to be institutionalized, to smoke, and to have atherosclerosis. There were no significant differences in baseline scores of MMSE.

Incidence of AD

After an average follow-up period of 25.2 months, 62 incident cases of AD were identified. The incidence of AD in the total group of study subjects was 20.0 per 1000 person-years and ranged from 14.0 per 1000 person years in those aged 75-84 years to 41.6 per 1000 person-years in those aged 85 years or older. Figure 1 shows the crude incidence rates of AD for successive stages of ARM among subjects in two age categories. The 2-year cumulative incidence risks, calculated from the incidence rates, for all subjects aged 75 years or older were 3.4% for those with no ARM, 3.2% for those with stage 1, 3.8% for those with stage 2, 9.4% for those with stage 3, and 10.0% for those with stage 4 ARM . Women developed incident AD more often than men did, but this difference did not reach statistical significance (RR 1.7; 95% CI 0.9, 3.3; adjusted for age).

Risk of comorbidity

After adjustment for age and gender, we found that the risk of incident AD was increased for subjects with stage 3 or 4, but not for subjects with stage 1 or 2 ARM (table 2). After additional adjustment for smoking and atherosclerosis, the point estimate of the relative risk for stage 3 or 4 decreased and the association became insignificant. Additional adjustment for presence of the apolipoprotein $\epsilon 4$ allele did not alter the risk estimates (data not shown).

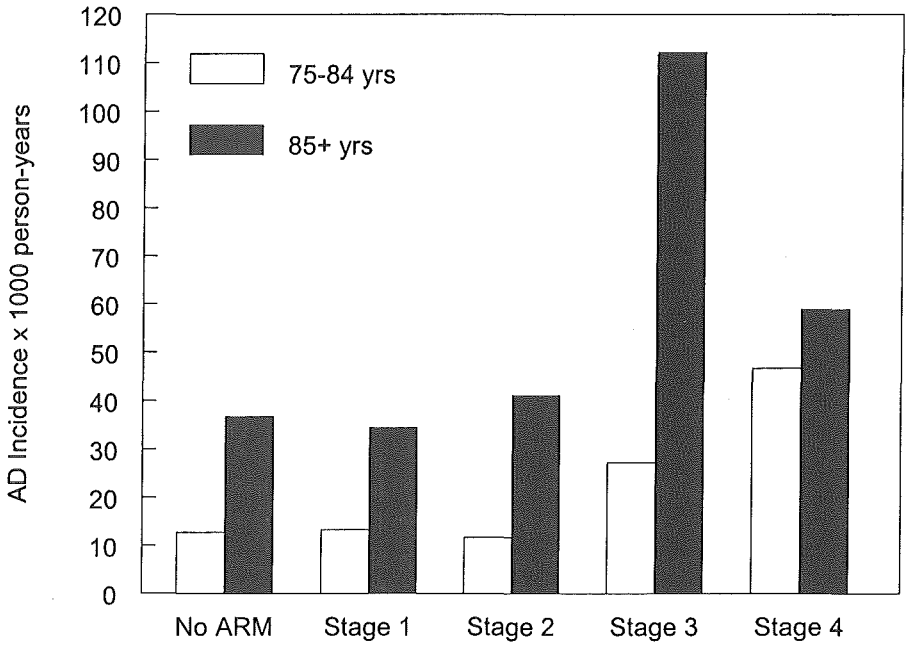

Figure 1. ***Incidence rates of Alzheimer's disease (AD), in person-years in two age strata, for successive stages of age-related maculopathy (ARM) as determined at baseline***

Numbers of person-years for age stratum 75-84 years: no ARM, 1418.7; ARM stage 1, 617,5; ARM stage 2, 255,6; ARM stage 3, 74,7; ARM stage 4, 64.4.
Numbers of person-years for age stratum 85+ years: no ARM, 362.1; ARM stage 1, 117.5; ARM stage 2, 97.8; ARM stage 3 26.8; ARM stage 4, 68.4.

To investigate whether the association between stage 3 or 4 ARM and incident AD resulted from a decline in visual function rather than ARM, we studied the association between poor visual acuity at baseline and incident AD. For subjects with a best-corrected visual acuity of less than 0.05 the relative risk was 0.96 (95% CI: 0.68, 7.05, adjusted for age, age^2, and gender), for subjects with a best-corrected visual acuity of more than or equal to 0.05 but less than 0.3, the relative risk was 1.01 (95% CI: 0.35, 2.88). It is therefore unlikely that the observed association between ARM and AD can be explained by visual impairment.

