ISBN 978-952-92-3711-1 (paperback)
ISBN 978-952-10-4641-4 (PDF)
Yliopistopaino
Helsinki 2008

Sanna Seitsonen

Molecular Genetics of Age-related Macular Degeneration

Molecular Genetics of Age-related Macular Degeneration

Sanna Seitsonen

Department of Ophthalmology
University of Helsinki
Helsinki, Finland

Department of Medical Genetics
University of Helsinki
Helsinki, Finland

Molecular Genetics of Age-related Macular Degeneration

By

Sanna Seitsonen

Academic Dissertation

To be publicly discussed, by permission of
The Medical Faculty of the University of Helsinki
In the Lecture Hall 3 of Biomedicum Helsinki
Haartmaninkatu 8, Helsinki,
On April 18th, 2008, at 12 o'clock noon.

Helsinki 2008

Supervised by

Ilkka Immonen, MD, PhD
Department of Ophthalmology
Helsinki University Hospital
Helsinki, Finland

Irma Järvelä, MD, PhD
Department of Medical Genetics
University of Helsinki
Helsinki, Finland

Reviewed by

Iris Sorri, MD, PhD
Department of Ophthalmology
Kuopio University Hospital
Kuopio, Finland

Markku Viander, MD, PhD
Department of Microbiology
University of Turku
Turku, Finland

Discussed with

Professor Usha Chakravarthy, MD
Centre for Vision Science
Queen's University Belfast
Belfast, UK

ISBN 978-952-92-3711-1 (paperback)

ISBN 978-952-10-4641-4 (PDF)

Yliopistopaino

Helsinki 2008

Memento vivere.

J.W. Goethe

To Topi, Pauli, and Aini-Maija

TABLE OF CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following original articles on genetics of age-related macular degeneration, referred to in the text by Roman numerals I-V.

I Sanna Seitsonen, Susanna Lemmelä, Juha Holopainen, Petri Tommila, Päivi Ranta, Antti Kotamies, Jukka Moilanen, Tapani Palosaari, Kai Kaarniranta, Seppo Meri, Ilkka Immonen, Irma Järvelä. Analysis of variants in the complement factor H, the elongation of very long chain fatty acids-like 4 and the hemicentin 1 genes of age-related macular degeneration in the Finnish population. Mol Vis 2006;12:796-801.

II Sanna Seitsonen, Irma Järvelä, Seppo Meri, Petri Tommila, Päivi Ranta and Ilkka Immonen. Complement factor H Y402H polymorphism and characteristics of exudative age-related macular degeneration lesions. Acta Ophthalmol Scand; 2007 Nov 7; [Epub ahead of print].

III S. P. Seitsonen, I. E. Järvelä, S. Meri, P. V. Tommila, P. H. Ranta, I. J. Immonen. The effect of complement factor H Y402H polymorphism on the outcome of photodynamic therapy in age-related macular degeneration. Eur J Ophthalmol 2007;17:943-9.

IV Matti Laine, Hanna Jarva, Sanna Seitsonen, Karita Haapasalo, Markus J. Lehtinen, Nina Lindeman, Don H. Anderson, Patrick T. Johnson, Irma Järvelä, T. Sakari Jokiranta, Gregory S. Hageman, Ilkka Immonen, and Seppo Meri. Y402H Polymorphism of Complement Factor H Affects Binding Affinity to C-Reactive Protein. J Immunol 2007;178:3831-6.

V Sanna P Seitsonen, Päivi Onkamo, Petri V Tommila, Päivi H Ranta, Juha M Holopainen, Jukka A Moilanen, Tapani Palosaari, Kai Kaarniranta, Seppo Meri, Ilkka J Immonen, Irma E Järvelä. Interaction between Complement Factor H Y402H and LOC387715 A69S in Age-related Macular Degeneration. Submitted.

ABBREVIATIONS

ABCA4	ATP-binding cassette (ABC) transporter 4
AMD	age-related macular degeneration
APOE	apolipoprotein E
AREDS	Age-Related Eye Disease Study
ASP	affected sib-pair
B	factor B
BCA	bicinchonic acid
BDES	Beaver Dam Eye Study
BMES	Blue Mountains Eye Study
C3	complement component 3
CAD	coronary artery disease
CFB	complement factor B
CFH	complement factor H
CI	confidence interval
CNBr	cyanogenbromide
CNV	choroidal neovascularization
CRP	C-reactive protein
DF	degrees of freedom
dGI	dietary glycemic index
DNA	deoxyribonucleic acid
ELOVL4	elongation of very long chain fatty acids-like 4
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	fluorescein angiography
FGF	fibroblast growth factor
FHL	factor H-like
FHR	factor H related
GLD	greatest linear dimension
HDL	high density lipoprotein
HPLC	high-performance liquid chromatograph
HTRA1	high-temperature requirement factor A 1
HUS	hemolytic uremic syndrome

ICG	indocyanine green (angiography)
LD	linkage disequilibrium
LOD	logarithm of odds
MAC	membrane-attack complex
MASP	mannan-binding lectin -associated proteases
MBL	mannan-binding lectin
MPGNII	membranoproliferative glomerulonephritis type II
mRNA	messenger ribonucleic acid
Na_2SO_4	sodium sulfate
OCT	optical coherence tomography
OD_{492}	optical density at 492 nm
OMIM	Online Mendelian Inheritance in Man
OR	odds ratio
PAR	population attributable risk
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDT	photodynamic therapy
PED	pigment epithelial detachment
PLEKHA1	pleckstrin homology domain-containing protein 1
RAP	retinal-angiomatous proliferation
RCA	Regulators of Complement Activation
ROI	reactive oxidative intermediates
RPE	retinal pigment epithelium
RS	Rotterdam Study
SCR	short consensus repeat
SD	standard deviation
SDS-Page	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SNP	single nucleotide polymorphism
TAP	Treatment of AMD with Photodynamic Therapy
TGF	transforming growth factor
TLR4	toll-like receptor 4
VEGF	vascular endothelial growth factor
VIP	Verteporfin in Photodynamic Therapy

ABSTRACT

Age-related macular degeneration (AMD; OMIM # 603075) is an eye disease of the elderly, signs of which appear after the age of 50. In the Western world it is a leading cause of permanent visual loss with a prevalence of 8.5% in persons under 54 years of age and of 37% in persons over 75 years of age. Early forms of AMD may be asymptomatic, but in the late forms usually a central scotoma in the visual field follows severely complicating daily tasks.

Smoking, age, and genetic predisposition are known risk factors for AMD. Until recently no true susceptibility genes had been identified though the composition of drusen deposits, the hallmarks of AMD, has suggested that the complement system might play a role in the pathogenesis of AMD. When four groups reported in March 2005, that, on chromosome 1q32, a Y402H variant in the *complement factor H* (*CFH*) gene confers risk for AMD in independent Caucasian samples, a new period in the field of genetic research of AMD started. CFH is a key regulator of the complement system. Thus, it is logical to speculate, that it plays a role in the pathogenesis of AMD.

We performed a case-control association study to analyse whether the *CFH* Y402H variant contain a risk for AMD in the Finnish population. Although the population of Finland represents a genetic isolate, the *CFH* Y402H polymorphism was associated with AMD also in our patient sample with similar risk allele frequencies as in the other Caucasian populations. We further evaluated the effects of this variant, but no association between lesion subtype (predominantly classic, minimally classic or occult lesion) or lesion size of neovascular AMD and the *CFH* Y402H variant was detected. Neither did the variant have an effect on the photodynamic therapy (PDT) outcome. The patients that respond to PDT carried the risk genotype as frequently as those who did not respond, and no difference was found in the number of PDT sessions needed in patients with or without the risk genotypes of *CFH* Y402H. Functional analyses, however, showed that the binding of C-reactive protein (CRP) to CFH was significantly reduced in patients with the risk genotype of Y402H.

In the past two years, the *LOC387715*/ *high-temperature requirement factor A1* (*HTRA1*) locus on 10q26 has also been repeatedly associated with AMD in several populations. The recent discovery of the LOC387715 protein on the mitochondrial outer membrane suggests that the *LOC387715* gene, not *HTRA1*, is the true predisposing gene in this region, although its biological function is still unknown. In our Finnish patient material, patients with AMD carried the A69S risk genotype of

LOC387715 more frequently than the controls. Also, for the first time, an interaction between the *CFH* Y402H and the *LOC387715* A69S variants was found.

The most recently detected susceptibilty gene of AMD, the *complement component 3* (*C3*) gene, encodes the central component of the complement system, C3. In our Finnish sample, an additive gene effect for the *C3* locus was detected, though weaker than the effects for the two main loci, *CFH* and *LOC387715*. Instead, the *hemicentin-1* or the *elongation of very long chain fatty acids-like 4* genes that have also been suggested as candidate genes for AMD did not carry a risk for AMD in the Finnish population.

This was the first series of molecular genetic study of AMD in Finland. We showed that two common risk variants, *CFH* Y402H and *LOC387715* A69S, represent a high risk of AMD also in the isolated Finnish population, and furthermore, that they had a statistical interaction. It was demonstrated that the *CFH* Y402H risk genotype affects the binding of CFH to CRP thus suggesting that complement indeed plays an important role in the pathogenesis of AMD.

1 INTRODUCTION

Life expectancy is continuously rising in the Western world. We all hope that these extra days of old age would be full of health and capability of managing with daily tasks. Age-related macular degeneration (AMD) is a leading course of irreversible visual loss in the industrialized countries, and thus one of the threats for freedom and activity in old age. Neovascular AMD, the most severe form of AMD, leads frequently to bilateral loss of central vision, which fundamentally troubles daily tasks, including reading, cooking, and driving a car.

The pathogenesis of AMD has been largely unknown and therefore the choices of preventive and therapeutic strategies are limited. A strong genetic component in AMD has been established, suggesting that it is a complex disease, probably several genes and environmental factors predispose individuals to it and, similarly protect them from it. Therefore, the methods of molecular genetics that need no prior knowledge of pathogenesis have been applicable. Besides, the results obtained from these studies have been exceptionally successful in AMD. First, genome wide scans showed repeatedly linkage to two chromosomal regions 1q32 and 10q26. Focused genotyping of single nucleotide polymorphisms (SNP) revealed the first real susceptibility gene, *complement factor H* (*CFH*) on 1q32. The Y402H polymorphism of *CFH* has been significantly associated with AMD in several populations. CFH plays a fundamental role in the regulation of the complement system, which is a vital part of innate immunity, and it thus offers a new biological pathway to understand the pathogenesis of AMD.

The three-year period, in which this study has been mainly performed, has been internationally a time of significant progress in the genetics of AMD. Since the finding of polymorphism in the *CFH* gene, several other susceptibility genes have been reported, probably the most significant of these being the *LOC387715*/*HTRA1* locus residing on 10q26. In contrast to many other complex diseases where each genetic variant contributes only to a relatively small percentage of cases this seems not to be the case in AMD. Namely, the *CFH* Y402H polymorphism and the *LOC387715*/*HTRA1* locus appear to explain jointly even 70 % of the AMD cases. Furthermore, a variant in the *complement component C3*, a crucial element in the complement system, has also appeared to confer a risk for AMD, thus further emphasizing the role of the complement cascade in the pathogenesis of AMD.

This study was undertaken to evaluate whether the recently identified candidate genes contain a risk for AMD also in the Finnish population that has characteristics of genetic isolation. The *CFH* Y402H polymorphism was further analysed since it is almost certainly one of the main contributors

in AMD pathogenesis. Especially, as the polymorphism is located at CRP binding site of CFH it may thus connect the genetic change with a protein-level risk factor for AMD. It was evaluated whether the *CFH* has an effect on lesion characteristics of neovascular AMD or on the outcome of the photodynamic therapy. In addition, the effects of the *CFH* Y402H polymorphism on the CFH protein function were analysed. Finally, the statistical interaction of the *CFH* Y402H, *LOC387715* A69S, and *C3* R102G polymorphisms, and smoking was evaluated.

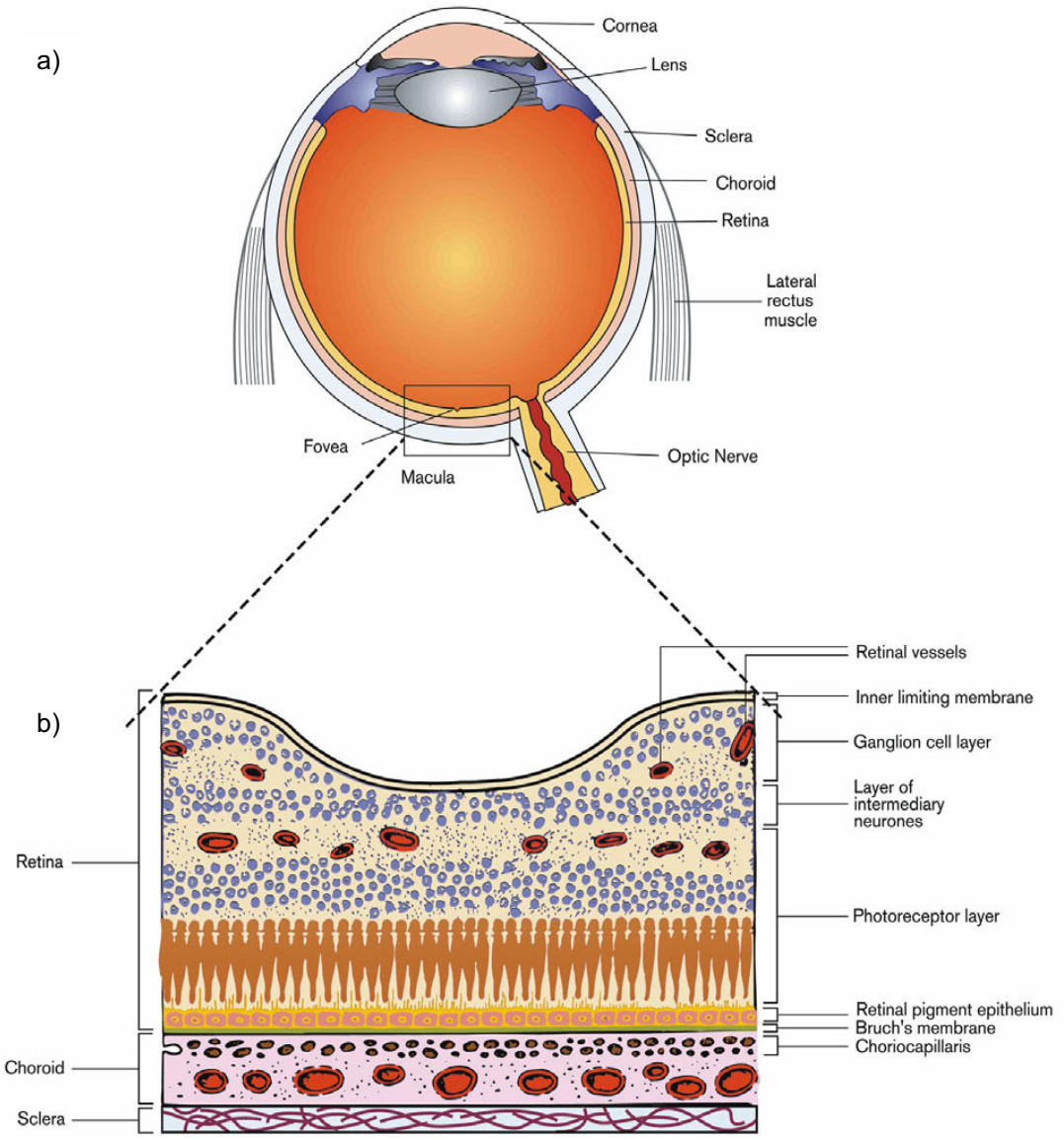
2 REVIEW OF THE LITERATURE

2.1 DIAGNOSIS OF AGE-RELATED MACULAR DEGENERATION

2.1.1 Overview of the anatomy

The retina is a transparent lining of the fundus of the eyeball with several layers of highly specialized cells, of which the rod and cone photoreceptors capture light rays and convert them into electrophysiological impulses. The ineer retina is adjacent to the vitreous and the outer retina to the choroid. The outer retina, the most essential structure in AMD, consists of outer segments of photoreceptors, retinal pigment epithelium (RPE), and Bruch's membrane. The RPE is a monolayer of cuboidal cells, rich of melanosomes from which the name is derived. It has a crucial waste-disposal function in the phagocytosis of shed photoreceptor outer segments. Bruch's membrane is a three-layered membrane under RPE: The innermost elastic layer is between two collagenous layers which are limited to the RPE basement membrane and to the choriocapillaris endothelial basement membrane. Both the RPE and Bruch's membrane play a pivotal role, on one hand as a barrier between the subretinal space and the choroid, and on the other hand, in the transport of molecules between these two spaces (Odgen 1994).

The macula, or macula lutea which means yellow spot in Latin, is the central area of the retina, which is responsible for the central visual field. It is 6 mm in diameter and is located temporal to the optic disc, and lined with temporal vascular arcades. The cones are the principal type of photoreceptor cells in the macula with the highest density in the fovea (Ø 1.5 mm), the centre of the macula and the area of the sharpest vision (Figure 1) (Odgen 1994).