Table 2. Relative Risk of incident Alzheimer's disease for subjects with successive stages of ARM

	No. at baseline	RR* (95% CI*)†	RR* (95% CI*)‡
No ARM	811	Reference	Reference
Stage 1 or 2	514	1.0 (0.6-1.8)	1.0 (0.6-1.9)
Stage 3 or 4	113	2.1 (1.1-4.3)	1.5 (0.6-3.5)

* RR, relative risk; CI, confidence interval
† Adjusted for sex, age, age^2
‡ Adjusted for sex, age, age^2, smoking, and atherosclerosis

DISCUSSION

Based on a general population of elderly subjects, this study shows an association between the most severe stages of ARM and incident AD. The nature of this association depends partly on smoking and atherosclerosis, which are important risk factors for both ARM and AD.

Strengths of this study include setting, methods of diagnosis and temporal design. The population-based setting warranted a valid comparison of study groups and reduced the possibility of information bias. The standardized methods of diagnosis of both ARM and AD were based on internationally accepted criteria,[15,16] which improved independent case finding. An important part of the design was the temporal sequencing of diagnosis of the two diseases. Poor cognitive functioning of patients with severe AD generally hampers the performance of extensive clinical investigations, and fundus photography is often difficult to carry out. Therefore, we considered a cross-sectional analysis of the association between ARM and AD to be unreliable. To reduce selection bias, we evaluated the diagnosis of ARM prior to the occurrence of AD. The overall incidence of AD in the Rotterdam Study was higher than the incidence of this disease in the present study cohort,[22] suggesting that subjects for whom ARM data were missing were at an increased risk of AD. Complete data on ARM for all subjects would have helped to characterize the actual association. Nevertheless, this does not explain the associations observed, since the lack of complete data generally tends to weaken any relation.

Both ARM and AD are complex disorders, in which genetic as well as environmental factors have been implicated. Smoking is an established risk factor for ARM; the increased risk of incident early or advanced ARM has been estimated to be approximately twofold.[23-25] Although former studies suggested an inverse relation with AD,[26] it has recently been shown that smoking is also associated with a two-fold increased risk of incident AD.[27] In our analysis, smoking partly explains the association between ARM and AD, which may imply that the neurotoxic effect of smoking is rather nonspecific. The exact mechanisms are unknown, but altered hemodynamic and vascular regulation,[28,29] and reduction of oxygen transport into the tissue,[30] are some of the destructive effects that may be involved.

Atherosclerosis also partly determines comorbidity of ARM and AD. Earlier findings from the Rotterdam Study suggested that generalized atherosclerosis is a risk factor for either disease; that is, it was associated with a twofold increased prevalence of ARM[31] and a 30% increased prevalence of AD.[19] Aside from more disease-specific effects such as thickening of Bruch's membrane in ARM[32] and increased amyloid angiopathy in AD,[33] decreased vascular flow and endothelial damage are candidate mechanisms by which atherosclerosis may alter both retinal and cortical cell function.

The apolipoprotein ϵ4 allele is associated with both disorders but in an opposite way. The presence of this allele has been shown to increase the risk of AD,[34, 35] but to decrease the risk of ARM.[36, 37] It is therefore unlikely that the apolipoprotein E genotype contributes to an association between ARM and AD. In our analyses, adjustment for presence of apolipoprotein ϵ4 allele did not distort the relative risk estimates.

In conclusion, the present study suggests that the neurodegenerative diseases of ARM and AD show comorbidity, and that smoking and atherosclerosis may be causal links. Whether other factors determine a common neurodegenerative pathogenesis remains to be elucidated.

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Part V

General discussion and summary

Chapter

10

General discussion

The aim of this thesis was to expand the epidemiologic knowledge on the etiology of age-related maculopathy. The studies that were performed provided data on disease occurrence, impact, and genetic and environmental risk factors. This chapter will focus on the most important findings, the clinical relevance, and the methodological issues of these studies. It will also provide suggestions for future genetic epidemiologic research.

Findings and Clinical Relevance

Disease frequency and impact

The occurrence of ARM's late stage, AMD, has been extensively studied in prevalence studies.[1-6] All studies found a strong rise with age, and there appeared to be a global difference in frequency, and type[7] of AMD. Incidence studies were, and still are, needed to confirm these findings. In the Rotterdam Study, we studied the incidence of AMD at 2-year follow up, and compared our data with two incidence studies from the United States. We found a lower incidence of AMD at all ages, confirming the trend that had been suggested by previous prevalence studies. In a recent 'three continent' consortium study, we could not find discrepancies in known risk factors to explain this issue ("Smith et al, submitted"). It may be a breakthrough for our understanding when these responsible factors are identified.

The majority of clinical and epidemiological articles on ARM that have appeared over the last two decades started with the sentence: 'Age-related macular degeneration is the most common cause of blindness in the Western world'. Recent population-based studies found justification for this statement,[8-10] but it was unclear which age groups were concerned. In the Rotterdam Study, we studied the importance of ARM in relation to visual decline as a function of age. Our data demonstrated that only after

the age of 75 did the end stages of ARM become the leading cause of blindness. The impact grew with further increasing age to a prevalence of 6% of bilateral blindness or visual impairment due to AMD in those aged 85 years and over. Whether this proportion is sufficient to entitle this disease as a public health problem is a matter of debate. Bilateral development of AMD has a great impact on quality of life,[11,12] and is an important reason for elderly to give up independent living. On the other hand, dementia and cardiovascular disease are very disabling geriatric diseases with much higher frequencies of occurrence[13-15] and more implications for nursing care and health costs.

Genetic risk

Unravelling the genetic basis has recently become a focus of attention in ARM research. New statistical methodologies make it possible to dissect complex genetic disorders once thought to be unapproachable. The key aim is to identify the susceptibility genes in order to gain insight into the molecular basis and perhaps create therapeutic starting-points. However, one must know the magnitude of the genetic risk to design this kind of research. We performed a familial aggregation study to quantify this factor and found that the relative risk of AMD for first degree relatives of AMD cases was 4.2 (95% CI 2.6-6.8). Moreover, relatives of cases developed all features of ARM at an earlier age, suggesting that presence of a genetic factor was associated with earlier onset of disease. We estimated the attributable risk of genetic factors to the overall occurrence of AMD to be approximately one fourth. In comparison, the strongest known environmental factor, smoking, had a prevalence odds ratio of 3.5 (95% CI 1.8, 7.0, Chapter 8) and an attributable risk of one fifth in the Rotterdam Study. Our genetic risk data will be the basis for the design of further genetic studies, and are of clinical importance in genetic counselling of patients.

Although some reports suggest otherwise,[16] it is rather unlikely that one single gene accounts for the overall genetic risk. True evidence of genetic heterogeneity requires the identification of all the genes involved - an accomplishment for the future. To enhance the current understanding, we attempted to find clues for familial differences in risk by calculating risk scores for each family. Our data showed that two thirds of the ARM families did not have an excess familial risk, but 17% appeared to have a low risk, 14% a moderately increased risk, and only 3% a high risk of disease. These findings suggest that only a small fraction of all ARM may be due to mono- or oligogenetic factors segregating in some families, and these families will be of particular interest to geneticists in the search for genes.

Different approaches can be used to dissect the genes determining susceptibility of a complex trait. Basically, they fall into two categories: positional cloning and candidate gene approach. The former establishes the location of susceptibility loci within the genome, while the latter specifically tests whether or not a particular gene contributes to the disease. We pursued the latter technique and performed a genetic association study to investigate whether the APOE gene contributes to the etiology of ARM.

APOE is a polymorph gene involved in neurodegeneration; the ϵ4 allele increases the risk of Alzheimer's disease and Creutzfeldt Jakob disease.[17,18] Contrary to our hypothesis, we found a decreased risk of AMD for subjects carrying the ϵ4 allele in our study. This unexpected finding was confirmed by a French study,[19] but not all other studies could replicate these findings.[20] Our demonstration of the apoE protein in drusen and basal laminar deposit provided preliminary evidence of a biological role of APOE in retinal neurodegeneration, but this evidence is now growing. Recent mouse studies have shown that apolipoprotein E transgenic mice on a high fat diet develop significant amounts of basal laminar deposits,[21] and that apolipopotein E deficient mice have abnormal retinal cell layers.[22] Evidence for retinal Müller cells as a production site of apolipoprotein E comes from our report as well as from others,[23] and it has been shown that these cells increase expression in case of retinal damage.[24]

There are various possibilities with regard to the underlying mechanism of the role of APOE in ARM. Firstly, apolipoprotein E may have a neurotrophic effect by its role of redistributor of lipids and cholesterol from degenerating neurons to form new cell membranes. The latter are necessary at a constant rate for remodelation of photoreceptors.[25] Secondly, apolipoprotein E may be needed to clear Bruch's membrane of lipids and other waste products from the retinal pigment epithelium which may otherwise facilitate the development of drusen and basal laminar deposit. Thirdly, apolipoprotein E has anti-oxidative capacities[26] and may interact with lipid peroxidation of photoreceptor membranes occurring in light absorption. At this moment we do not have an explanation for the direction of the genetic risk association, but isoform differences in binding capacity, internalization and degradation of lipids, as well as differences in anti-oxidant activity may be important.