Reproduced with permission from Novartis

Figure 1. *Anatomic structures of the eyeball (a) and cross section of layers of the eye (b) in the macula.*

2.1.2 Classification

In early AMD, drusen that are hallmarks of AMD appear between the RPE basal lamina and the inner collagenous layer of Bruch's membrane. They are pathological lipoproteinaceous deposits which are funduscopically seen as yellow or white spots in the macula. The composition of drusen has been constantly under investigation and they are known to be comprised of many immunological molecules (Anderson et al. 2002). Drusen are classified according to their morphology (hard/soft/distinct/indistinct), to their predominant type, to number, to size, to main location, and to the percentage of area they cover. Pigment epithelial changes are early signs of AMD, as well. Either hypo- or hyperpigmentation is seen along with drusen (Bird et al. 1995). Early AMD may proceed to late AMD, but it is not uncommon that a patient with no major signs of early AMD presents first with late wet AMD. Interestingly, drusen may disappear when early AMD proceeds to late AMD (Klein et al. 1997, Mitchell et al. 2002a).

Late AMD has two forms, neovascular (exudative/wet/disciform) AMD and central geographic atrophy (dry late AMD). In wet AMD, vessels grow from the choriocapillaris through abnormal Bruch's membrane to the space between the neural retina and the RPE (subretinal space) or between the RPE and Bruch's membrane (sub-RPE space) forming neovascular membranes. These novel vessels easily leak leading to accumulation of fluid and exudates or even haemorrhage in the subretinal and the sub-RPE spaces. Detachment of the neurosensory retina or the RPE follows. The final form of neovascular AMD is called disciform macular degeneration, which is characterized by a scar tissue under the retina.

The early neovascular AMD lesions can further be graded as predominantly classic, minimally classic, or occult according to fluorescein angiographic pattern, where changes in retinal and choroidal hemodynamics are analysed. Leakage refers to a presence of fluorescein outside blood vessels with increasing density in the late phases of angiography. Sometimes the term pooling has been used to describe increased leakage of fluorescein into delineated anatomical space. Diffuse leakage to tissues, such as subretinal fibrosis or drusen, is staining. In the window effect an intense choroidal fluorescence is seen because of atrophy of the RPE. In the classic type, the choroidal neovascularization (CNV) more predominantly grows to the space between the RPE and the neural retina. In an angiogram, the classic lesion is seen as a hyperfluoresence with a lacy pattern, unrelated to the window effect, in the early fluorescein angiogram, and leakage of fluorescein with indistinct edges in the late phase of angiogram. The occult lesion type grows more frequently to the sub-RPE-space and shows no or minimal early hyperfluoresence in the early phase, while it

hyperfluoreseces with indistinct edges, unrelated to the window effect, in the late phase (Macular Photocoagulation Study Group 1991b, Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group 1999). Frequently, the neovascular lesions consist of both classic and occult components. In the predominantly classic lesion, the classic component covers ≥50% of the total CNV lesion area and in the minimally classic lesion less than 50% (Blinder et al. 2003). These classifications are used in cases, where the combined classical and occult CNV lesion covers ≥50% of the total lesion area. Typically, other lesion components than CNV are serous pigment epithelial detachment or haemorrhage. Central geographic atrophy, dry form of late AMD, is characterized by central areas of hypopigmentation or depigmentation or absence of RPE, in which the underlying large choroidal vessels become visible with the window effect (Bird et al. 1995). Of all AMD patients, approximately 10 % are suffering from neovascular AMD, and the rest from early AMD (80%) or central geographic atrophy (10%) (Friedman et al. 2004), but neovascular AMD accounts for the majority (80-90%) of severe visual loss (Ferris et al. 1984).

In this thesis a commonly accepted term “age-related macular degeneration (AMD) ” is used to cover the spectrum of the disease. “Early age-related macular degeneration” or “late age-related macular degeneration” are used when referring to different stages of the disease, the former including drusen and pigment epithelial changes, the latter neovascular (exudative/wet) AMD and central geographic atrophy (dry late AMD).

2.1.3 Clinical diagnosis

Patients with early AMD are often asymptomatic. Moderate decrease of visual acuity or minimal distortion may occur, however. In the wet AMD distortion of lines and images (also called metamorphopsia) is usually the first sign while in initial stages of late dry AMD there may be gaps in images or the disappearance of letters while reading. At the final stage of both type of late AMD, there is typically a central scotoma in the central visual field seriously disturbing many essential daily tasks, including reading a newspaper, preparing meals, driving a car, and recognizing faces of acquaintance.

Diagnosis of AMD is based on macular findings irrespective of the visual acuity. Biomicroscopy of the fundus, fundus photographs, fluorescein (FA) and/or indosyanine green (ICG) angiography, and increasingly, optical coherence tomography (OCT) have been used as diagnostic tools. An Amsler Grid is especially helpful for patients monitoring vision at home (Figure 2).

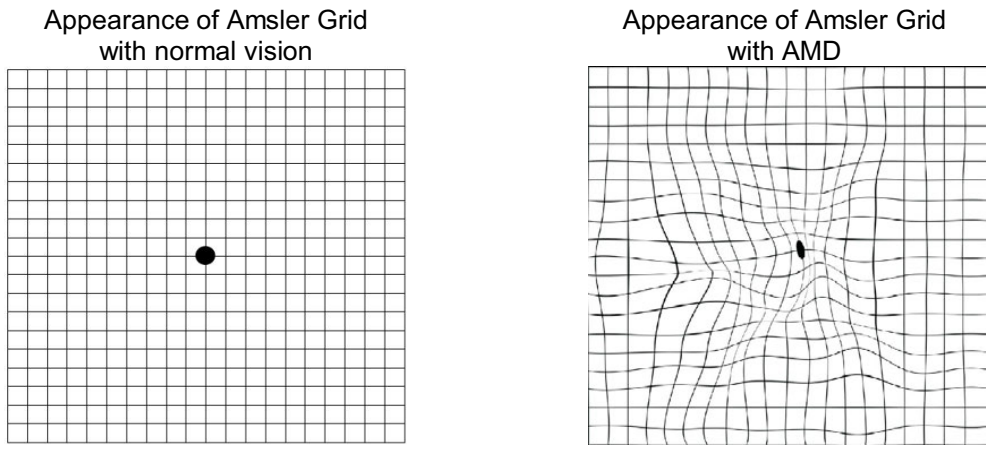


Figure 2. *Patient is asked to stare at the dot in the center of the grid. Wavy lines or missing squares in the grid suggest irregularity of the photoreceptor layer, which is typically caused by a wet AMD lesion.*

2.2 EPIDEMIOLOGY

2.2.1 Prevalence and incidence

Age-related macular degeneration is the leading cause of irreversible visual loss in the elderly in the Western world. While the elderly population is continuously growing in number, AMD is becoming a remarkable public health problem.

Several population-based studies have been performed to study the actual prevalence and incidence of AMD. Three major studies, the Beaver Dam Eye Study (BDES) from the US, The Blue Mountains Eye Study (BMES) from Australia, and the Rotterdam Study (RS) from the Netherlands have used similar protocols, the Beaver Dam Eye Study and the Blue Mountains Eye Study protocols' are almost identical, to evaluate the stage of AMD (Mitchell et al. 1995), which permits direct comparison of these three studies (Klein et al.1992, Mitchell et al. 1995, Vingerling et al. 1995).

Prevalence rates of early AMD (soft drusen and retinal pigmentary abnormalites) are clearly higher than the rates of late AMD. In the Beaver Dam Eye Study the prevalence of early AMD was 15.6% in persons aged ≤86, which was, nevertheless, higher than in the Blue Mountains Eye Study with a prevalence of 7.2 %. The authors propose that this might reflect a real difference between the two

populations, US and Australian, rather than a grading difference (Mitchell et al. 1995). The prevalence of early AMD increases with age as shown in Table 1.

The prevalence of late AMD (neovascular AMD or central geographic atrophy) in all persons ≤86 years of age has been fairly similar in these three studies (1.6%, 1.9% and 1.7%, respectively) (Klein et al. 1992, Mitchell et al. 1995, Vingerling et al. 1995). The same applies to the ratio of AMD subclasses with 2:1 for neovascular to atrophic AMD (Mitchell et al. 1995). However, in the age group 55-86, a lower neovascular AMD prevalence (which remained almost unchanged after adjusting for age and smoking-status) was detected in the Rotterdam study with OR of 3.07 for the Beaver Dam Eye Study and OR of 2.61 for the Blue Mountains Eye Study when compared to the Rotterdam study (Smith et al. 2001). The difference may reflect differing environmental or genetic factors between the European and US or Australian populations. Generally, a steep increase can be seen after 75 years of age in the prevalence of late AMD with percentages in each age group being: 0.2% (55-64 yrs), 0.9% (65-74 yrs), 4.6% (75-84 yrs) and 13.1% (>85 yrs) (Smith et al. 2001).

In all three studies the incidence of AMD seems also strongly to be influenced by the age of patient plus the stage of AMD at the baseline. In the Rotterdam Study the maximum 5-year risk of 42% was for persons ≥80 years of age who had soft indistinct drusen and retinal pigmentary abnormalities at the baseline (van Leeuwen et al. 2003b). In the Blue Mountains Eye Study it was estimated that 20-30% of eyes with late lesions in one eye will also develop late AMD in the second eye in five years (Mitchell et al. 2002a). However, eyes with only small hard drusen at the baseline did not develop to late AMD in five years regardless of the area involved (Klein et al. 1997).

In a Finnish population based study of 478 persons aged ≥70, the prevalence of AMD was as follows: early AMD 32%, geographic atrophy 5.5% and, exudative AMD 3.8%. The prevalence of any AMD was 41% which was slightly higher than 37% in patients ≥75 years of age in the Beaver Dam Eye Study. In the Finnish study the participants were older (range 70-95 years) than in the Beaver Dam Eye Study (range 43-86 years) which may explain the minor difference (Laatikainen et al. 1995).

Seven population-based studies of the prevalence of AMD from the US, Australia and Europe (Klein et al. 1992, Mitchell et al. 1995, Schachat et al. 1995, Vingerling et al. 1995, West et al. 1997, Friedman et al. 1999, VanNewkirk et al. 2000) were used in a meta-analysis which was undertaken to estimate the overall prevalence of AMD in the US (Friedman et al. 2004). The prevalence of late AMD (neovascular AMD or central geographic atrophy) was estimated to be

1.47% in persons ≥40 of age and over 11% in persons older than 80 years, which indicates that in the year 2000 a total of 1.75 million individuals were suffering from central visual loss caused by AMD in the US and the number is estimated to increase to 2.95 million by 2020 (Friedman et al. 2004). If these estimations are extrapolated to Finnish population, it means roughly that in 2006 over 37 000 persons aged ≥40 and over 26 000 persons aged ≥80 years had advanced AMD (Statistics Finland, Population Structure 29.3.2007; http://www.stat.fi/tup/suoluk/suoluk_vaesto.html). With the longer life expectancy of Western populations these figures are quite a challenge for future health care.

Table 1. *Prevalence and incidence of age-related macular degeneration (AMD) in the three population-based studies. Percentages for two age groups and for a total patient material of the study are presented. Ranges in the age groups differ since prevalences are reported differently. (Klein et al. 1992, Klein et al. 1997, Klein et al. 2002, Klein et al. 2007, Mitchell et al. 1995, Mitchell et al. 2002a, Wang et al. 2007a, Vingerling et al. 1995, van Leeuwen et al. 2003b)*

		BDES n=4926			**BMES** n=3654			**RS** n=6251		
		55-64	**75-86**	**Total**	**55-64**	**75-86**	**Total**	**55-64**	**75-84**	**Total**
Prevalence	Early AMD	13.8%	29.7%	15.6%	2.6%	15.5%	7.2%	*	*	*
	Late AMD	0.6%	7.1%	1.6%	0.2%	5.7%	1.9%	0.2%	3.7%	1.7%
		55-64	**75-86**	**Total**	**60-69**	**80+**	**Total**	**60-64**	**80+**	**Total**
5-year incidence	Early AMD	4.7%	22.8%	8.2%	7.4%	14.8%	8.7%	2.6%	22.5%	7.9%
	Late AMD	0.3%	5.4%	0.9%	0.6%	5.4%	1.1%	0.1%	3.4%	0.9%
10-year incidence	Early AMD	10.7%	36.7%	12.1%	14.7%	32.5%	14.1%	NA		
	Late AMD	1.0%	9.5%	2.1%	3.0%	24.3%	3.7%			
15-year incidence	Early AMD	12.7%	24.4%	14.3%	NA			NA		
	Late AMD	2.6%	7.6%	3.1%						

BDES= Beaver Dam Eye Study

BMES= Blue Mountains Eye Study

RS= Rotterdam Study

*In RS study drusen and pigmentary abnormalities are analysed separately while in BDES and BMES studies "early AMD" is reported as an entity

NA= Not available

2.2.2 Risk factors

Several factors have been identified as potential risk factors for AMD, the most consistent of these being age, tobacco smoking (Thornton et al. 2005), and heredity (will be discussed in sections 2.5-2.6), which have been repeatedly and strongly associated with increased risk of AMD.

The incidence and prevalence of AMD increases with age (Table 1). In the pooled analysis of the Beaver Dam Eye Study, the Blue Mountains Eye Study and the Rotterdam Study (14752 participants, aged 43 to 99 years) the prevalence of AMD was 0.2% in persons aged 55 to 64 years but rise up to 13% in those older than 85 years (Smith et al. 2001). Age (>70 versus ≤65 years of age) was a risk factor also in all four case groups of the Age-Related Eye Disease Study (AREDS) study with ORs of 1.47 (Group 2), 2.80 (Group 3), 3.12 (Group 4) and 4.11 (Group 5) (Age-Related Eye Disease Study Research Group 2000).

Tobacco smoking, the most significant preventable lifestyle exposure, has been found to increase the risk for AMD from 1.7 to 3.2 -fold in ever smokers and from 1.9 to 4.5-fold in current smokers (Smith et al. 1996, Delcourt et al. 1998, Seddon et al. 2006). An increased 5-year incidence of late AMD was detected when comparing current smokers to never smokers (OR 2.35, p=0.008) or to past smokers (OR 1.82, p=0.04) (Tomany et al. 2004b). A dose-response relationship has been found with a 6.5-fold risk for neovascular AMD in persons who have smoked more than 10 pack-years (Vingerling et al. 1996) when compared to persons who had never smoked. Past smokers´ risk stayed higher for 20 years after cessation of smoking (Vingerling et al. 1996, Delcourt et al. 1998) and even passive smokers may have an increased, although not statistically significant, risk (OR=1.42, 95%CI 0.62-3.26)) of late AMD (Smith et al.1996). Current smokers developed late AMD at an earlier age than ever smokers or non-smokers (mean ages for cases with incident late AMD: current smokers: 67; past smokers: 73 and; non-smokers: 77 years) (Mitchell et al. 2002b). Furthermore, smoking appears to increase the risk of bilateral AMD. The risk of bilateral AMD was 5-fold (OR 5.08, p=0.03) in heavy smokers (median of pack-years 18.5), but quitting smoking more than 20 years ago decreased the risk of bilateral AMD (OR 0.49, p=0.0004) (Chakravarthy et al. 2007).

At present, the rest of the risk factors are less consistent. Female gender has been associated with increased risk of AMD in many studies. However, it has been speculated that only population based studies are reliable in considering this issue since women have a tendency to use health care services more than men (Evans 2001). From this point of view, in the pooled data of three large population based studies (Smith et al. 2001), no overall difference between female and male gender existed (OR 0.95, 0.95% CI 0.70-1.28), though a higher 5-year and 10-year incidence of neovascular AMD was detected in women in the Blue Mountains Eye Study (Mitchell et al. 2002a, Wang et al. 2007a).

Being a member of the white race may predispose people to AMD. Rates of neovascular disease have been lower in black populations but differences in prevalence of early AMD have not been that clear (Pieramici et al. 1994, Friedman et al. 1999, Age-Related Eye Disease Study Research Group 2000). Generally, variations between different ethnic groups do exist, but it would be challenging to evaluate whether they really represent racial differences rather than differences in life style factors (Klein et al. 1999a).

Studies of alcohol consumption are conflicting, as well. A protective effect of moderate wine consumption has been reported (Obisesan et al. 1998), while in a study of female nurses and male health professionals (Cho et al. 2000) or in the Beaver Dam Eye Study (Knudtson et al. 2007) evidence of an inverse or positive effect of alcohol was not substantial. However, there was an increased risk of early and dry form of AMD in a former study among women with two or more white wine drinks per day (RR 2.03, 95% CI 1.24-3.30) and in a latter study the cumulative 15-year incidence of geographic atrophy (OR 9.2, 95% CI 1.7-51.2) was increased among heavy drinking men (four or more drinks daily). No association was found neither with coffee nor caffeine consumption and early AMD (Tomany et al. 2001).

Several dietary factors have been suggested to have an effect on AMD. The authors of the AREDS study recommended that zinc and antioxidant supplementation (vitamin C: 500mg, vitamin E: 400IU beta carotene: 15 mg, and zinc: 80 mg) should be considered for persons with intermediate size drusen, at least one large drusen, noncentral geographic atrophy in one or both eyes, or advanced AMD or vision loss due to AMD in one eye, based on the finding that persons with these lesions and supplementation had 25 % reduction in 5-year progression of advanced AMD. The doses used were 5 to 15 times the daily value but no significant adverse effects were reported, though the supplementation with beta carotene was not recommended for smokers (Age-Related Eye Disease Study Research Group 2001). The result was supported by a population based cohort with even a 35% reduction in incident AMD in cases with above-median dietary intake of vitamins compared to below-median intake (van Leeuwen et al. 2005). In contrast, the authors of the Blue Mountains Eye Study reported that high beta carotene intake was associated with an increased risk of neovascular AMD (also after adjusting the smoking status). However, they found zinc, lutein and zeaxanthin to have protective effect on AMD (Tan et al. 2007b), which had also been suggested in an earlier study (Gale et al. 2003).