Environmental risk and comorbidity

An earlier report of the Rotterdam Study showed that smoking was a risk factor for AMD in subjects aged 55-84 years, but not in subjects aged 85 years and over.[27] This was an unexpected finding for which we did not have a good explanation. After we improved the diagnosis of ARM by regrading baseline fundus transparencies using

ophthalmic history data and criteria of the International Classification System,[28] we reanalyzed the smoking data. Now, we also found a relation with smoking among those aged 85 years and older.

Smoking is undoubtedly the most established environmental risk factor of ARM to date. A large number of epidemiologic studies have reported a strong relation between smoking and prevalent AMD,[29-31] two studies showed a relation with incident ARM as reported by medical physicians,[32,33] and a recent study by Klein et al. reported an association with incident early ARM as diagnosed on fundus transparencies.[34] Several mechanisms have been suggested to explain the association. Because smoking decreases plasma antioxidant levels[35,36] as well as luteal pigments in the human retina,[37] it may increase the risk of damage to the macula by light and oxidative stress. Another explanation may be that smoking causes maculopathy by promoting atherosclerotic damage to the choroidal vessels; atherosclerosis has been shown to be associated with AMD.[38] Lastly, smoking may aggravate retinal degeneration by liberation of arachidonic acid, with subsequent initiation of an inflammatory response.[39] In line with this pathway is the finding that nicotine and cotinine activate phospholipase A2 present in photoreceptor membranes with formation of arachidonic acid.[40]

The pathogenesis of ARM shows some striking similarities with Alzheimer's disease (AD). In fact, some researchers refer to ARM as 'the Alzheimer of the eye'. Both disorders are characterised by precursor lesions that are presumably derived from degenerating neurons, subsequently leading to neural malfunction and cell loss. Moreover, vascular insufficiency, smoking, apolipoprotein E and oxidative stress appear to be causal links in both disorders. Our studies showed that subjects with severe stages of ARM had an increased risk to develop Alzheimer's disease, but it appeared that most of this relation could be ascribed to the shared risk factors smoking and atherosclerosis. These findings do not have clinical consequences since adequate therapy is not available, but it may be relevant for caretakers to be cognisant of the relationship. Deducing from our data, it is not to be expected that the two disorders have significant genetic factors in common.

METHODOLOGICAL CONSIDERATIONS

Study design

All studies described in this thesis were observational studies based on a large study population derived from a general Dutch population. One of the great benefits of this type of design is that a great variety of different diseases as well as risk factors can be

studied, that their general distribution can be evaluated, and that study results can easily be generalized. Among the drawbacks are the large enterprise, and the relative infrequency of outcomes despite the substantial number of study subjects.

We applied a *cross-sectional* design in chapters 3, 7, and 8. Cross-sectional studies are generally less preferable than longitudinal studies, because they are limited in the interpretation of causality. In practice, they are often the initial phase of a longitudinal study, and can be used to generate hypotheses which can be tested in follow up phases later on. In chapter 4, the aim was to assess disease impact, and a cross-sectional design was therefore appropriate. In chapter 7, the aim was to assess the association between the APOE gene and AMD. The APOE genotype is an exposure that does not alter over time, so the current information on the APOE gene is as useful as any. However, because this gene may be related to survival,[41] it is recommended that our investigation is replicated with incident AMD cases. In chapter 8, the aim was to assess evidence for a causal relation between smoking and AMD. Smoking is not a stable exposure, and ideally there should have been a time-interval between the measurements of exposure and disease corresponding to the latency period. Although the consistency of findings among studies adds to the evidence of a true relation, replication by many more well described incidence studies is needed to confirm the findings and to establish a better estimate of the strength of the association.

We applied a *longitudinal* design in chapters 3 and 9, using incident data from 2 years follow up. Obtaining stable estimates of incidence requires a substantial person-time experience, and a more lengthy follow up time is generally preferred. In chapter 3, the aim was to describe the natural course of ARM, and incidence of late stages. The short follow up period was very suitable to investigate all the steps involved in the development of ARM. However, a longer follow up time will be needed to increase the number of incident cases and the precision of the incidence rate estimate. In chapter 9, the aim was to assess the relation between ARM and incident Alzheimer's disease. Although the association will be reinvestigated with more incident cases in the future, the substantial number of incident Alzheimer's cases allowed for reasonable risk estimates.

We applied a so-called *reconstructed cohort* design[42] in the genetic-epidemiologic studies described in chapters 5 and 6. This design is a true hybrid of a case-control and a cohort design. In chapter 5, the familial aggregation study was initiated with the selection of index cases and controls, while their relatives were subsequently assembled in cohort form. Relatives of cases were considered to be the exposed cohort, relatives of controls the non-exposed cohort, and the two cohorts were analyzed in life tables. For this procedure, it was necessary to pool relatives from different families, to

disregard the family unit, and to ignore the possibility of heterogeneity. In chapter 6, we performed a supplementary analysis on the same data to make up for these drawbacks. We now respected the family unit and calculated family scores based on observed and expected number of affected relatives. Small families sizes, as well as a small number of families, create a problem for this approach, because it decreases the power to achieve statistical significance across risk strata.

Validity

Selection bias occurs when participation in a study is influenced by correlates of the disease or the determinant. It causes distortion of the true relation; in other words, the relation between exposure and disease differs for those who participate in the study from those who are eligible but do not participate. Lost to follow up is an important source of selection bias for cohort studies, especially when response rates are low. The overall response in the Rotterdam Study was reasonable to good with 78% and 79% at baseline and at 2-years follow up, respectively. However, comparison between participants and nonparticipants learned that nonparticipants were older, more likely to be diseased, and more severely affected. Assuming that we selected a relatively healthy cohort, the prevalence and incidence of ARM may be higher in reality than in our studies. We do not think that non-response has influenced the direction of the risk for the associations with apolipoprotein E, smoking, and Alzheimer's disease, because the associations are likely to be more pronounced in more severely affected subjects. However, it may have caused an underestimation. In the familial aggregation study, the response rate was 84% and similar for cases and controls. Nonparticipants did not visit ophthalmologists more often than participants did. Thus, we think it is unlikely that non-response has distorted estimates of the genetic risk.

Misclassification of outcome or determinants may lead to *information bias*. Misclassification that is non-differential, that is, which is randomly distributed over the compared groups, leads to dilution of the effect towards the null-value. In this thesis, we made a great effort to classify the outcome as correct as possible. We collaborated with the Beaver Dam Eye Study to learn the specifics about the ARM grading rules, and regraded all the baseline macular transparencies using the protocol of the International Classification System and all existing ophthalmologic information to diagnose ARM. Our diagnoses of AMD were judicated by investigators from the Beaver Dam Eye Study and Blue Mountain Eye Study. Furthermore, we regularly tested inter- and intra-observer variation of ARM grading, and repeated consensus training when weighted kappa values were unacceptable. These procedures improved the baseline ARM classification especially in the older subjects, which enabled us to

detect an association with smoking in this age group. Most determinants were measured using standardized and generally approved protocols, but non-differential misclassification of risk factors and confounders cannot be totally excluded.

We do not think that differential misclassification distorted results, because the assignments of determinants and outcome were independent of each other and data-collection occurred in a symmetrical fashion. An exception to this may be the results of the logistic regression analysis in the familial aggregation study. Here, misclassification of the disease status among control relatives may have affected the prevalence odds ratio's, because these relatives were slightly younger and developed ARM at a later age than case relatives. In the Kaplan-Meier analysis, non-affected subjects were censored at age at examination, and we therefore think that it is unlikely that differential misclassification has influenced these risk estimates.

Confounding bias occurs when the association under study can be explained by an extraneous factor that is not an intermediary in the causal chain. A confounder must be associated with the disease under study as well as with the exposure under study to be confounding. In all the studies described in this thesis, age was probably the most important confounder. It is the strongest risk factor of ARM, and related to most determinants that we studied. We controlled for the effect of age in most analyses by stratification in various age groups and by adding this factor in a multivariate model. Where appropriate, we dealt with other confounding factors as atherosclerosis, smoking, and APOE in the same way. Theoretically, the factors smoking and atherosclerosis may be intermediate factors of the relations under study in chapters 5, 7, and 9. Adjustment for these factors may then have led to overadjustment, taking away most of the effect. We handled this problem by comparing the results with and without these factors. Only in chapter 9 did adjustment for these factors attenuate our estimates, which may suggest that smoking and atherosclerosis are causally important in the relation between ARM and Alzheimer's disease. In future analyses, more sophisticated techniques as G-estimation[43] may be used to further study these factors.

FUTURE RESEARCH

Disease frequency and impact

The prevalence of AMD is now quite well established in western Caucasian populations, but not in many other populations. Reports among subjects from Chinese origin suggesting a high registry of pigment epithelial detachments[7] call for well-

designed surveys in Asian populations. The racial differences in early and late signs of ARM[44,45] need further investigation. In addition, incidence studies with long follow up periods are needed throughout the world. Eventually, global comparisons of these studies registering occurrence, life styles, environmental influences, and genetic background will provide more insights into the causal pathway.

Our findings regarding disease impact indicate that blindness due to the end stages of ARM is common in a population of those aged 75+ years. With the growing life expectancy this is a rather grey perspective, especially when there are no means to change this fate. There is a clear need for preventative and therapeutic strategies, but until research has advanced to that stage, efforts should now be made to improve the impaired functional status, independence, and quality of life of AMD patients. Optical magnifiers using new computer and television technology, or even intra-ocular devices, are a way of alleviating impairment,[46] but teaching patients how to cope with their handicap using the remaining visual field and other senses for daily tasks may improve quality of life just as well. Investigations evaluating these modalities and their cost-effectiveness will lead to better management of patients in the near future.