High total fat and high linolenic acid intake have been associated with increased risk of AMD (Cho et al. 2001), and high fish consumption by itself (Smith et al. 2000, Cho et al. 2001, Seddon et al.

2006) or with low linolenic acid intake with decreased risk of AMD (Seddon et al. 2001, Seddon et al. 2003c). The protective effect of fish (OR 0.61, 95%CI 0.37-1.00) and omega-3 long chain fatty acid (OR 0.61, 95%0.41-0.90) intake on neovascular AMD was confirmed in a recent AREDS report (SanGiovanni et al. 2007). Interestingly, a high intake of vegetable, monounsaturated and polyunsaturated fat may also increase the risk of AMD (Seddon et al. 2001) while no association between fat intake and AMD was found in a large epidemiolgical study (Heuberger et al. 2001).

Results related to dietary fat have been inconsistent and the same applies to other common cardiovascular risk factors (except smoking) and the relationship between cardiovascular diseases and AMD. Hypertension, for instance, has been suggested to increase the risk of AMD in some (Age-Related Eye Disease Study Research Group 2000, Hyman et al. 2000, Klein et al. 2003, van Leeuwen et al. 2003a, Hogg et al. 2007), but not in all studies (Klein et al. 1993, Tan et al. 2007a). Recently, persons with early AMD appeared to have a higher risk of incident stroke (Wong et al. 2006a), and a history of cardiovascular disease (stroke, myocardial infarction or angina) was demonstrated to predict incident early AMD (Tan et al. 2007a), and neovascular AMD or late AMD appeared, on the other hand, to increase the risk of incident myocardial infarction (Duan et al. 2007, Wong et al. 2007). However, in previous reports the associations have been not been quite that clear (Klein et al. 1999b, Tomany et al. 2004b, Clemons et al. 2005).

Obesity (Hirvelä et al. 1996, Age-Related Eye Disease Study Research Group 2000, Seddon et al. 2003b, Clemons et al. 2005, Hogg et al. 2007) and high cholesterol levels, major cardiovascular risk factors, have also been associated with AMD. Increased serum high density lipoprotein (HDL) levels have been related to late AMD both positively (Hyman et al. 2000) and inversely (Tan et al. 2007a). In one report, an increased total serum cholesterol level predicted a higher risk of geographic atrophy but not neovascular AMD (Tomany et al. 2004b), whereas in the other report elevated total/HDL ratio was associated with both neovascular AMD and geographic atrophy (Tan et al. 2007a), and in the third report the highest quartile of serum cholesterol was associated with neovascular AMD (Hogg et al. 2007). Dietary carbohydrates may also be associated with AMD. In a study of AREDS patients, patients with higher dietary glycemic index (dGI) had a higher risk for the progression of AMD. It was estimated that 20% of prevalent cases and in five years, 7.8% of new advanced AMD cases could be prevented with low dGI-diets (Chiu et al. 2007a, Chiu et al. 2007b).

A high level of C reactive protein (CRP), as an indicator of systemic inflammation, may also be a risk factor for AMD. Persons with advanced AMD had significantly higher levels of CRP than

those with no AMD (OR 1.65, CI 95% 1.07-2.55, p=0.02; the highest quartile of CRP versus the lowest quartile) (Seddon et al. 2004) and high CRP levels predicted progression of AMD to more advanced forms (RR=2.10, CI 95%1.06-4.18, p=0.046) (Seddon et al. 2005b). On the other hand, Cardiovascular Health Study showed no assocation between AMD and high CRP levels (McGwin et al. 2005).

Finally, sunlight exposure has been suggested to play a role in the development of AMD yet several studies could not have confirmed the sun as a risk (West et al. 1989, Darzins et al. 1997, Delcourt et al. 2001, Khan et al. 2006). However, in the BDES study, persons who had been exposed to summer sun more than 5 hours a day (in their teens, 30s and at baseline) had a higher 10-year incidence of early AMD than those exposed less than two hours. If a person had more than 10 sun burns in his youth the risk to develop large drusen (≥250 um) was 2.5-fold when compared to those who had ≤1 burns (Tomany et al. 2004a).

2.3 PATHOGENESIS

The pathogenesis of AMD is poorly understood, but it is generally accepted that AMD does not merely represent a normal aging process. No major single pathogenic cascade has been established and it is yet to be resolved whether different forms of AMD, neovascular AMD and central geographic atrophy, represent the outcomes of the same pathogenic process. Of the several theories of pathological changes, more or less connected with each other, in the outer retina and retinal pigment epithelium (RPE)- Bruch's membrane- choriocapillaris complex, the general ones are presented here, more as a list than a comprehensive theory.

2.3.1 Oxidative stress

Oxidative stress is not suggested only in the pathogenesis of AMD but also in many other age-related diseases. It refers to cellular damage caused by reactive oxidative intermediates (ROI), which include free radicals, hydrogen peroxide (H_2O_2), and singlet oxygen, all involved in oxygen metabolism of cells and generated in cellular metabolism and photochemical reactions. The main targets of ROI are cell membranes that contain polyunsaturated fatty acids with double bonds, which are then exposed to lipid peroxidation (Beatty et al. 2000, Ambati et al. 2003).

Oxidative hypothesis in the pathogenesis of AMD is supported by several facts: 1) The oxygen metabolism of the retina is one of the most active in the body. 2) The outer segments of

photoreceptors are rich in polyunsaturated fatty-acids. 3) The retina is exposed to radiation. 4) Free radicals are generated by RPE in the phagocytosis of photoreceptor outer segments (Beatty et al. 2000, Ambati et al. 2003).

Lipofuscin, a group of lipid and protein aggregates in RPE cells, plays an important role in the oxidative stress theory. Lipofuscin increases with age and accumulates in the macula, concentrated especially in the parafoveal ring where the rods are the densest. It is presumably a product of the incomplete processing of phagocytosized photoreceptor outer segments in RPE cells. Lipofuscin disturbs RPE function by reducing the phagocytosis capacity, by potentiating phototoxicity and thus aggravating oxidative damage, and by changing the cell architecture (Beatty et al. 2000, Ambati et al. 2003). The relevance of oxidative stress in AMD is supported by studies that showed that anti-oxidant supplementation (vitamin C, vitamin E, beta carotene and zinc) decreases the incidence of AMD (Age-Related Eye Disease Study Research Group 2001).

2.3.2 Inflammation

Though no clinically visible inflammation can be seen in eyes with AMD, one of the key pathophysiological mechanism undoubtedly is subclinical, local inflammation. The strongest evidence comes from the studies of the composition of drusen, the hallmark of AMD, located between the RPE and Bruch's membrane. Several studies have identified drusen to contain inflammatory molecules such as vitronectin, complement components C5 and C5b-9, HLA-DR, and immunoglobulin light chains (Hageman et al. 2001, Anderson et al. 2002).

Since the early 2000's (Hageman et al. 2001, Anderson et al. 2002), inflammation and/or immune-mediated processes with complement activation have been suggested to play a role in drusen formation. A series of events was suggested to lead finally to the buildup of drusen, with RPE cell dysfunction as a trigger. Several mechanisms including genetic effects, lipofuscin toxicity and oxidative injury have been hypothesized to contribute to the RPE pathology. Dysfunction leads to accumulation of cellular debris between RPE basal lamina and Bruch's membrane. As a consequence of failure to dispose of this extra material, a series of suggested inflammatory events ensues, among others activation of the complement cascade and upregulation of inflammatory mediators (e.g.cytokines). Proteins and lipids that are involved in the inflammation are encapsulated with the aforementioned primary RPE debris, thus assisting in drusen formation (Hageman et al. 2001, Anderson et al. 2002). Complement attack induces further damage to bystander macular

tissues and additional RPE atrophy, photoreceptor degeneration and CNV follows (Gehrs et al. 2006).

2.3.3 Choroidal neovascularization

In neovascular AMD, vessels penetrate abnormally from the choroid through Bruch's membrane to the subretinal space. Probably the most powerful inducer of neovascularization is vascular endothelial growth factor (VEGF), which has also been identified in choroidal new vessels in AMD. A myriad of the proangiogenetic mediators are known, as well as, local (such as pigment epithelial-derived factor [PEDF]) or systemic (such as angiostatins) inhibitors of neovascularization. In exudative AMD the balance between pro- and anti-angiogenetic factors combined with a loss of integrity of the Bruch's membrane may favour the growth of new vessels into the RPE and the subretinal space (Ambati et al. 2003).

2.4 TREATMENT AND PROGNOSIS

2.4.1 Photocoagulation

Until recently the therapeutic options of AMD, not to speak of prevention, have been very limited. Besides, most of them have been almost exclusively targeted at neovascular AMD.

Laser photocoagulation as a therapy for AMD was first presented in 1980's but it has proven to have serious weaknesses. Neovascular membranes are ablated thermally and hence the neuroretina adjacent to treated lesion is also damaged. Consequently, only extrafoveal lesions (>200 μm from the fovea) can be treated without visual loss. In juxtafoveal (1-200 μm from the fovea) and especially in subfoveal lesions, the scotoma that follows the treatment affects central vision substantially. Although the prognosis was better than the natural course, the immediate scotoma was not well accepted by the patient, especially not with recurrence rates of 50% in photocoagulation treatment (Macular Photocoagulation Study Group. 1991a, Schmidt-Erfurth et al. 2007).

2.4.2 Photodynamic therapy using verteporfin

In photodynamic therapy (PDT), a chemical photosensitizer (verteporfin) is administered intravenously before non-thermal laser. Light is absorbed by the verteporfin molecules attached to

endothelial cell walls of neovascular membranes. Subsequent release of free radicals from the drug leads to damage of endothelial cells and other structures in the neovascular and normal choriocapillaris vessels. PDT induces photothrombosis of the choroidal neovascularization and the normal choriocapillaris, followed by a varying degree of recovery and recanalization. (Schmidt-Erfurth et al. 1994, Kramer et al. 1996, Husain et al. 1999, Schmidt-Erfurth et al. 2000, Schmidt-Erfurth et al. 2002).

For the Treatment of AMD with Photodynamic Therapy (TAP) study, a patient was eligible if he had a subfoveal CNV lesion with a classic component with the greatest linear dimension of ≤5400 mm and visual acuity ranging from 20/40 to 20/200 (TAP Study Group 1999). After two years, 53% of the PDT-treated patients and 38% of the sham-treated patients ($p<0.001$) had lost less than 15 letters (3 lines). In patients with predominantly classic lesions the corresponding percentages were 59% and 31% ($p<0.001$). However, only 8% of patients had gained 15 or more letters at the two-year follow-up. In the first year the average number of treatment session was 3.4 and in the second year 2.2 (Bressler 2001). Visual acuity seemed to remain stable between follow-ups at two years and four years with only 0.4 treatment needed in the fourth year (Bressler et al. 2005).

In the Verteporfin in Photodynamic Therapy (VIP) study, patients with occult-only CNV types were analysed. At two-year follow-ups, in the PDT-group 55% and in the sham-group 68% lost at least 15 letters ($p=0.032$), and 29% and 47%, respectively, at least 30 letters ($p=0.004$) (VIP Study Group 2001). A combination of PDT with intravitreal injection of triamcinolone acetate has also given favourable results (Arias et al. 2006). Studies on combination therapy of anti-VEGF drugs and PDT are under way.

2.4.3 Anti-angiogenic therapy

Using PDT, it was possible to slow down or stop the progression of visual decline with improvement in only 8% (gain of 15 letters or more) of patients (TAP Study Group 1999, Bressler 2001). In this respect, the results obtained with vascular endothelial growth factor (VEGF) inhibitors have provided better results. At present, three anti-VEGF drugs are available, pegaptanid (Macugen®), an aptamer, and two recombinant, humanized, monoclonal antibodies, ranibizumab (Lucentis®) and bevacizumab (Avastin®). Pegaptanid and ranibizumab are approved both in US and Europe for ocular use while bevacizumab is an off-label drug. They are administered repeatedly intravitreally, pegaptanid every six weeks and ranibizumab and bevacizumab every four to every six weeks. Since the lesion size or lesion composition are irrelevant in terms of maintenance of vision

or vision gain, optical coherence tomography (OCT), not necessarily an angiogram, can be used for monitoring the treatment response (Schmidt-Erfurth et al. 2007). Results from studies of pegaptanid have not been as favourable as expected (VEGF Inhibition Study in Ocular Neovascularization [V.I.S.I.O.N.] Clinical Trial Group et al. 2006). At two years, 59% of eyes that have been treated with 0.3 mg pegaptanid lost less than 15 letters, the corresponding persentage for PDT monotherapy being 53%. Thus the effect of six-weekly injections of pegaptanid were not clearly superior to the effect of the PDT (Schmidt-Erfurth et al. 2007).

In the MARINA study (Rosenfeld et al. 2006) patients with occult or minimally classic CNV lesions received either ranibizumab or sham injections monthly, and in the ANCHOR study (Brown et al. 2006) patients with predominantly classic lesions received either ranibizumab injections monthly or standard care PDT treatment. The MARINA two-year results showed that in the ranibizumab-group (0.5mg) 94.6% and in sham-group 62.2% lost fewer than 15 letters. Improvement of visual acuity was seen in 33.8% of the ranibizumab-group (0.5mg) and in 5.0% of the sham-group. Furthermore, patients in the ranibizumab-group gained 7.2 letters whilst in the sham group they lost 10.4 letters (Rosenfeld et al. 2006). In the ANCHOR study the results were similar, all differences in the aforementioned comparisons were also statistically significant (Brown et al. 2006). The PIER study enrolled all lesion subtypes, but the ranibizumab injections were given first monthly three times, and then in three months interval. This study has been suggested that injection every three months may be insufficient (Schmidt-Erfurth et al. 2007).

Bevacizumab is approved for therapy of metastatic colorectal cancer but not for AMD. Because its molecular structure resembles that of ranibizumab, it is hypothesized to have similar effects. The low costs of bevacizumab and preliminary promising results in a few studies have made it attractive, though no prospective randomized studies are available. Its systemic safety is also largely unknown, and it is reported to cause a mild rise in systolic blood pressure (Schmidt-Erfurth et al. 2007).

2.5 GENETICS

2.5.1 Twin studies and family aggregation analyses

The genetic basis of AMD was first demonstrated 30 years ago (Gass 1973). A clearly higher concordance rates in monozygotic than in dizygotic twins (Gass 1973, Melrose et al. 1985, Meyers et al. 1988, Klein et al. 1994, Meyers et al. 1995, Grizzard et al. 2003) in several small studies

suggested a role of heredity in AMD, which was confirmed in two larger twin cohorts. In a British study of 226 monozygotic and 280 dizygotic twin pairs, an estimated heritability was 45% (95%CI 35%-53%), most heritable phenotypes being soft drusen (57%; 95%CI 50%-64%) and ≥20 hard drusen (81%; 95%CI 77%-84%) (Hammond et al. 2002), while in a US study of 210 monozygotic and 181 dizygotic twin pairs, estimates of heritability ranged from 46% to 71%, the highest rates being for advanced AMD (Seddon et al. 2005a). The first study comprised only of early AMD that may explain differing results in these two studies (Hammond et al. 2002). Family aggregation analyses have further indicated that genetic factors play a role in the aetiology of AMD. The prevalence of AMD was higher in first-degree relatives of patients with AMD when compared to first-degree relatives of controls (OR=2.4, 95%CI 1.2-4.7, p=0.013), with even greater risk in patients with neovascular AMD (OR=3.1, 95%CI 1.5-6.7, p=0.003) (Seddon et al.1997). In the population based Rotterdam study an increased risk was detected both for early (OR=4.8, 95%CI 1.8-12.2) and in late AMD (OR=19.8, 95%CI 3.1-126) when relatives of patients with late AMD were compared to relatives of controls. An earlier onset of disease was also suggested. Lifetime risk estimate of late AMD was significantly different ($p<0.001$) for the relatives of patients and for the relatives of controls, with risk of 50% (95%CI 26-73%) and of 12% (95%CI 2-16%), respectively (Klaver et al. 1998b).

2.5.2 Molecular genetic studies

The development of molecular genetic methods has made it possible to identify genes for Mendelian disorders, and increasingly also predisposing genes for complex diseases. In genetic mapping analyses are made for whether a certain allele of a genetic marker and a disease phenotype are inherited together. It is based on a phenomenon called linkage. This means that during meiosis adjacent genetic loci on the same chromosome segregate together and are said to be linked to each other. On the other hand, if two loci (genetic marker and disease phenotype) are far away from each other, there is a greater risk of recombination to occur in homologous chromosomes during meiosis that suggests that a marker and disease are not inherited together i.e. not linked with each other. Application of genetic mapping does not require any knowledge about the underlying cause of the disease and can thus be applied to diseases whose biochemical backgrounds are totally unknown.

For genome-wide linkage-analysis, families with two or more affected individuals are collected and the pedigrees are genotyped with 400-1000 markers. Thereafter, it is statistically determined whether the marker of some locus is inherited with the disease i.e. is in linkage with the disease phenotype. In further analysis, regions with evidence of linkage may be fine-mapped with denser

maps of markers, either with microsatellite markers and/or with single nucleotide polymorphisms (SNPs) to define the region of candidate genes more precisely.