Genetic Risk

Our findings provide clues for the magnitude of the genetic risk and its heterogeneity. It is now a challenge to dissect the genes that are responsible for this risk. Conventional parametric linkage analysis ideally requires large pedigrees with multiple generations affected. Using this technique, a potential ARM-locus was detected on chromosome 1 in a large multigenerational family with ten subjects with either large confluent drusen or geographic atrophy.[56] Current research is aiming at refinement of this locus and identification of the gene, and others are momentarily investigating this locus for confirmation. Linkage analysis using multigenerational families with ARM will continue to be a powerful tool to identify genes in the future, but these families are difficult to find.

Other techniques known as allele-sharing methods have been developed to overcome this problem.[48] The aim is to show that affected relatives inherit identical copies of a genomic region more often than expected by chance. A great benefit is that this method is non-parametric: it requires no assumptions about mode of inheritance or number of genes. Affected sib-pair analysis is the simplest form of this method, but newer versions take more distantly related relatives into account. At present, several research groups are collecting affected relative pairs, and all have difficulty in estimating the necessary size of the study population. In the complex diseases Alzheimer's disease and non-insulin dependent Diabetes Mellitus, a size of 150-300

sib-pairs was sufficient to show linkage, but, presumably, even more are required for ARM.[49]

Association studies investigating candidate genes do not concern familial inheritance patterns at all. These studies handle the genes of interests as regular exposures using epidemiologic designs like the case-control study. All the pitfalls that affect validity in traditional epidemiologic research play a role in these studies, and especially the choice of a control population is crucial in avoiding selection bias.

Examples of this candidate gene approach is the study by Allikmets et al.[52] reporting associations with variations of the Stargardt's disease gene ABCR. In this study, the coding regions of the ABCR gene were screened for sequence variations in a group of unrelated ARM subjects, and these variations were compared with a control group consisting of non age-matched subjects from the general population collected for other studies. Overall, there was no statistically significant difference in frequency of variants between the two groups (36% vs 31%, P=0.3). However, 13 of the 18 sequence variations were found more frequently in the ARM group, and the authors claimed that these were 'AMD-associated'. Moreover, they suggested that ABCR may be a dominant susceptibility locus for AMD because a heterozygous older adult in a Stargardt family had developed AMD. A major discussion started after the publication of this study concerning flaws in design, analysis, and far-reaching conclusions, which highlighted the challenges of candidate gene analysis.[51,52] The debate accrued even more when two other research groups,[53,54] failed to find an association with ABCR in their case-control studies, while a French study confirmed heterozygous ABCR mutations in three grandparents of Stargardt families.[55] Allikmets et al. are momentarily involved in an international survey using more accepted diagnostic criteria to investigate the frequencies of the two most pronounced ABCR variants.[56] More studies will be needed to establish the role of ABCR in AMD; these could be well-designed association studies, family studies investigating cosegregation of the specific ABCR variant and disease, molecular studies showing affected protein structure, function, or expression, and perhaps in vitro or laboratory studies demonstrating altered gene effects which could be related to ARM.

Research regarding the apolipoprotein E gene should basically follow the same course. Already laboratory studies are evolving to investigate the retinas of APOE deficient or transgenic animals. More clinical as well as laboratory studies are needed to investigate the retina-specific effects of the $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ alleles, for this is as yet not understood.

Considering the fast genetic progress, it is to be expected that at least some of the ARM susceptibility genes will have been identified in the near future. This will then

start off a whole new cascade of research, including genetic epidemiology. Once a gene has been identified in a specific family or patient population, the next step may be to investigate the distribution of this gene in more general populations. If the gene occurs at a considerable rate in those populations, one may want to know if gene expression can be modified, and investigate the relation with environmental factors. This will lead to a better understanding of the mechanisms involved, and may provide 'life-rules' for high-risk individuals.

Environmental risk

Future environmental research should focus on identification of risk factors that provide insight into the etiology of ARM, and preferably on those that are amenable to change. It is important to know how smoking increases the risk of end stage ARM, and at what stage of early ARM one should stop smoking to alter this risk. Nutrition comprehends a large range of environmental risk factors that are potentially modifiable. Most current knowledge is based on cross-sectional analyses of data, and future analyses should make use of incident ARM. When certain food factors or supplements show a stable association in these analyses, intervention research as randomized clinical trials may be initiated to establish a definite causal relationship. Another risk factor that can be influenced is cataract extraction. Well-designed prospective studies are necessary to study the causality of this relation, and to investigate whether any surgical procedures may prevent the increased risk of subretinal neovascularization.

Only little susceptible to change, but interesting from an etiologic point of view are the vascular factors hypertension, atherosclerosis, and estrogens. Incidence studies are also necessary to verify their etiologic role in ARM, and to learn about the mediating mechanism. Although other ARM research areas are investigating this relation,[57-59] more studies are needed which incorporate histopathologic and pathofysiologic data in good clinical epidemiologic studies. This may help elucidate the consequences of atherosclerosis, such as decreased choroidal vascular flow, diminished passage of nutrients and oxygen, increased lipid deposition in Bruch's membrane, accumulation of deposits and membranous debris, clumping of pigment, new vessel formation and loss of photoreceptors.

FINAL REMARK

I hope our studies help improve the insights into the causes of ARM, and stimulate research to further investigate the pathogenesis of ARM. What may be the benefits? The challenges are knowledge of pathologic mechanisms, insight into gene functions, identification of individuals at risk, development of public health measures, better patient management, and new therapeutic options.

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Chapter

11

Summary

Part I - Background

Chapter 1 describes the aims of our studies. ARM is a retinal disease causing a central scotoma in the visual field. There are currently no therapeutic modalities that restore this loss of vision. The ultimate goal of this thesis was to gain more insight into the causes of ARM. For this purpose, we performed genetic epidemiologic investigations based on subjects selected from the Rotterdam Study.

Chapter 2 provides a review of the previous epidemiologic studies on ARM. The general notion is that genetic factors are important in the disease etiology, but to what extent is unclear. Smoking is the most-established environmental factor associated with the disease to date.

Part II - Disease frequency and impact

Chapter 3 reports on the incidence of the end stages of ARM, also called AMD, and on the natural course of the disease. In 2 years, 2 per 1000 subjects of our total study population developed AMD. Subjects older than 85 years, and subjects with indistinct drusen and RPE-alterations in the macular area were most at risk. The early stages of ARM appeared to progress in a distinct pattern at a stable rate.

Chapter 4 shows data on the consequences of ARM with regard to visual function. The end stages were the most frequent cause of bilateral blindness after the age of 75 years, and the proportion of subjects blind due to AMD increased significantly with further rising age. In subjects aged 85 years and older, one fourth of all blindness was caused by AMD.

Part III - Genetic risk of ARM

Chapter 5 reports on the magnitude of the genetic component. In a familial aggregation study, we estimated that the lifetime relative risk of AMD for first degree relatives of affected subjects was 4.2 (95% CI 2.6, 6.8). Furthermore, we calculated that approximately one fourth of all AMD in the general population can be attributed to genetic factors.

Chapter 6 describes the familial risk in greater detail. The increased risk of ARM was not the same among families. On the contrary, we demonstrated that there are low, intermediate and high risk families. The data suggest that only a small fraction of all ARM is caused by a strong genetic component.
Chapter 7 provides evidence for a genetic association with the APOE gene. APOE had been associated with other neurodegenerative disorders, which prompted us to study its relation with AMD. Subjects with the $\epsilon 4$ allele were at a significantly decreased risk of AMD, while subjects with the $\epsilon 2$ allele had a slightly increased risk. Since the association was not immediately understood, we investigated whether the apoE protein was present in the macula of the eye. In an immunohistochemical study in human post-mortem eyes, we demonstrated that apoE was particularly present in soft drusen and basal laminar deposit, the pathological hallmarks of ARM.

Part IV - Environmental risk and comorbidity
Chapter 8 gives a supplement to an earlier investigation in the Rotterdam Study. In this analysis, we demonstrated that the relation between smoking and AMD is independent of age. The relation was also present in subjects aged 85 years and over.
Chapter 9 shows that ARM and Alzheimer's disease have certain risk factors in common. Subjects with severe ARM had an increased risk of incident Alzheimer's disease, but this relation was mostly determined by smoking and atherosclerosis.

Part V - General discussion and summary
Chapter 10 provides the general discussion on all the studies described in this thesis. In this chapter, strategies for future research are discussed.

Samenvatting

Deel 1 - Achtergrond

Hoofdstuk 1 beschrijft de doelstellingen van dit promotie-onderzoek. Leeftijd-gebonden maculopathie, ook wel ouderdoms maculopathie genoemd, is een ziekte van het netvlies die een zwarte vlek in het centrum van het gezichtsveld veroorzaakt. Er zijn momenteel nog geen therapeutische mogelijkheden om dit gezichtsverlies te herstellen. Het doel van dit proefschrift was om de kennis omtrent de oorzaken van leeftijd-gebonden maculopathie te vergroten. Hiertoe verrichtten wij genetisch epidemiologische studies gebaseerd op personen die deelgenomen hadden aan het *ERGO*-onderzoek (Erasmus Rotterdam Gezondheid voor Ouderen).