Recently, significant advances in the identification of predisposing genes have been made in AMD. Thus far, a total of 11 genome wide-scans have been performed (Klein et al. 1998, Weeks et al. 2000, Weeks et al. 2001, Majewski et al. 2003, Schick et al. 2003, Seddon et al. 2003a, Abecasis et al. 2004, Iyengar et al. 2004, Weeks et al. 2004, Santangelo et al. 2005, Barral et al. 2006). Three studies should not be considered to have used independent samples hence their patient material is overlapping with earlier studies (Weeks et al. 2004, Santangelo et al. 2005, Barral et al. 2006). It is noteworthy that in several genome scans the ethnicity of the patients is not clearly stated but Caucasian origin might be assumed. (Table 2)

In the first scan a small family with 10 affected members with dry form of AMD was analysed (Klein et al. 1998) whereas the following scans comprised of patients with both dry and wet forms of AMD (Weeks et al. 2000, Weeks et al. 2001, Majewski et al. 2003, Seddon et al. 2003a, Iyengar et al. 2004, Abecasis et al. 2004, Weeks et al. 2004, Santangelo et al. 2005, Barral et al. 2006). Assuming that different forms of AMD have distinct pathogenesis/genetics, the variety of phenotypes in many scans might have biased the linkage analysis. Some evidence for linkage have been found in nearly every chromosome, though in most of the scans the LOD (logarithm of odds) scores for the three best loci have not reached the standard accepted value 3.3 (general pedigrees) or 3.6 (sib-pairs) for significant linkage. Instead, they frequently have reached the suggestive evidence for linkage with a LOD score 1.9/2.2 (Lander et al. 1995) (Weeks et al. 2001, Majewski et al. 2003, Seddon et al. 2003a, Abecasis et al. 2004, Iyengar et al. 2004, Weeks et al. 2004, Barral et al. 2006). However, the most often replicated loci with the strongest evidence of linkage have been on the long arms of chromosomes 1 and 10 (Weeks et al. 2001, Majewski et al. 2003, Abecasis et al. 2004, Iyengar et al. 2004, Weeks et al. 2004, Barral et al. 2006), which were confirmed in the meta analysis of 6 genome scans, as well (Fisher et al. 2005). The strongest evidence for linkage ($p=0.00025$) was on chromosome 10q26 while a nominal significance also appears on chromosomes 1q, 2p, 3p, and 16 (Fisher et al. 2005). In a study of specific chromosomal regions on 1q, 9p, 10q, and 17q, a total of 70 multiplex families (133 affected sib pairs [ASP]) with different forms of AMD were analysed. Once again the best linkage was identified on chromosome 10q26 with LOD score 1.52 (Kenealy et al. 2004).

Table 2. *Genome-wide scans in AMD. The three best loci with LOD >1.5 are presented. The LOD score for the chromosomes 1q or 10q are mentioned whenever reported in a study.*

Study	Material	Ethnicity	Phenotype	Best loci	Maximum LOD score
Klein et al. 1998	Single family (n=21) 10 affected members	Not stated	Dry AMD	1q25-q31	LOD 3.00
Weeks et al. 2000	225 families 212 affected sib pairs	Not stated	Dry AMD >60% wet AMD	Chr9 Chr10	HLOD 1.87 HLOD 1.42
Weeks et al. 2001	391 families 452 affected sib pairs of which 30 individuals also genotyped in Weeks et al. 2000	97% Caucasian	Dry AMD 65% Wet AMD	17q25 1q31 9p13 10q26	HLOD 3.16 HLOD 2.46 LOD 2.04 LOD 2.10
Majewski et al. 2003	70 families 344 affected members	Caucasian	Extensive drusen Late AMD	10q26 4q32 3p13 1q31	HLOD 3.06 HLOD 2.66 HLOD 2.19 HLOD 2.07
Schick et al. 2003	102 families 263 sib pairs	Caucasian	Quantitative trait from early AMD to late AMD	Chr12 Chr5 Chr6 Chr15	P=0.004 P<0.01 P<0.01 P<0.01

Study	Material	Ethnicity	Phenotype	Best loci	Maximum LOD score
Seddon et al. 2003	158 families 511 affected sib pairs	Not stated	Extensive intermediate drusen or any large drusen Geographic atrophy Exudative AMD	Chr22 Chr2 Chr8 Chr10 Chr1	LOD 2.00 LOD 1.81 LOD 1.67 LOD 1.61 LOD 1.33
Weeks et al. 2004	736 affected sib pairs of which 452 also genotyped in Weeks et al. 2001	Not stated	Dry AMD 65% Wet AMD	17q25 1q31 10q26	HLOD 3.53 HLOD 2.72 LOD 2.69
Abecasis et al. 2004	113 families 331 affected	Caucasian	Early AMD 59% Neovascular AMD/ Geographic atrophy	22q 5p 1q	LOD 2.59 LOD 2.55 LOD 2.13
Iyengar et al. 2004	34 families 297 affected	Not stated	Quantitative trait from early AMD to late AMD	15q21 1q31 Chr2	$P=1.98x10^{-7}$ P=0.0052 P=0.0024
Santangelo et al. 2005	40 families 110 sib pairs A subset of material of Seddon et al. 2003	Not stated	Extensive intermediate drusen or any large drusen Geographic atrophy Exudative AMD	1q 2q	LOD 1.64 LOD 1.60
Barral et al. 2006	124 families of which 70 families also genotyped in Majewski et al. 2003	Not stated	Extensive drusen Neovascular AMD Geographic atrophy	6q25.2 10q 1q	HLOD 3.70 HLOD 2.78 HLOD 2.26

2.5.3 Association studies

Association studies are becoming increasingly useful tools for detecting causal variants of common diseases. The frequency of alleles in genetic marker/markers (SNP/SNPs) is compared in affected and unaffected individuals; the collection of family pedigrees is not needed. In association mapping, two possible ways are available, direct and indirect. For direct analysis to be used, the functional variant that predisposes individuals to disease has to be known. However, as this situation is rare an indirect method is more frequently utilized. This is based on a phenomenon called linkage disequilibrium (LD), which means that a certain haplotype (an array of polymorphisms) has been inherited together with the disease variant. So, an SNP can be an indicator of the causal gene/SNP that lies in neighbourhood and will be associated with the disease due to LD (Collins et al. 1997). The International Hap Map Project, the goal of which is to compare genetic similarities and differences in human beings, has given tools for genome-wide association analyses by mapping SNPs in several populations (Nigeria, China, Japan and US). Most recently, The International HapMap Consortium reported that it has characterized over 3.1 million SNPs that are publicly available for research community (International HapMap Consortium et al. 2007). In the research of AMD, linkage analyses have led the way, and later on association analyses were used to detect susceptibility genes.

2.5.4 Candidate genes

2.5.4.1 Complement system related susceptibility genes

Not surprisingly, in 2005, the first notable susceptibility gene of AMD was found on 1q32, the region that had been replicated in several genome-wide scans. The finding of Y402H polymorphism in the *complement factor H* (*CFH*) gene was a significant breakthrough not only in the field of ophthalmic research but also in the field of complex diseases in general. Thus, a full section is devoted only to the complement system, *CFH* and other complement system related susceptibility genes (Section 2.6).

2.5.4.2 LOC387715/HTRA1 locus

When the other major candidate locus on 10q26 was further analysed with focused SNP genotyping, two adjacent genes were first identified as risk factors for AMD: The hypothetical *LOC387715* gene and the *pleckstrin homology domain-containing protein 1* (*PLEKHA1*) gene

(Jakobsdottir et al. 2005). Later studies suggested that the T allele of rs10490924 in the *LOC387715* gene (other designation *ARMS2*), resulting in a nonsynonymous A69S change in exon one, is the causal variant (Rivera et al. 2005, Conley et al. 2006, Schmidt et al. 2006). *LOC387715* is a hypothetical gene with unknown biological function and only weak expression in human tissues. However, a recent study strengthened the *LOC387715* A69S`s role as a possible causal variant of AMD by demonstrating that *LOC387715* mRNA could be found in the human retina and that it encodes a mitochondrial outer membrane protein (Kanda et al. 2007).

The *LOC387715* A69S variant has been shown to confer a risk for AMD both in Caucasian and Asian populations. The risk allele frequency has reached 36-42 % in Caucasian (Rivera et al. 2005, Schmidt et al. 2006, Ross et al. 2007, Schaumberg et al. 2007) and 59% in Japanese populations (Tanimoto et al. 2007). Corresponding percentages for controls have been 19-26% (Rivera et al. 2005, Schmidt et al. 2006, Ross et al. 2007, Schaumberg et al. 2007) and 35% (Tanimoto et al. 2007), respectively. In all these populations the difference in the risk allele frequency between cases and controls has been highly significant. Interestingly, in the population-based Blue Mountains Eye Study the risk allele frequency was lower with 27%, which reflects the difference in patient materials: BMES had more early and intermediate AMD cases than the clinic-based samples (Ross et al. 2007). Overall, the *LOC387715* A69S variant has been associated more often with late AMD than with the earlier stages (Rivera et al. 2005, Conley et al. 2006, Shuler et al. 2007a, Shuler et al. 2007b). Several studies have also demonstrated an allele-dosage effect in A69S with ORs ranging from 1.7 to 2.7 for heterozygous (TG) and from 5.7 to 8.6 for homozygous (TT) individuals when compared to a non-risk genotype (GG) (Rivera et al. 2005, Conley et al. 2006, Schmidt et al. 2006, Ross et al. 2007). Schmidt et al. (2006) have reported gene-environment interaction between the *LOC387715* A69S and smoking, which has not been replicated since (Conley et al. 2006, Hughes et al. 2007, Ross et al. 2007, Wang et al. 2007b). Even their own later study failed to demonstrate interaction, though the study was not aimed at analyzing smoking (Shuler et al. 2007a).

The role of the *LOC388715* as a susceptibilty gene on 10q26 has been controversial since associations that have been found between late AMD and a promoter variant (rs11200683) of the *high-temperature requirement factor A1* (*HTRA1*) gene (other designation *PRSS11*) have been highly significant, as well (Dewan et al. 2006, Yang et al. 2006, Cameron et al. 2007, Lu et al. 2007, Mori et al. 2007b, Weger et al. 2007, Yoshida et al. 2007). *LOC387715* and *HTRA1* are adjacent genes, which are in complete linkage disequibrillium, *HTRA1* is only 6 Kb downstream from *LOC387715*. The *HTRA1* promoter variant risk allele A frequency in cases (36-44%;

Caucasian [Yang et al. 2006, Cameron et al. 2007], 69%; Japanese [Yoshida et al. 2007] and 78%; Chinese populations [Lu et al. 2007]) and controls (25%; Caucasian [Yang et al. 2006, Cameron et al. 2007], 32%; Japanese [Yoshida et al. 2007] and 44%; Chinese populations [Lu et al. 2007)]) has been similar to that of the T allele frequency of *LOC387715*. The same applies to the differences between genotypes, which have been highly significant. HtrA1 belongs to the human HtrA family of proteases HtrA1, HtrA2, HtrA3, and HtrA4. The precise function of HtrA1 is unknown, but some suggestions of its role in the pathogenesis of various disease have been made: It has been reported to have a role as a tumour suppressor in ovarian carcinoma (Shridhar et al. 2002) and melanoma (Baldi et al. 2002), and HtrA1 levels in synovial fluids of patients with osteoarthritis and rheumatoid arthritis have been 3-7-fold when compared to non-arthritic individuals (Grau et al. 2006). Recently, HtrA1 was detected in mature mucosal human and murine mast cells (Gilicze et al. 2007) and in the pigment retina of mouse embryo (Tocharus et al. 2004), and has been shown to act as an inhibitor of transforming growth factor β (TGF β) signalling (Oka et al. 2004, Tocharus et al. 2004). HtrA1 has also been detected in the human retina (drusen) of AMD patients (Yang et al. 2006), although Kanda et al. recently showed that rs11200638 did not change the *HTRA1* promoter activity (Kanda et al. 2007). This might once again speak for the fact that rs10490924 of the *LOC387715* gene, not the *HTRA1*, is the true causal variant of AMD on 10q26.

2.5.4.3 Others

Both positional and functional candidate gene approaches have been used in the research of AMD. Genes of juvenile macular dystrophies that have similar clinical characteristics with AMD have been hypothesized as candidates, but this approach has failed. The genes of Best disease or Sorsby disease could not have been associated with AMD (De La Paz et al. 1997, Allikmets et al. 1999), and though the *ATP-binding cassette* (*ABC*) *transporter* (*ABCA4*) gene of Stargardt disease has been under considerable investigation its role in AMD has not been ascertained. In addition to positive results (Allikmets et al. 1997, Allikmets 2000, Shroyer et al. 2001), several groups have not detected any clear association between the *ABCA4* gene and AMD (Rivera et al. 2000, Guymer et al. 2001, Webster et al. 2001, Schmidt et al. 2003). Earlier, variants in the *elongation of very long chain fatty acids-like 4* (*ELOVL4*) gene on 6q14 have been identified in autosomal dominant macular dystrophy (Bernstein et al. 2001, Edwards et al. 2001, Zhang et al. 2001, Maugeri et al. 2004), but not in AMD (Ayyagari et al. 2001). When Conley et al. analysed several candidate genes, they found that a M299V variant in exon 6 of the *ELOVL4* gene was associated with AMD

(Conley et al. 2005), but later studies failed to replicate their finding (Conley et al. 2006, DeAngelis et al. 2007).

The relationship between the *apolipoprotein E* (*APOE*) gene and AMD is also quite controversial. Allele 2 of the *APOE* gene at chromosome 19q may be associated with an increased risk and allele 4 with a decreased risk for AMD (Klaver et al. 1998a, Souied et al. 1998, Schmidt et al. 2002, Baird et al. 2004, Zareparsi et al. 2004), however, results giving no association have also been reported (Schultz et al. 2003a, Schmidt et al. 2005, Wong et al. 2006b).

After the linkage on chromosome 1q was detected, a sequence variant Q5345R in exon 104 of the *hemicentin-1* gene on 1q31 was identified in a large family with AMD (Schultz et al. 2003b), but replication has never again been successful (Abecasis et al. 2004, Hayashi et al. 2004, Iyengar et al. 2004, Fuse et al. 2006).

Several other candidate genes (Haddad et al. 2006) have been hypothesized as a risk for AMD including functional candidates *fibulin-5* (Stone et al. 2004), *vascular endothelial growth factor* (*VEGF*) (Haines et al. 2006), and *HLA*-genes (Goverdhan et al. 2005), as well as, a positional candidate *toll-like receptor 4* gene (TLR4) (Zareparsi et al. 2005b). The individual contribution of these genes as a risk for AMD appears, after all, to be relatively minor.

2.6 COMPLEMENT SYSTEM

2.6.1 Overview of the complement system

The complement system, comprised of about 35 components in the plasma and on cell surfaces, is a part of the innate immune system with three main physiologic activities: acting as a host defence against bacterial infection and as a link between adaptive and innate immunity and having a waste-disposal function in the clearance of immune complexes and apoptotic cells. In complement activation, three distinct activation pathways are involved, the classical, mannan-binding lectin and alternative pathways, all of which converge in the cleavage of C3 to C3b and C3a by a C3 convertase enzyme. Later steps of complement activation (terminal pathway) are similar in the three pathways leading to formation of the membrane-attack complex (MAC) and to complement-mediated cell lysis (Figure 3) (Walport 2001).

The classical pathway is initiated when the C1 complex (consisting of one C1q, and two C1r and C1s molecules) first binds to the antibody-antigen complex on a bacterial cell surface. C1s, an

active enzyme of the C1 complex, then cleaves C4 and C2, leading to the formation of a C4b2a enzyme complex, the C3 convertase of the classical pathway. C-reactive protein (CRP), nucleic acids and damaged cell membranes can also act as activators of classical pathway in the absence of antibodies (Figure 3) (Walport 2001).

The lectin pathway is initiated by binding of mannan-binding lectin (MBL) to mannose groups on the surface of a target cell. MBL is associated with two serine protease, MBL-associated proteases 1 and 2 (MASP1 and MASP2). MASP2 acts in a similar fashion to the C1s, leading to the formation of the C3 convertase enzyme, C4b2a (Figure 3) (Walport 2001).

The alternative pathway is initiated by low-grade cleavage of C3 in plasma and by the covalent binding of a small amount of C3b to a cell-surface. The environment of the surface on which C3b is deposited determines whether factor B, a protein homologous to C2, or complement factor H (CFH), which is a regulator of the complement pathway, binds to C3b. On microbial surfaces, factor B binds to C3b with a higher affinity than CFH. Factor B, when bound to C3, is further cleaved by factor D to Bb and Ba. The formed C3bBb complex is the alternative pathway C3 convertase. Properdin stabilizes this enzyme. The C3 convertase enzymes cleave many molecules of C3 to C3b, which initiate the formation of the membrane-attack complex. On host cell surfaces polyanions such as sialic acid or glycosaminoglycans favour the binding of CFH, instead of factor B, to C3b. CFH binds to C3b and acts as a cofactor to factor I, which cleaves C3b into an inactive product, iC3b. The iC3b can no longer participate in the formation of the C3 convertase enzyme. Thus, the terminal steps leading to the MAC formation are interrupted (Figure 3). Normally, complement activity is under strict control with the defence against invading organisms and the control of excess attack against host tissues in fine balance (Walport 2001).

Disturbances in complement regulation may lead to “innate autoreactivity” which means an attack against self-tissues in the absence of a specific autoimmune response (Meri 2007). Examples of diseases with complement autoattacks are hereditary angioedema and paroxysmal nocturnal hemoglobiuria as well as two rare kidney diseases with acute renal failure; membranoproliferative glomerulonephritis type II (MPGNII) and recurrent atypical hemolytic uremic syndrome (aHUS). The cause of latter two diseases is complement factor H (CFH) deficiency or mutation, which lead to excessive consumption of C3 and thus to severe depletion of plasma C3 (MPGNII) or to a misdirected complement attack against blood vessel endothelia and blood vessels (aHUS) (Meri 2007). Interestingly, patients with MPGNII develop drusen at young age, the composition and the structure of which are generally similar to that in patients with AMD (Mullins et al. 2001).

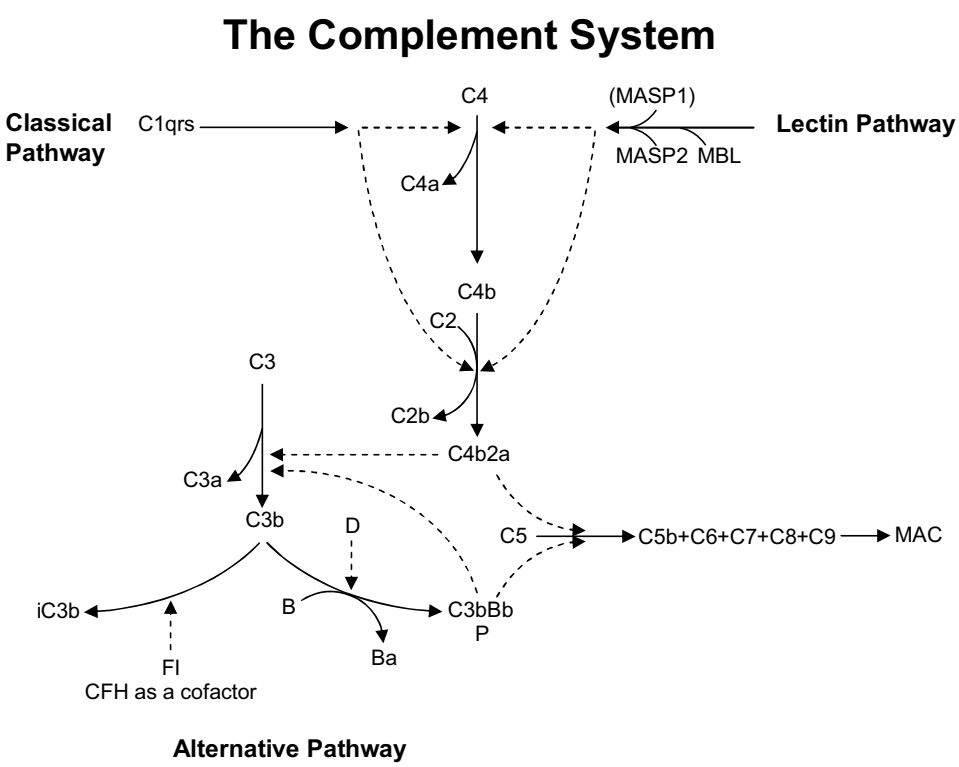


***Figure 3**. Complement activation pathways. Enzymatic activity is indicated by broken arrows. MASP= mannan-binding lectin-associated proteases, MBL=mannan-binding lectin; CFH= complement factor H; FI=factor I; D=factor D; B=factor B, P=properdin, MAC=membrane attack complex.*

2.6.2 Complement factor H

2.6.2.1 Biological function

CFH is a multifunctional glycoprotein, predominantly synthesized in the liver. It plays a vital role in the protection of host cells from inappropriate attack of complement by regulating of the alternative pathway in the fluid-phase and on cellular surfaces. CFH consists 20 repetitive units of 60 amino acids called short consensus repeats (SCRs). Each of the 23 exons of *CFH* gene encodes for a single SCR, except for SCR 2, which is encoded by exons 3-4. In addition, exon one encodes a signalling peptide and exon 10 is utilized only in factor H-like (FHL)-1 transcript. The complement regulatory functions, decay acceleration, and cofactor activity, are located in N-terminus of the protein, in SCR 1-4. The C-terminus also contributes to the host protection function on cell and tissue surfaces (Hellwage et al. 2002, Jozsi et al. 2007) (Figure 4).

CFH plasma levels vary significantly (110-615 ug/ml) in the population, representing the combined effect of genetic and environmental factors (Rodriguez de Cordoba et al. 2004). Levels have been shown to decrease in smokers and to increase with age (Esparza-Gordillo et al. 2004).

2.6.2.2 Factor H protein family

Factor H family is comprised of eight proteins with common structural elements (Figure 4). Their genes are all located on the Regulators of Complement Activation (RCA) gene cluster on chromosome 1q32 (Rodriguez de Cordoba et al. 2004). In addition to CFH, factor H-like (FHL-1) protein and factor H related (FHR) proteins FHR1-FHR5 belong to this family. FHL-1 represents an alternative spliced product of the *CFH* gene with its complement regulatory activity. In its N-terminal it has seven SCRs identical to CFH SCR 1-7 and in the C-terminal end four unique amino acids. Five *FHR* genes possess high sequence homology with *CFH* and are, in all likelihood, duplications of a common ancestor (Zipfel et al. 2002). The precise functions of products of these five genes are poorly understood though the high sequence homology with *CFH* suggests common functions (Figure 4).

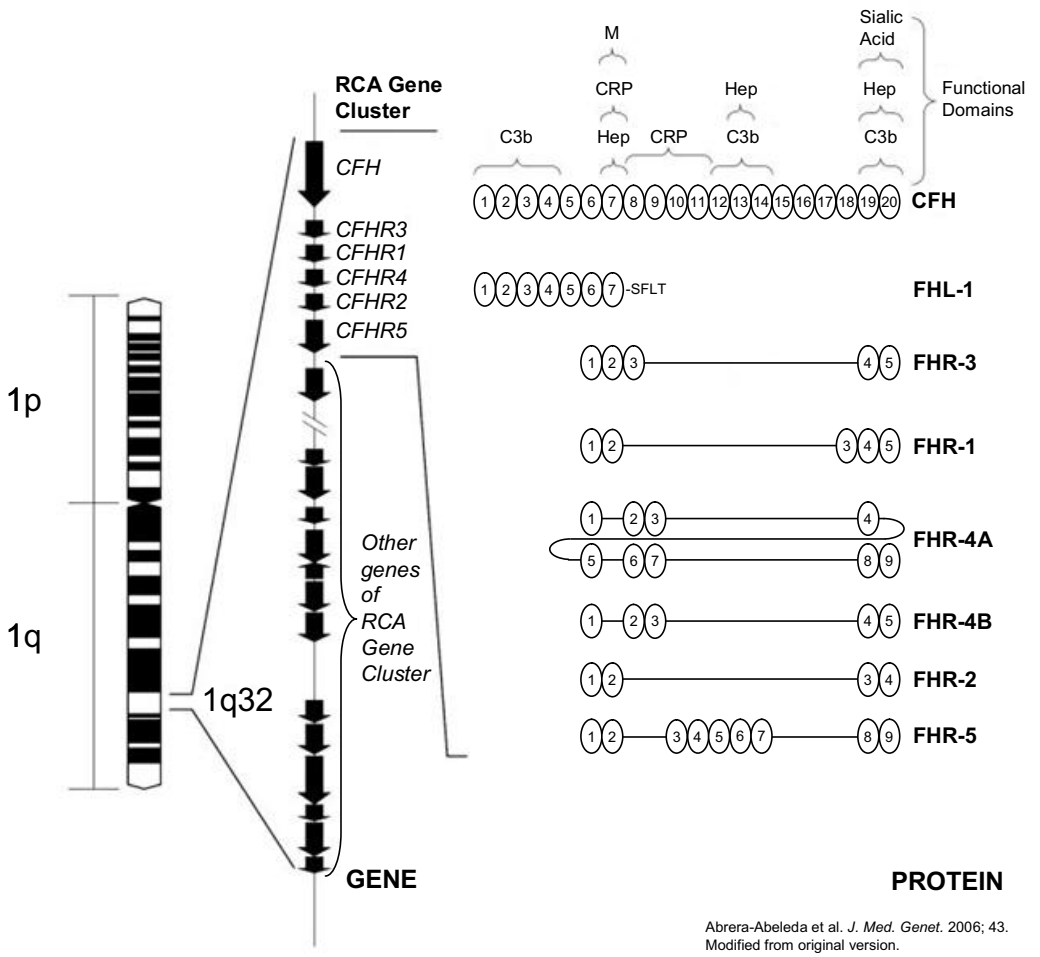


Figure 4. *The complement factor H (CFH) family. Genes of the complement factor H family, CFH and five complement factor H related (CFHR) genes, are located in the Regulators of Complement Activation (RCA) cluster on chromosome 1q32. Factor H-like 1 (FHL-1) protein is a product of alternatively splicing of the CFH gene, and FHR4A and FHR4B are products of alternatively splicing of the CFHR4 gene* (Jozsi et al. 2005)*. CFH is composed of 20 consecutively numbered short consensus repeats (SCR). A schematic structure of complement factor H, FHL-1, and factor H related (FHR) proteins and localization of functional domains in each of them are shown, SCRs aligned according to the highest homology with SCRs of CFH. The interaction sites with C3, heparin (Hep), C-reactive protein (CRP), and the streptococcal M protein (M) are indicated. The CFH Y402 polymorphism is located in SCR domain number 7.*

2.6.2.3 CFH Y402H association studies

Four independent groups reported in early 2005 that a 1277T>C change, resulting in the substitution of tyrosine to histidine, in exon 9 (SCR 7) of the *complement factor H* (*CFH*) gene was associated with AMD (Edwards et al. 2005, Hageman et al. 2005, Haines et al. 2005, Klein et al. 2005). The starting points for three of the studies (Edwards et al. 2005, Hageman et al. 2005, Haines et al. 2005) were genome-wide linkage-analyses where a consistent linkage on 1q25-32 had been found. Then, SNPs flanking only this region were selected. Hageman et. al even narrowed their study to only *complement factor H* gene, a probable candidate gene, located in that region. In the fourth study a whole-genome association study was performed (Klein et al. 2005). Regardless of the research frame, all the groups concluded that *CFH* Y402H was highly significantly associated with AMD.

Since then, the finding has been confirmed in several studies and populations (Conley et al. 2005, Rivera et al. 2005, Souied et al. 2005, Zareparsi et al. 2005a, Baird et al. 2006, Conley et al. 2006, Kaur et al. 2006, Lau et al. 2006, Magnusson et al. 2006, Sepp et al. 2006, Simonelli et al. 2006, Narayanan et al. 2007, Tedeschi-Blok et al. 2007) with relatively similar allele frequencies in many Caucasian populations. In contrast, the frequencies of the risk allele C have been lower in Japanese (Fuse et al. 2006, Gotoh et al. 2006, Okamoto et al. 2006, Uka et al. 2006), Chinese (Chen et al. 2006, Lau et al. 2006) and Hispanic populations (Tedeschi-Blok et al. 2007) (Table 3 and Figure 5). Despite lower risk allele frequency, an association between the *CFH* Y402H polymorphism and AMD has also been found in Chinese population (Lau et al. 2006).

Table 3. *Association between age-related macular degeneration (AMD) and complement factor H (CFH) Y402H polymorphism in various populations. Shown are odds ratios (OR), 95% confidence intervals (CI), and p-values for allelic (C versus T) and/or for genotypic comparisons (CT versus TT and CC versus TT). Subphenotypic comparisons are shown in the corresponding row.*

Study	Ethnicity	Material	Allelic comparison		Genotypic comparison		
		number of AMD cases AMD subfenotypes % number of controls	p-value C versus T	OR (95%CI) C versus T	OR (95%CI) CT versus TT	OR (95%CI) CC versus TT	OR (95%CI) CC or CT versus TT
Baird et al. 2006	Caucasian	236 cases	<0.001		1.86 (1.10-3.16)	9.26 (4.52-18.98)	2.98 (1.81-4.93)
		exudative AMD 39 %			2.79 (1.21-6.43)	12.43 (4.61-33.49)	4.34 (1.94-9.71)
		GA 11 %			1.01 (0.28-3.67)	9.61 (2.63-35.09)	2.35 (0.74-7.41)
		early AMD 50 %			1.78 (0.95-3.32)	6.52 (2.90-14.65)	2.54 (1.40-4.61)
		144 healthy controls					
Chen et al. 2006	Chinese	163 cases	NS				
		exudative AMD 100 %					
		244 healthy controls					
Conley et al. 2005	Caucasian	196 sporadic cases	<0.0001	3.67 (2.56-5.28)			
		exudative AMD 66 %	<0.0001	3.46 (2.33-5.13)			
		GA 29 %	<0.0001	4.76 (2.87-7.91)			
		120 healthy controls					
Conley et al. 2006 (CHS study)	Caucasian	126 cases	≤0.00001		1.82 (1.13-2.92)	4.22 (2.39-7.42)	2.26 (1.45-3.53)
		exudative AMD 7 %					
		GA 12 %					
		mixed exudative AMD/GA 2 %					
		early AMD 79 %					
		1051 healthy controls					
Conley et al. 2006 (AREDS study)	Caucasian	701 cases	≤0.00001		2.66(1.81-3.92)	6.69 (4.08-10.98)	3.73 (2.60-5.34)
		exudative AMD 40 %			2.48 (1.57-3.93)	5.60 (3.21-9.78)	3.31 (2.16-5.07)
		GA 21 %			2.54 (1.44-4.48)	7.04 (3.69-13.41)	3.73 (2.21-6.31)
		mixed exudative AMD/GA 22 %					
		early AMD 17 %			1.93 (1.04-3.60)	4.95 (2.46-9.95)	2.73 (1.55-4.83)
		175 healthy controls					

Study	Ethnicity	Material number of AMD cases AMD subfenotypes % number of controls	Allelic comparison p-value C versus T	 OR (95%CI) C versus T	Genotypic comparison OR (95%CI) CT versus TT	 OR (95%CI) CC versus TT	 OR (95%CI) CC or CT versus TT
Edwards et al. 2005	Caucasian	400 cases exudative AMD 37 % GA 16 % early AMD 47 % 202 healthy controls	4.95 X 10^{-10}				
Fuse et al. 2006	Japanese	80 cases dry AMD 100 % 196 healthy controls	NS				
Hageman et al. 2005 (Iowa cohort)	Caucasian	403 cases 131 healthy controls	2.09 X 10^{-12}	2.82 (2.11-3.78)			
Hageman et al. 2005 (Columbia cohort)	Caucasian	549 cases 272 healthy controls	1.64 X 10^{-13}	2.25 (1.79-2.75)			
Haines et al. 2005	Caucasian	182 families and 495 cases with AMD >grade 3 of AREDS grading system NA % 185 healthy controls			2.45 (1.41-4.25)	3.33 (1.79-6.20)	
Kaur et al. 2006		100 cases exudative AMD 52 % GA 13 % early AMD 35 % 120 healthy controls	1.19 X 10-7		1.51(0.82-2.80))	11.52 (5.05-26.28)	
Klein et al. 2005	Caucasian	96 cases exudative AMD 52 % GA 48 % 50 healthy controls	4.1 X 10-8		4.6(2.0-11)	7.4 (3.0-19)	
Lau et al. 2006	Chinese	163 cases exudative AMD 100 % 232 healthy controls	<0.00001	4.44 (2.3-8.5)			3.9 (2.0-7.8)

Study	Ethnicity	Material	Allelic comparison		Genotypic comparison		
		number of AMD cases AMD subfenotypes % number of controls	p-value C versus T	OR (95%CI) C versus T	OR (95%CI) CT versus TT	OR (95%CI) CC versus TT	OR (95%CI) CC or CT versus TT
Magnusson et al. 2006	Caucasian	581 late AMD cases	5.9×10^{-12}	2.39 (1.86-3.07)			
(Icelandic cohort)		exudative AMD 48 %	2.1×10^{-9}	2.32 (1.75-3.07)			
		GA 35 %	7.4×10^{-8}	2.27 (1.67-3.08)			
		mixed exudative AMD/GA 17 %	3.6×10^{-9}	2.92 (2.03-4.20)			
		435 early AMD cases					
		soft drusen 51 %	8.5×10^{-10}	2.52 (1.87-3.40)			
		hard drusen only 21 %	1.0×10^{-2}	1.57 (1.07-2.30)			
		pigments only 28 %	0.16	1.21 (0.83-1.76)			
		171 healthy controls					
Magnusson et al. 2006	Caucasian	322 late AMD cases	2.0×10^{-9}	2.14 (1.66-3.07)			
(Utah cohort)		exudative AMD 76 %	8.5×10^{-9}	2.17 (1.66-2.84)			
		GA 24 %	1.1×10^{-4}	2.05 (1.40-3.00)			
		109 early AMD cases					
		soft drusen 100 %	8.4×10^{-6}	2.10 (1.50-2.95)			
		203 healthy controls					
Rivera et al. 2005	Caucasian	794 cases			1.76 (1.35-2.28)	6.43 (4.58-9.01)	
(Initial cohort)		exudative AMD 53 %					
		GA 19 %					
		mixed exudative AMD/GA 15 %					
		early AMD 13 %					
		612 healthy controls					
Rivera et al. 2005	Caucasian	373 cases (replication cohort)			2.49 (1.72-3.61)	7.37 (4.72-11.50)	
(Replication cohort)		subphenotypes NA %					
		335 healthy controls					
Sepp et al. 2006	Caucasian	443 cases	<0.0005		3.1 (2.0-4.6)	6.3 (3.8-10.4)	
		exudative AMD 60 %			2.7 (1.7-4.2)	5.1 (2.9-8.9)	
		GA 24 %			2.6 (1.4-5.0)	6.0 (2.9-12.5)	
		mixed exudative AMD/GA 16 %					
		262 healthy controls					