Hoofdstuk 2 geeft een overzicht van voorgaande epidemiologische studies naar maculopathie. De huidige mening is dat deze ziekte genetische oorzaken heeft, maar het is nog onduidelijk in welke mate genen het risico op de ziekte bepalen. Roken is momenteel de belangrijkste omgevings factor die geassocieerd is met maculopathie.

Deel 2 - Frequentie en gevolgen van de ziekte

Hoofdstuk 3 rapporteert de incidentie van de eindstadia van leeftijd-gebonden maculopathie, ook wel maculadegeneratie genoemd. Tevens wordt het natuurlijk verloop van de ziekte beschreven. In 2 jaar ontwikkelden 2 op de 1000 personen in onze studie maculadegeneratie. Personen ouder dan 85 jaar, en personen met onscherp begrensde *drusen* (witte vlekjes) op het netvlies met veranderingen van het retinaal pigmentepitheel, hadden het grootste risico om maculadegeneratie te krijgen. De progressie van de vroege stadia van maculopathie bleek volgens een vast stramien te verlopen.

Hoofdstuk 4 geeft de consequenties van de ziekte voor het zien weer. Maculadegeneratie was de meest frequente oorzaak van bilaterale blindheid na de leeftijd van 75 jaar, en het aandeel van maculadegeneratie in de totale blindheid nam nog verder toe met de leeftijd. In personen ouder dan 85 jaar was maculadegeneratie verantwoordelijk voor een kwart van alle blindheid.

Deel III - Genetisch risico op leeftijd-gebonden maculopathie

Hoofdstuk 5 beschrijft in welke mate maculadegeneratie genetisch bepaald is. Wij berekenden in een familie aggregatie onderzoek, dat het risico op de ziekte voor eerste graads familieleden van aangedane personen ruim vier keer verhoogd is. Tevens schatten wij, dat ongeveer een kwart van alle maculadegeneratie in de algemene bevolking door genetische factoren veroorzaakt wordt.

Hoofdstuk 6 geeft een meer gedetailleerde beschrijving van dit genetisch risico. Niet alle maculadegeneratie families bleken dezelfde kans op de ziekte te hebben. Integendeel, wij toonden aan dat er families waren met een laag risico, een middelmatig verhoogd risico, en een sterk verhoogd risico. Onze gegevens suggereren dat slechts een klein deel van alle maculadegeneratie door een sterke genetische factor bepaald wordt.
Hoofdstuk 7 levert bewijs voor een genetische associatie met het apolipoproteine E gen. Uit eerder onderzoek bleek dit gen belangrijk voor neurodegeneratieve ziekten, zoals de ziekte van Alzheimer. Dit inspireerde ons om de rol van apolipoproteine E bij maculadegeneratie te onderzoeken. Wij vonden dat personen met het $\epsilon 4$ allel een lager risico op de ziekte hadden dan personen met het normale allel. Omdat ons niet geheel duidelijk was of dit gen daadwerkelijk bij de ziekte betrokken is, onderzochten wij of het apolipoproteine eiwit aanwezig is in de macula. In een immunohistochemische studie op post-mortem verkregen humane ogen, toonden wij het eiwit aan in die structuren, die bepalend zijn voor leeftijd-gebonden maculopathie. Onze gegevens suggereren dat het apolipoproteine E gen een rol speelt in het ontstaan van maculopathie.

Deel IV - Omgevings risico en comorbiditeit
Hoofdstuk 8 is een aanvulling op een eerder verschenen artikel van het *ERGO*-onderzoek. In deze analyse lieten wij zien, dat de relatie tussen roken en maculadegeneratie onafhankelijk is van leeftijd. Ook rokers ouder dan 85 jaar hadden een verhoogd risico op maculadegeneratie.
Hoofdstuk 9 laat zien dat het ontstaan van leeftijd-gebonden maculopathie overeenkomsten heeft met de ziekte van Alzheimer. Personen met ernstige vormen van maculopathie hadden een verhoogd risico om de ziekte van Alzheimer te ontwikkelen, maar deze relatie was met name bepaald door roken en aderverkalking. Deze factoren lijken gezamenlijke risico factoren te zijn.

Deel V - Algemene discussie en samenvatting
Hoofdstuk 10 bediscussieert alle bevindingen van dit proefschrift. Ook worden in dit hoofdstuk aanbevelingen gedaan voor verder onderzoek.

Chapter
12

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Laat die eeuw nu maar komen.

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