Study	Ethnicity	Material number of AMD cases AMD subfenotypes % number of controls	Allelic comparison p-value C versus T	 OR (95%CI) C versus T	Genotypic comparison OR (95%CI) CT versus TT	 OR (95%CI) CC versus TT	 OR (95%CI) CC or CT versus TT
Simonelli et al. 2006	Caucasian	104 cases exudative AMD 52 % GA 18 % early AMD 30 % 131 healthy controls	<0.001		1.4 (0.7-2.6)	3.9 (1.9-8.2)	
Souied et al. 2005	Caucasian	81 familial cases 60 sporadic cases 141 cases in total exudative AMD 100 % 91 healthy controls	<0.0001 <0.0001 <0.0001		3.54 (1.66-7.56) 2.47 (1.12-5.45) 3.00 (1.60-5.62)	8.08 (3.24-20.14) 5.71 (2.23-14.63) 6.93 (3.11-15.46)	
Tedeschi-Blok et al. 2007	Hispanic	285 cases early AMD 100 % 570 healthy controls	NS				1.2 (0.93-1.59)
Uka et al. 2006	Japanese	67 cases exudative AMD 100 % 107 healthy controls	NS				
Zareparsi et al. 2005	Caucasian	616 cases exudative AMD 39 % GA 23 % mixed exudative AMD/GA 22 % large drusen 16 % 275 healthy controls	$<1 \times 10^{-24}$			5.52(3.54-8.59)	4.36 (3.13-6.08)

AREDS= Age-Related Eye Disease Study; CC patients homozygous for the *CFH* Y402H risk allele; CT patients heterozygous for the *CFH* Y402H risk allele; TT patients with normal genotype; CHS= Cardiovascular Health Study; CI= confidence interval; GA= geographic atrophy; NA=not available; NS= not significant

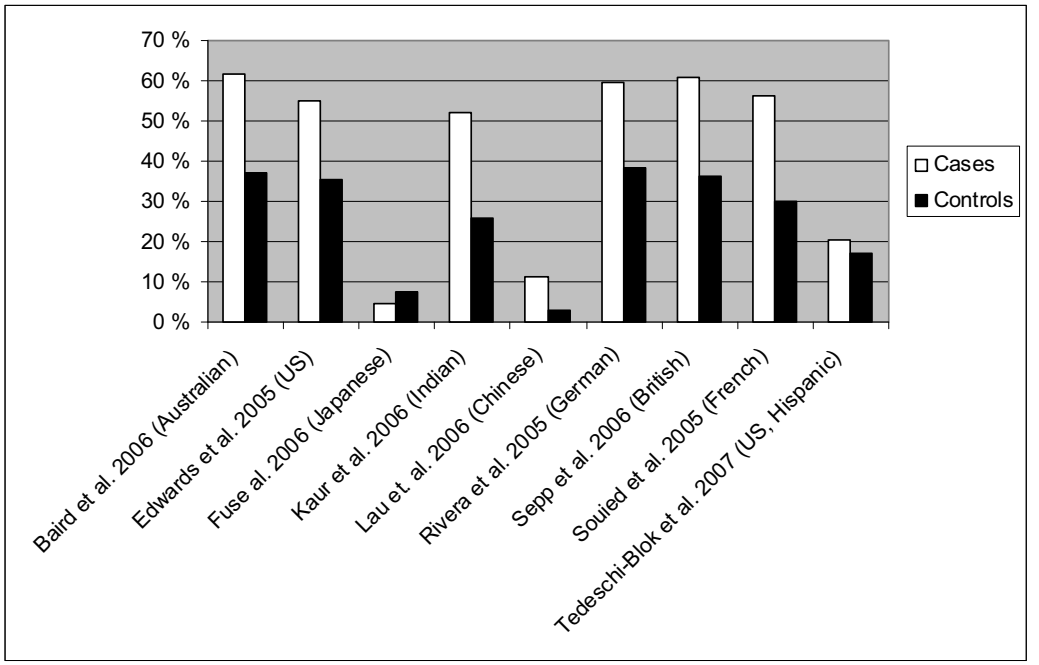


Figure 5. *Allele frequencies of the Y402H polymorphism of the complement factor H gene in different populations. Frequencies of the risk allele C are presented in cases with age-related macular degeneration and in healthy controls. C-allele frequencies in Japanese, Chinese and Hispanic populations are notably lower than in Caucasian populations.*

CFH Y402H polymorphism has been associated with all forms of AMD: early AMD (Baird et al. 2006, Conley et al. 2006, Despriet et al. 2006, Magnusson et al. 2006, Postel et al. 2006, Schaumberg et al. 2007, Zareparsi et al. 2005a), central geographic atrophy (Zareparsi et al. 2005a, Baird et al. 2006, Conley et al. 2006, Magnusson et al. 2006, Postel et al. 2006), and neovascular AMD (Zareparsi et al. 2005a, Baird et al. 2006, Conley et al. 2006, Lau et al. 2006, Magnusson et al. 2006, Postel et al. 2006, Schaumberg et al. 2007), though in some studies the effect has been stronger in late AMD than in early AMD (Baird et al. 2006, Despriet et al. 2006, Francis et al. 2007). Subphenotype analysis of neovascular AMD (predominantly classic, minimally classic and occult) by two groups has suggested that the CC genotype of the *CFH* Y402H polymorphism is associated with predominantly classic CNV lesion configuration (Brantley et al. 2007a, Wegscheider et al. 2007), while no statistically significant association between neovascular lesion size and genotype was detected (Brantley et al. 2007a).

2.6.2.4 Functional analyses

Identification of CFH and other complement components in ocular tissues have provided further evidence of CFH´s role in the pathogenesis of AMD. Repeatedly, drusen deposits, the hallmark feature of AMD have been shown to contain CFH amongst other complement proteins including C3b and MAC (Anderson et al. 2002, Hageman et al. 2005, Donoso et al. 2006). Furthermore, in human and mouse ocular tissues, CFH has been detected in the distal optic nerve, the sclera, the RPE-choroid, the retina, the lens, and the ciliary body, and in mouse eyes CFH levels have shown an increase with age (Mandal et al. 2006). Recently, when CFH-deficient (cfh-/-) mice were compared to age-matched normal mice, they showed significantly reduced visual acuity and anomalies in electroretinography. Moreover, in their neural retina accumulation of C3 was detected (Coffey et al. 2007).

The *CFH* Y402H polymorphism is in exon 9 that codes SCR domain 7, which has binding sites for C-reactive protein (CRP) (Jarva et al. 1999), heparin (Meri et al. 1990, Blackmore et al. 1996) and streptococcal M-protein (Horstmann et al. 1988, Blackmore et al. 1998) (Figure 4). Thus, CRP would easily offer a link between *CFH* Y402H polymorphism and AMD. CRP levels of patients with AMD have been shown to be elevated (Seddon et al. 2004, Seddon et al. 2005b), and individuals with homozygous (CC) risk genotype of *CFH* Y402H had 2.5-fold levels of CRP in the choroidal stroma when compared to TT non-risk individuals. It should be noted, however, that this difference was independent of disease status (Johnson et al. 2006). A haplotype in the CRP gene that is associated with increased serum CRP levels also increased the risk of AMD in the CC individuals but not in the TT individuals (Despriet et al. 2006). Reduced binding of CRP, as well as heparin and RPE cells by CFH, was detected in CFH heterozygous CT and homozygous CC risk variants (Skerka et al. 2007), but the binding of C3b was similar, irrespective of genotype. In this study the difference was also seen in FHL-1, which also possesses the SCR7, where the polymorphism is located (Skerka et al. 2007).

2.6.2.5 Gene- gene and gene- environment interactions

With regard to the nature of complex diseases, several gene-gene and gene-environment interactions are assumed to be involved in their pathogenesis. These interactions can be approached by statistical and cell biological methods. Statistically, the degree of biological interaction of the susceptibility genes and environmental risk factors can be assessed by the departure-from-additivity model (Andersson et al. 2005). In this model, it is assumed that odds ratios can be used in lieu of relative risks. Relative risk due to interaction (RERI), attributable proportion due to interaction (AP)

and, synergy index (S) can be calculated by an Excel program freely available on the Internet (www.epinet.se). The regression coefficients (odds ratios) received from logistic regression model are exploited in the calculations. If no statistical biological interaction exists, RERI and AP are equal to 0 and S equal to 1 (Andersson et al. 2005). Detecting a biological interaction of two factors means that at least one pathophysiological pathway towards disease exists, where both of the risk factors play a role. A statistical interaction can be evaluated by the logistic regression model (departure-from-multiplicative model).

In AMD, it is attractive to hypothesize whether the two most consistently replicated risk factors, smoking and *CFH* Y402H, could turn out to be related to a common pathogenetic mechanism. Especially, because smoking itself decreases CFH plasma levels (Esparza-Gordillo et al. 2004). However, smoking and *CFH* Y402H have appeared independently to increase statistically the risk of all forms of AMD (Sepp et al. 2006, DeAngelis et al. 2007, Scott et al. 2007). In a population based material, a suggestive biological interaction was detected between smoking and Y402H. Current smoking alone increased the risk of AMD to 3.4-fold, carrying both risk alleles of *CFH* Y402H to 12.5-fold, and the combination of current smoking and the homozygous risk genotype to 34-fold (Despriet et al. 2006).The association analyses have indicated that the *LOC387715* A69S and *CFH* Y402H variants have independent effects on AMD (Rivera et al. 2005, Conley et al. 2006, Maller et al., 2006, Schmidt et al. 2006).

2.6.2.6 Other variants of the complement factor H gene

Already in their first study of *CFH* Y402H polymorphism, in 2005, Hageman et al. (Hageman et al. 2005) reported other AMD-associated variants, as well. They used eight SNPs in their haplotype analysis and found one common risk haplotype H1 (OR 2.46, $p<0.00001$) and two protective haplotypes, H2 (OR 0.54, $p=0.00003$) and H4 (OR 0.48, $p=0.00008$). Li et al. reported 20 SNP out of 84, they examined in and around the *CFH* gene, possessing stronger association with AMD than the Y402H polymorphism. In addition, they found two susceptibility and two protective haplotypes (5-SNP haplotypes). The frequency of protective haplotype 1 was 14% in cases and 44% in controls and the frequency of haplotype 2 was 3% in cases and 11% in controls (Li et al. 2006). An Indian study, in which haplotype block consisted of 6 SNPs, revealed a risk haplotype, similar to the H1 in the study of Hageman et al., and a protective haplotype, similar to the H2 in the study Hageman et al. (Hageman et al. 2005, Kaur et al. 2006). The presence of two protective haplotypes, again similar to those in the study of Hageman et al. (H2 and H4), was confirmed in a family-based

dataset. Moreover, an interaction between smoking and one of the protective haplotype (H4 in study of Hageman et al.) was suggested (Spencer et al. 2007b).

Most recently, an association study of three different patient samples (familial AMD cases, sporadic advanced AMD cases and cases from AREDS study) showed, in addition to Y402H, a highly significant association between a synonymous A473A (rs2274700) polymorphism in exon 10 and AMD. A haplotype containing this polymorphism, Y402H, and A307A in exon 7 was also significantly associated with AMD (Francis et al. 2007). Though, the *CFH* Y402H polymorphism is not a risk factor in the Japanese, two SNPs in *CFH* that are in almost complete LD have been associated with AMD, the aforementioned SNP A473A alongside with intronic SNP (rs1410996) (Mori et al. 2007a).

In a study of factor H family genes, a novel protective haplotype was detected with a frequency of 20% on chromosomes of controls and of 8% on the chromosomes of patients with neovascular AMD (Hughes et al. 2006), and with a frequency of homozygous deletion in 5.7% in controls and in 1.1.% in cases with AMD ($p=1.6 \times 10^{-9}$) (Hageman et al. 2006). This haplotype carries a deletion of *complement factor related* (*CFHR*)*1* and *CFHR3* genes. In sera of homozygous individuals no protein coded by these genes could either be detected (Hughes et al. 2006). It was suggested that FHR1 and FHR3 might interfere with the decay acceleration and cofactor functions of CFH and lead to the uncontrolled action of complement. Thus, patients with a deletion of *FHR1* and *FHR3* might have a more unrestricted function of CFH than those with normal *FHR1* and *FHR3*. Importantly, this deletion and *CFH* Y402H polymorphism seemed independently to confer a significant risk for AMD (Hughes et al. 2006).

2.6.2.7 CFH Y402H polymorphism in other diseases

Since inflammation plays an important role in coronary artery disease (CAD) and AMD, and atherosclerotic plaques and drusen deposits have both been found to contain complement factor H (Oksjoki et al. 2003, Hageman et al. 2005), it has been reasonable to hypothesize whether the *CFH* Y402H polymorphism may also be associated with CAD. In the Rotterdam study, persons carrying the homozygous risk genotype (CC) of the *CFH* Y402H polymorphism had a hazard ratio of 1.77 (95% CI 1.23-2.55) for myocardial infarction when compared to non-risk homozygous genotype (TT), but the risk of heterozygous genotype (CT) was not increased (Kardys et al. 2006). In a recent report of the Nurses's Health Study and the Health Professional Follow-up Study, an inverse association between *CFH* Y402H risk genotype and CAD was found in women (risk ratio [RR]

0.51, 95% CI 0.29-0.89) and in persons < 65 of age at onset of CAD (RR 0.30, 95% CI 0.15-0.61), but not in men or in persons ≥65 at onset of CAD (Pai et al. 2007). The rest of the studies have failed to detect any association between *CFH* Y402H and CAD, ischaemic stroke or venous embolism (Goverdhan et al. 2006, Zee et al. 2006, Nicaud et al. 2007, Stark et al. 2007). Nor was the polymorphism associated with common cardiovascular risk factors such as diabetes, hypercholesterolaemia, hypertension, obesity, smoking or CRP serum levels, in a German study of patients with a familial background of CAD (Stark et al. 2007). No association between the *CFH* Y402H variant and pre-eclampsia was detected in a recent study from Finland (Kaare et al., in press).

2.6.3 Other susceptibility genes of the complement system

Since the association between *CFH* Y402H and AMD was found, researchers have logically been interested in, whether there might be other AMD associated variants in the complement system. First, an association between AMD and *C2* and *complement factor B* (*CFB*) genes was found (Gold et al. 2006). Both genes are located in the major histocompability complex class III region of chromosome 6, only 500 kb from each other. C2 and factor B are homologous proteins that play a role in the initial steps of complement pathways, C2 in the classical pathway and factor B in the alternative pathway (Figure 3). Four protective variants of *C2* and *CFB* that confer significantly reduced risk of AMD were detected in two independent cohorts: The L9H variant of *CFB* and the E318D variant of *C2*, in strong LD with each other, and the R32Q variant of *CFB* and the intronic variant (rs547154) of *C2*, also in strong LD with each other. Interestingly, it appeared that controls carrying the *CFH* risk allele had a high frequency of the protective allele(s) in *CFB*/*C2* (Gold et al. 2006). Later, the inverse associations between AMD and *CFB* R32Q, *CFB* L9H and *C2* E318D variants have been replicated (Maller et al. 2006, Spencer et al. 2007a), though after controlling for age, *CFH* Y402H, *LOC387715* A69S, and smoking in the logistic regression model, the association of *C2* E318D was no longer statistically significant (Spencer et al. 2007a). This might suggest that *complement factor B* is the true causal gene in this region.

Most recently, a common polymorphism (rs2230199) in exon three of the *complement factor 3* (*C3*) gene on 19p13.3 was associated with AMD in two case-control sets (Yates et al. 2007), and confirmed in a large case-control sample (Maller et al. 2007). C3 is hypothesized to be very well conserved phylogenetically (Zarkadis et al.2001), and as earlier described, it plays a vital role in the complement system in all three pathways (Figure 3). Like CFH, C3 has been found in drusen deposits, in choroid, and in Bruch's membrane (Anderson et al. 2002, Hageman et al. 2005) of

human donors, and in the CFH deficient mice, an accumulation of C3 was detected in the neural retina (Coffey et al. 2007). Cigarette smoke, confirmed environmental risk factor of AMD, activates complement (Robbins et al. 1991) by possibly modifying the C3 protein (Kew et al. 1985, Kew et al. 1987). Also, high plasma levels of C3 have been detected in patients with other complex diseases, such as coronary artery disease, incident diabetes and, metabolic syndrome (Ajjan et al. 2005, Engstrom et al. 2005, van Oostrom et al. 2007).

3 AIMS OF THE PRESENT STUDY

1. To investigate whether the risk alleles Y402H of the *complement factor H* gene, M299V of the *elongation of very long chain fatty acids-like 4* gene, or Q5345R of the *hemicentin-1* gene contain a risk for age-related macular degeneration in the genetically isolated Finnish population.

2. To analyse whether a connection exists between the *complement factor H* Y402H polymorphism and the variation in age-related macular degeneration lesion characteristics in eyes with a recent exudative AMD.

3. To analyse whether the *complement factor H* Y402H polymorphism might be related to the outcome of photodynamic therapy in patients with exudative age-related macular degeneration.

4. To explore the possible functional changes in complement factor H caused by the Y402H polymorphism.

5. To investigate whether the risk alleles of the *complement factor H, LOC387715*, and *complement component C3* genes and smoking contain a risk for age-related macular degeneration in the Finnish population, and whether the polymorphisms of these genes and smoking might have an interaction.

4 MATERIALS AND METHODS

4.1 Patient material

The patient material of study I consisted of 335 Finnish patients with AMD attending the Departments of Ophthalmology of Helsinki (n=203), Oulu (n=10) and Kuopio (n=40) University Hospitals, or private offices and outpatient clinics (n=82) between 1999 and 2005. Patients with AMD fell into two groups: sporadic cases, who reported no known relatives with AMD (n=154) and familial cases, who had at least one close relative with AMD (n=181). Of the familial cases, 66 index patients had a total of 75 affected relatives. A total of 40 index cases from Kuopio University Hospital were classified as familial cases based on a detailed interview about having relatives with AMD (these relatives were not included in genotyping).

Two separate control groups were analysed in this study. The "non-AMD group" comprised of 105 Finnish subjects and the "blood donor group" of 350 anonymous Finnish blood donors. The inclusion criteria for control subjects of the "non-AMD group" were >65 of age with no large drusen and no or minimal focal pigmentary abnormalities in the macula. In addition, the ocular media had to be sufficiently clear for fundus photography. Subjects filling these criteria and attending the Department of Ophthalmology of Helsinki University Hospital or private clinics were asked to participate in the study as control subjects. In this setting it is not possible to evaluate how our cases and controls differ from the entire population of AMD patients and subjects >65 years of age without AMD in Finland. This is less of problem in genetic analyses but we cannot exclude a possible selection bias in relation to non-genetic risk factors, e.g. smoking. (Figure 6, Table 4).

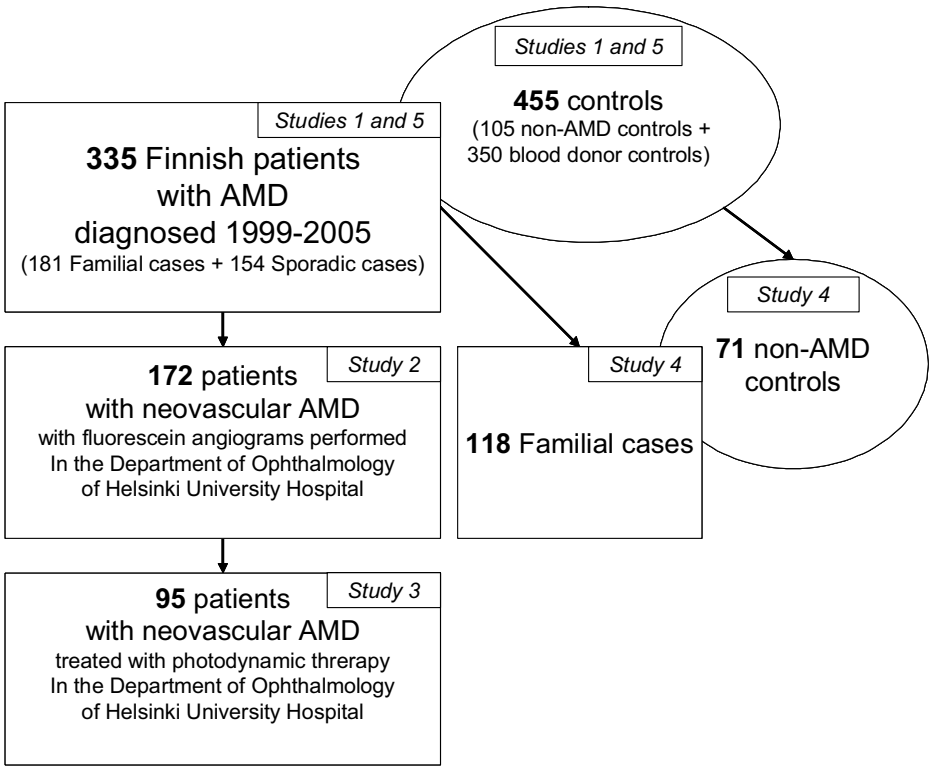


Figure 6. *Patient material of Studies I-V.*

Table 4. *Clinical characteristics of the eyes of the patients of Studies I and V, indicated I/V in each column. For the type of the most advanced AMD lesion, the lesions were graded in the order of increasing severity: drusen or geographic atrophy not involving the fovea, geographic atrophy involving the fovea and an acute exudative or a disciform lesion. For bilateral involvement, the term 'late AMD' indicates the presence of geographic atrophy involving the fovea or an acute exudative or a disciform lesion.*

				The type of the most advanced AMD lesion in the eyes of a study participant		
Patient group	n	Age mean (range)	Large drusen	Geographic atrophy involvolving fovea	Acute exudative or a disciform lesion	Bilateral late AMD
Sporadic AMD	154/151	75.4 (52.4-88.5)/ 75.5 (52.4-88.5)	13.0%/12.6%	0.0%/0.0%	87.0%/87.4%	33.8%/33.1%
Familial AMD index patients	106/106	76.1(56.3-92.7)/ 75.8 (56.3-92.7)	16.0%/17.0%	12.3%/12.3%	71.7%/70.7%	42.8%/42.5%
Familial AMD relatives	75/75	77.7 (52.9-91.5)/ 77.7 (52.9-91.5)	17.3%/17.3%	13.3%/13.3%	69.4%/69.4%	48.6%/48.6%
Non-AMD controls	105/105	76.9 (66.8-87.7)/ 76.9 (66.8-87.7)	0.0%/0.0%	0.0%/0.0%	0.0%/0.0%	0.0%/0.0%

Of these 335 cases with AMD, a total of 172 patients attending the retina clinic of the Department of Ophthalmology, Helsinki University Hospital, for a recent exudative AMD, were enrolled to Study II. Thus, all the patients of Study II had acute wet AMD lesion. Out of the 172 eyes enrolled, 8 eyes were excluded because more than 50% of the total lesion area consisted of subretinal hemorrhage. In the remaining 164 eyes the mean age (SD) of the patients was 75.0 (6.6) years. In patients with bilateral wet AMD, the eye which had been evaluated for the first time for a recent wet AMD at a date closest to 31 March 2003 was selected as the study eye.

Further, for Study III, we selected 95 patients out of these 172 who had been treated with photoynamic therapy (PDT) in 1999 -2005 at the Department of Ophthalmology, Helsinki University Hospital. Seven PDT- treated AMD patients were excluded from the final analysis: Six patients were scheduled for further PDT sessions and one of the patients had been given an intravitreal injection of triamcinolone acetonide. The mean age (SD) of these 88 patients was 75.2 (6.4) years. If a patient had bilateral exudative AMD, the eye which had PDT therapy for the first time at a date closest to 31 March 2003 was selected as the study eye. The final material consisted of 88 PDT-treated eyes in which no further PDTs were planned.

For Study IV, 118 familial AMD cases seen in the Department of Ophthalmology of the Helsinki University Hospital were collected from the case sample of Study I. A control group that comprised of 71 patients was collected from the non-AMD controls of Study I. For CRP-CFH binding studies a random cohort of 46 AMD patients and 33 control patients was selected out of the original Study IV patient material. The mean age of the AMD cases was 77.0 (range 58.1-92.4) years and that of the control subjects 76.6 (range 69.8-87.5) years. Sections of human eyes that were studied were obtained, stained and analyzed by laser scanning confocal immunofluorescence microscopy as described by Johnson et al. (Johnson et al. 2006).

In Study V, three patients out of 335 cases of Study I were excluded: There was a genotyping failure in two of the 154 sporadic cases and one of the sporadic cases was reported to have a novel AMD case in his family and was thus transferred to the familial group. Of the 181 previous familial cases, one from Kuopio University Hospital could not be genotyped because of the insufficient amount of DNA. The final patient material for Study V comprised of 332 Finnish patients with 151 sporadic cases and 181 familial cases. Otherwise the patient material and the controls were the same as in Study I (Table 4).

Blood samples for genotyping were obtained from all the patients with AMD and control individuals. The study was approved by the Ethics Committee of the Helsinki University Eye and Ear Hospital and performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all of the subjects after explanation of the nature and possible consequences of the study.

4.2 Ophthalmologic investigations

For Studies I and V, the AMD stage was verified in the family members of the index patients from fundus photographs or angiograms in 45 (60.0%) (I and V), from the medical records in 28 (37.3%) (I and V) and from an examination by a retinal specialist belonging to the study group in two of the patients (2.7%) (I and V). In the rest of the subjects (index cases, sporadic cases and non-AMD controls) the AMD stage was verified from fundus photographs or angiograms in 341 (93.4%) (I)/ 338 (93.4%) (V), from an examination by a retinal specialist belonging to the study group in four (1.1%) (I and V), and from medical records in 20 (5.5%) (I and V) of the subjects. A more detailed description of the AMD stage is given in Table 4.

In Studies II and III visual acuity (VA) assessment, using Snellen or ETDRS (Early Treatment Diabetic Retinopathy Study) tables and transformed into logMAR values (III), biomicroscopy of the anterior and posterior parts of the eye, and fluorescein angiography (FA) were performed for all patients (II and III). The angiograms were recorded using either the Topcon Imagenet (Topcon Inc., Tokyo, Japan) or Heidelberg retinal angiograph (Heidelberg Engineering, Heidelberg, Germany) systems. The area measurements were performed with the software within the respective imaging systems.

The records and the angiograms of the patients were later reviewed by a retina specialist masked to the identity and the *CFH* Y402H genotype of the patients. The following parameters were recorded: Total lesion area included the occult and classic choroidal neovascular (CNV) lesion components, serous pigment epithelial detachment (PED), and haemorrhage, dense enough to cover underlying fluorescence. The CNV lesion area consisted of the classic and occult lesion components but excluded serous PED and haemorrhage. The presence and size of a serous PED and the presence of a retinal-angiomatous proliferation (RAP) were also recorded. Only one eye of each subject was analysed, except for the data presented on the fellow eyes. The choroidal neovascularization (CNV) category was further classified in to lesions where the CNV lesion was ≥50% of the total lesion area. These lesions were graded as predominantly classic, minimally classic or occult according to

the Treatment of Age-related Macular Degeneration with Photodynamic Therapy (TAP) study guidelines (TAP Study Group 1999) (II and III).

In the Study III, the patients were classified as “PDT-responders” or “PDT-non-responders” based on the outcome of PDT after the last treatment session. In the “PDT-responders” the PDT-treatment was considered to be successful (anatomic response), if the treating physician had deemed the lesion to be clinically dry without active leakage from CNV in FA at a visit scheduled at least 12 weeks after the last PDT treatment. In the “PDT-non-responders” the PDT sessions had been discontinued by the treating retina specialist due to a still exudative lesion after several PDT-sessions. Our clinic is the only referral centre for AMD patients in the district where the patients came from.

Information about the smoking history of the study participants was obtained by telephone (V). If a participant has ever smoked, the age at which he started and quit (if he had quit) smoking was recorded, as well as the number of cigarettes smoked per day during that period. Then, the number of pack-years was calculated (cigarettes smoked per day X years smoked / 20 [cigarettes per pack]). A binary variable never/ever was based on the information whether a study participant had smoked < one pack-year or > one pack-year in his lifetime.

4.3 PCR-sequencing

DNA was extracted from 10 ml of peripheral blood using a phenol-chloroform method. The DNA of the study subjects was amplified by the polymerase chain reaction (PCR) and sequenced using primers shown in Table 5 to determine the genotypes. Polymerase chain reaction conditions were as follows: 5 min at 94 °C followed by 35 cycles of denaturation step: 30 s at 94 °C; annealing step: 30 s at 52-60 °C; elongation step: 45 s at 72 °C; and final extension for 7 min at 72 °C. Sequencing was performed using cycle sequencing with the Big Dye Terminator kit (version 3.1) supplied by Applied Biosystems (ABI, Foster City, CA, USA), and reactions were run on an ABI 3730 capillary sequencer according to the manufacturer's instructions.

Table 5. *Primers for evaluation of variant of the hemicentin-1 gene and polymorphisms of the complement factor H (CFH), the elongation of very long chain fatty acids-like 4 (ELOVL), the LOC387715, the high-temperature requirement factor A1 (HTRA1), and the complement factor 3 (C3) genes. Numeral of study is indicated in parenthesis.*

Polymorphism	Primer sequencies
Hemicentin Q5345R (I)	5'-cctgtgttttgtgtgtgtatgtatg-3' (F) 5'-gagagcccacagaaaggaaa-3' (R)
CFH Y402H (rs1061170) (I-V)	5'-ctttgttagtaactttagttcg-3' (F) 5'-ttagaaagacatgaacatgctagg-3 (R)
ELOVL4 M299V (rs3812153) (I)	5'-atgcactgggctctaattgc-3' (F) 5'-ccaagctctcctttgcttct-3' (R)
LOC387715 A69S (rs10490924) (V)	5-ggtggttcctgtgtccttca-3 (F) 5-ggggtaaggcctgatcatct-3 (R)
HTRA1 (rs11200638) (V)	5-atgccacccacaacaacttt-3 (F) 5-cgcgtccttcaaactaatgg-3 (R)
C3 R102G (rs2230199) (V)	5-ggaacagacccctgacaatg-3 (F) 5-cttgtggttgacggtgaaga-3 (R)

4.4 C-reactive protein- CFH Y402H protein interaction analyses

4.4.1 Immunohistochemistry

Sections of human eyes were obtained, stained and analysed by laser scanning confocal immunofluorescence microscopy as described by Johnson et al. (Johnson et al. 2006).

4.4.2 SDS-PAGE and Immunoblotting

To determine whether the *CFH* Y402H polymorphism influenced the molecular nature of serum CFH, serum samples from 54 AMD patients and controls either non-risk (genotype TT) or risk homozygous for *CFH* Y402H (genotype CC) or heterozygous for the Y402H polymorphism (genotype CT) were analysed by immunoblotting using polyclonal and monoclonal (86X and 90X) antibodies directed against CFH (Jokiranta et al. 1996). Serum samples from patients and controls were separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Cleveland et al. 1977) under nonreducing conditions. The proteins were transferred onto a nitrocellulose membrane, and nonspecific binding sites were blocked. Polyclonal goat anti-human CFH (Calbiochem) or monoclonal antibodies and peroxidase-conjugated donkey anti-goat IgG (Jackson ImmunoResearch laboratories) or peroxidase-conjugated rabbit anti-mouse IgG (Jackson) were used for the detection of CFH and CFH-related proteins.

4.4.3 Quantification of CRP

CRP levels were determined using a highly sensitive immunoturbidometric assay (Orion Diagnostica, Espoo, Finland). CRP concentration was not available for three TT controls and one CT control.

4.4.4 Purification of CFH402Y and CFH402H

The IgG fraction of goat antiserum to human CFH (Quidel) was precipitated with 18% Na_2SO_4. The precipitate was washed with 18% Na_2SO_4 in phosphate buffered saline, pH 7.4 (PBS) and resuspended in sodium carbonate buffer. The precipitate was then coupled to cyanogenbromide (CNBr)-activated Sepharose 4B (Amersham Biosciences) according to the manufacturer's instructions. Sera from ten controls with the TT genotype were pooled for isolation of CFH402Y and sera from ten patients with the CC genotype were pooled for isolation of and CFH402H. Both serum pools were incubated with Sepharose for 1 hour and the column was washed with 0.5 M NaCl in PBS. The bound CFH proteins were eluted with the chaotropic agent sodium thiocyanate, 3M, and finally dialyzed against veronal-buffered saline. The purity of the proteins was checked by SDS-PAGE and both silver staining and Western blotting. The concentrations of purified proteins were determined by using absorbance measurements at 280 nm, the bicinchonic acid (BCA) Protein Assay (Pierce) and silver staining.

4.4.5 Expression of recombinant CFH SCR5-7 and construction of CFH SCR5-7402H variants

Recombinant CFH SCR5-7 constructs were generated as described by Jokiranta et al. (Jokiranta et al. 2006) using specific primers for SCRs 5, 6 and 7 and pPICZα expression vectors. The Y402H mutation was introduced to *CFH* SCR5-7 sequence by using the QuikChange Multi-Site Mutagenesis Kit (Stratagene).

4.4.6 Binding of complement factor H to C-reactive protein

Maxisorp microtiter plates (Nunc) were coated with CRP (1 µg/ml, Calbiochem), whose purity was confirmed with silver staining. After blocking, sera (1:1,000) from patients and controls, purified CFH proteins or recombinant SCR5-7 variants were added. The plates were incubated for one hour at 37°C and washed. Polyclonal goat anti-human CFH (1:5,000) was added. Peroxidase-conjugated donkey anti-goat IgG diluted 1:10,000 was used as the secondary antibody. Chromogenic substrate

and absorbance measurement at 492 nm were used for detection and results indicated as direct optical density (OD) values. All the CFH-CRP binding experiments were repeated at least twice on duplicate samples.

4.4.7 Binding of complement factor H to heparin

Binding of purified CFH proteins (CFH402Y and CFH402H) and respective SCR5-7 fragments to heparin was analysed using heparin affinity chromatography in a high-performance liquid chromatograph (HPLC) system (LaChrom L-7100, Hitachi). 10 μg of proteins were diluted in 1/2xPBS and applied to a heparin-Sepharose affinity column (HiTrap, Amersham Biosciences) at a flow rate of 0.5 ml/min. The column was extensively washed with 1/2xPBS, and the bound proteins were eluted using a linear salt gradient ranging from 75 to 500 mM NaCl, in a total volume of 10 ml and at a flow rate of 0.5 ml/min.

4.4.8 Molecular modeling

The schematic molecular display model depicting binding between CFH and CRP presented in Figure 4 (Study IV) is based upon the crystal structure of CRP (Shrive et al. 1996) and 10 copies of nuclear magnetic resonance structures of the CFH SCR15-16 domain pair as display units (Barlow et al. 1993). The model was created using InsightII 2000 (Accelrys Inc., San Diego, CA).

4.5 Statistical analysis

For skewed continuous variables the differences between groups were evaluated by non-parametric tests, the Kruskal-Wallis H test and Mann-Whitney U test. The median and the 25% and 75% quartiles are given as descriptive statistics. For the normally distributed continous variables ANOVA test and two-tailed Student's t-test were used. Post hoc comparisons between individual groups were performed with the Games-Howell test as the variances of different groups were not homogeneous. Genetic associations between the disease status and the SNPs were tested by means of Chi-square test (and Fisher's exact test, where necessary). Hardy Weinberg equilibrium was tested in cases and controls separately, with the standard Chi-square test, to identify possible genotyping errors.

Statistical analyses were performed with Tixel (version 8.1), a VBA-program for Excel (I), and with SPSS (SPSS Inc., Chicago, Illinois; release 13.0; 2005 and 15.0; 2006) statistical software (II-V). Odds ratios (OR) along with their confidence intervals were estimated with R scripts freely

available on the Internet (R Packages Epitools, http://www.epitools.net). Furthermore, population attributable risks (PAR) were estimated with Levin's formula.

Logistic regression was used for estimating the marginal effects of the individual SNPs and covariates (age, sex, smoking), and for dissecting potential statistical gene-gene and gene-environment interactions. This was carried out with the SPSS Binary logistic regression modelling procedure, in which stepwise backward variable selection procedure was used to screen out the informative covariates from the uninformative. The coding for SNP genotypes (also called design variables or dummy variables) followed the notation presented by Cordell and North et al. (Cordell 2002, North et al. 2005), to be able to assess the additive and dominance effects separately. The stepwise backward conditional method was used for evaluating the significance of the explanatory variables for the model.

The degree of biological interaction of the polymorphisms of the susceptibilty genes and smoking was assessed by the departure-from-additivity model (Andersson et al. 2005), where two dichotomous risk factors (carrier/non-carrier of a risk allele, smoker/non-smoker) were tested at a time. Thus, four different risk factor combinations were generated with the no-risk-factors combination as a reference. Relative risk due to interaction (RERI), attributable proportion due to interaction (AP) and, synergy index (S) were calculated by an Excel program freely available on the Internet (www.epinet.se).

The power of Study II was estimated by using the PS (version 2.1.31, 2004) software at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize, and the power of Study III by the `power calculator` at http://calculators.stat.ucla.edu/powercalc/.

5 RESULTS

5.1 Complement factor H gene (I-IV)

5.1.1 Association study (I)

In the *complement factor H* (*CFH*) gene the Y402H polymorphism (1277T>C) in exon 9 (rs1061170) was significantly associated with AMD in Finnish patients. We found that the frequency of the AMD risk allele C was over-represented both in the familial cases compared to the non-AMD ($p=1.18x10^{-10}$, Chi-square test) or to the blood donor controls ($p=1.85\ x10^{-11}$) and in the sporadic cases compared to the non-AMD ($p=3.01x10^{-8}$) or to the blood donor controls ($p=4.21\ x10^{-8}$) (Tables 6 and 7). No difference existed in the C allele frequency between familial and sporadic cases (p=0.419). Also, both familial and sporadic cases more often carried the homozygous risk genotype (CC) than the non-risk genotype (TT) when compared to the non-AMD controls ($p=4.83\ x\ 10^{-10}$; familial cases and $p=4.83\ x\ 10^{-10}$; sporadic cases) or to the blood donor controls ($p=2.64\ x\ 10^{-10}$; familial cases and $p=4.52\ x\ 10^{-8}$; sporadic cases), but again, no difference could be detected between familial and sporadic cases (p=0.814). Overall, the risk of AMD of the homozygous patients was higher than that of the heterozygous patients, though a statistically significant association was found also between AMD and the heterozygous genotype (CT) both in familial and sporadic cases. (Table 7)

Table 6. *Allele and genotype frequencies of the Y402H polymorphism of the complement factor H (CFH) gene. C allele is the risk allele.*

	CFH Y402H				
	Allele distribution		Genotype distribution		
Case group (n)	C (%)	T (%)	CC (%)	CT (%)	TT (%)
Familial cases (181)	66.3	33.7	45.3	42.0	12.7
Sporadic cases (154)	63.3	36.7	38.3	50.0	11.7
All AMD cases (335)	64.9	35.1	42.1	45.7	12.2
Non-AMD controls (105)	38.6	61.4	12.4	52.4	35.2
Blood donor controls (350)	44.6	55.4	20.0	49.1	30.9

Table 7. *Association between AMD and the Y402H polymorphism of the complement factor H (CFH) gene. AMD cases are compared to non-AMD controls (n=105) and to blood donor controls (n=350). Shown are odds ratios (OR) with their 95% confidence intervals (95% CI) for case-control comparisons. C allele is the risk allele.*

	Non-AMD controls (105)			Blood donor controls (350)		
CFH	OR (95% CI)			OR (95% CI)		
Case group (n)	(C versus T)	(CT versus TT)	(CC versus TT)	(C versus T)	(CT versus TT)	(CC versus TT)
Familial cases (181)	3.13 (2.20-4.46)	2.22 (1.19-4.16)	10.1 (4.64-22.2)	2.45 (1.89-3.19)	2.07 (1.23-3.51)	5.50 (3.17-9.55)
Sporadic cases (154)	2.75 (1.91-3.94)	2.88 (1.49-5.57)	9.33 (4.10-21.3)	2.15 (1.63-2.83)	2.69 (1.52-4.73)	5.06 (2.75-9.28)
All AMD cases (335)	2.95 (2.14-4.06)	2.51 (1.46-4.31)	9.79 (4.76-20.1)	2.30 (1.85-2.86)	2.34 (1.54-3.57)	5.31 (3.35-8.40)

5.1.2 CFH Y402H polymorphism and AMD lesion characteristics (II)

Medians of lesion sizes of patients with neovascular AMD (n=164) were similar in the *CFH* Y402H genotype groups, with lesion size of 8.15 mm^2 in patients homozygous for the risk allele (CC, n=58), 7.50 mm^2 in heterozygous patients (CT, n=80), and 7.05 mm^2 in those with the homozygous non-risk genotype (TT, n=26), (p=0.599, Kruskal-Wallis H test). The corresponding medians of CNV (classic and occult CNV together without serous PED and haemorrhage) lesion areas were 6.37 mm^2, 5.00 mm^2 and, 5.18 mm^2, respectively (p=0.407).

To further analyse whether the genotype had an effect on the lesion size, patients were divided into four quartiles according to the total lesion size. When the smallest or the largest quartile was compared to the remaining three quartiles, no difference existed even in this comparison between the genotypes. In a comparison of the CC and TT genotypes, the p-value was 0.916 when the largest quartile (n=41) was compared to the three smallest (n=123) and 0.946 when the smallest quartile (n=41) was compared to the three largest quartiles (n=123).

The CNV category was analysed only in lesions where the CNV lesion was 50% or more of the total lesion area (n=144). These lesions were classified as predominantly classic, minimally classic, or occult according to the Treatment of Age-related Macular Degeneration with Photodynamic Therapy (TAP) study guidelines (TAP Study Group 1999). A tendency for the minimally classic lesions to be more prevalent in patients with the CC genotype than with the TT genotype (p=0.105, Chi-square) was seen, but generally, no statistically significant genotype differences could be detected (Figure 7).

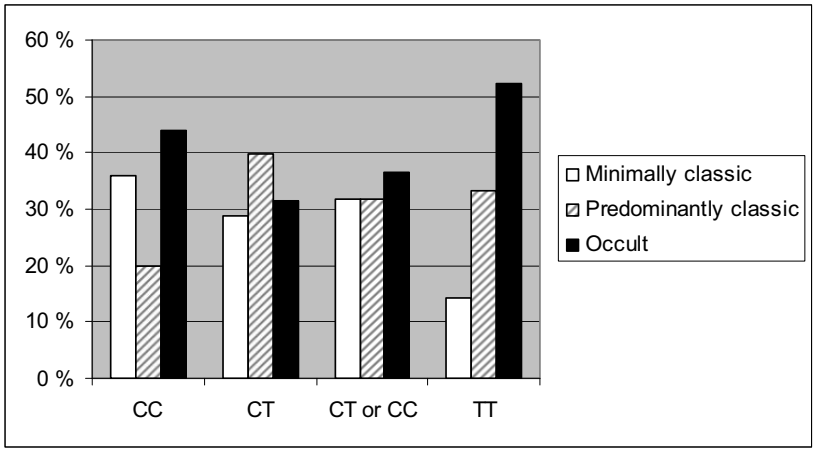


Figure 7. *Prevalence of the choroidal neovascularization (CNV) subtype categories (minimally classic lesion, predominantly classic lesion, and occult lesion) in the different genotypes of the complement factor H gene Y402H polymorphism, percentage within each genotype (CC, CT and TT) and in CC and CT genotypes combined. Only patients with neovascular lesions ≥50% of the total lesions are included.*

Patients carrying the TT genotype had the RAP lesions more often (23.1%) than the CT (17.5%), or CC (10.3%) patients, but the difference did not reach statistical significance (p=0.257, Fisher's exact test, TT compared to CT or CC). No difference in the proportion of patients with a serous pigment epithelium detachment (PED) between the genotypes (TT: 15.4%, CT: 10.0%, and CC: 13.8%, p=0.528, Fisher's exact test, TT compared to CT or CC) existed.

The age of onset of exudative AMD did not vary between *CFH* Y402H genotypes. The age of onset of AMD in the first eye was rather similar in different genotypes both in unilateral (p=0.517, Kruskall-Wallis H test) and bilateral (p=0.295) cases. The mean age of onset in the first eye was the same at 74.8 years in TT patients and CC patients in unilateral cases and nearly the same at the age of 73.3 (CC) and 73.0 (TT) in bilateral cases. Neither could a difference in the age of presentation of the second eye between the genotypes (p=0.364) be found.

5.1.3 CFH Y402H polymorphism and outcome of photodynamic therapy (III)

Genotype of the *CFH* Y402H polymorphism did not determine, whether a patient was a "PDT-responder" or a "PDT-non-responder". Of the PDT-treated patients, 59 out of 88 (67.0%) were "PDT-responders": 18/26 (69.2%) of patients carrying the homozygous risk allele (CC), 34/50 (68.0%) of heterozygous patients (CT), and 7/12 (58.3%) of those with the normal genotype (TT) (p= 0.520, Fisher's exact test, TT compared to CT or CC).

Median number (interquartile range) of PDT sessions of the "PDT-responders" was 3 (2) in all the genotypes separately, as well as, in all the "PDT-responders". In all the "PDT-non-responders" median number of PDT sessions was 2 (1). A bias produced by the various number of PDT sessions for the PDT-responders and for the PDT-non-responders (p=0.048 Mann-Whitney U test) was excluded by performing an analysis where *CFH* Y402H genotypes were compared between the PDT-responders with ≤2 PDT sessions and the PDT-non-responders with ≥2 PDT sessions (p=0.406, Chi-square test, df=2) and between the PDT-responders with ≤3 PDT sessions and the PDT-non-responders with ≥3 PDT sessions (p=0.182, Chi-square test, df=2). Thus, neither of these comparisons showed any difference in genotypes between PDT-responders and PDT-non-responders.

Within lesion type categories (predominantly classic, minimally classic, occult), no significant difference was found in response rates to PDT between genotypes. In occult lesions, the PDT-responder eyes with the CC genotype had received more treatments (3.5) than those with the CT genotype (1.0) (p= 0.012, Mann-Whitney U test). The number of treatments of the patients with the TT genotype (2.0) was between that with the CC genotype and that with the CT genotype, thus suggesting that the difference was not true.

The final visual acuity was better and ($p < 0.001$) and the change of visual acuity smaller ($p < 0.001$) in all the PDT-responders than in all the PDT-non-responders. PDT-responders with the CC genotype had a better final VA (20/80) than the patients with the CT genotype (20/200) (p=0.020, Mann-Whitney U test), but again the TT genotype (20/125) was between the CC the CT genotypes. Generally, no difference was detected in the final visual acuity or in change of visual acuity in the "PDT-responders" or the "PDT-non-responders" between different genotypes of the *CFH* Y402H.

5.1.4 Functional analysis of complement factor H (IV)

5.1.4.1 Location of C-reactive protein and complemen factor H in drusen

The expression of CFH and CRP were analysed by confocal immunofluorescence microscopy. In two representative cases, CFH was shown to be present throughout the drusen in individuals with either the TT or CC genotype, and CRP was present in small spheroid particles inside the drusen.

5.1.4.2 Reduced binding affinity of CFH_{402H} to C-reactive protein but binding to heparin not affected

An ELISA assay was set up by coating plate wells with CRP to analyse the binding of the different variants of the complement factor H to CRP. Binding of serum-derived CFH, purified CFH, and recombinant CFH SCR5-7 fragments to CRP were analysed. A significantly reduced binding of CFH to CRP was found in the sera of AMD patients homozygous for the Y402H risk allele (CC) (OD_{492}: 0.369±0.102, n=20) when compared to patients with the non-risk (TT) genotype (OD_{492}: 0.532±0.102, n=16, $p=4.9x10^{-4}$) (ANOVA). A similar difference in the sera of healthy controls indicated that the functional difference in CRP binding is genotype, not disease, associated (CC controls: OD_{492}: 0.324±0.098, n=9, TT controls: OD_{492}: 0.697±0.199, n=14, $p=1.0x10^{-4}$). Binding of serum CFH to CRP also was reduced in the heterozygous patients (CT). Also, when data from the patient and control groups were pooled according to genotype, a significant difference in CFH binding to CRP was observed ($F=27.4$, $p=1.1x10^{-9}$) with the greatest difference between the CC (OD_{492}: 0.355±0.101, n=29) and TT genotypes (OD_{492}: 0.609±0.173, n=30, $p=3.9x10^{-8}$) and between the CT (OD_{492}: 0.499±0.100, n=20) and TT genotypes ($p=4.2x10^{-4}$), but the difference between CC and CT genotypes was also significant ($p=6.8x10^{-3}$) (ANOVA). By measuring CFH concentrations of the samples, varying CFH levels as the cause for these results were excluded. When data of AMD patients were pooled regardless of genotype (OD_{492}: 0.445±0.129, n=46) and compared with pooled control data (OD_{492}: 0.518±0.214, n=33), no significant difference in CFH binding to CRP was observed ($p=0.062$) (Student's t-test). Binding of purified full length CFH_{402H} protein to CRP (OD_{492}: 0.470±0.033) was significantly weaker than that of the CFH_{402Y} protein (OD_{492}: 0.668±0.103, $p=0.034$) (Student's t-test). An even more marked difference was observed between the two recombinant fragments of CFH in binding to CRP than using the full-length proteins.

Heparin-Sepharose affinity chromatography was used to assess the binding of the two full-length CFH variants, CFH_{402Y} derived from controls and CFH_{402H} derived from patients, to heparin. Both protein variants eluted from the column at the same ionic strength indicating that the *CFH* Y402H variation does not markedly affect the binding of CFH to heparin. Similar results were obtained using recombinant fragments of CFH SCR5-7 containing either the 402Y or 402H residues.

5.2 Other candidate genes (I,V)

5.2.1 LOC387715/HTRA1 locus

The two SNPs (*LOC387715* rs10490924 and *HTRA1* rs11200638) are located only 6.1 kb from each other and are therefore in almost complete linkage disequilibrium. Due to the close proximity of the two genes we observed only 5 genotypes out of 787 inconsistent with perfect LD in these two genes. We decided to focus on the *LOC387715* gene, since there is cumulating evidence for *LOC387715* A69S to be the actual causal variant in AMD (Kanda et al. 2007).

In our Finnish patient material, both familial and sporadic cases had the AMD-associated *LOC387715* A69S risk allele T more often than the non-AMD ($p= 2.32 \times 10^{-14}$; familial cases and $p=1.54 \times 10^{-8}$; sporadic cases, Chi-square test) or the blood donor controls ($p=6.11 \times 10^{-18}$; familial cases and $p=6.90 \times 10^{-9}$; sporadic cases) (Tables 8 and 9). Both familial ($p =2.73 \times 10^{-12}$) and sporadic cases ($p=9.95 \times 10^{-7}$) also carried more often the *LOC387715* risk genotype TT than the GG genotype when compared to non-AMD controls, or to blood-donor controls ($p=1.35 \times 10^{-15}$; familial cases and $p=8.48 \times 10^{-7}$; sporadic cases) (Tables 8 and 9). No differences between the genotype frequencies of familial and sporadic cases were detected (p=0.09).

Table 8. *Allele and genotype frequencies of the A69S polymorphism of the LOC387715 gene. T allele is the risk allele.*

	LOC387715				
	Allele distribution		Genotype distribution		
Case group (n)	T (%)	G (%)	TT (%)	GT (%)	GG (%)
Familial cases (181)	51.4	48.6	27.6	47.5	24.9
Sporadic cases (151)	43.4	56.6	17.9	51.0	31.1
All AMD cases (332)	47.7	52.3	23.2	49.1	27.7
Non-AMD controls (105)	19.5	80.5	2.9	33.3	63.8
Blood donor controls (350)	24.7	75.3	6.4	36.5	57.1

Table 9. *Association between AMD and the A69S polymorphism of the LOC387715 gene. AMD cases are compared to non-AMD controls (n=105) and to blood donor controls (n=350). Shown are odds ratios (OR) with their 95% confidence intervals (95% CI) for case-control comparisons. T allele is the risk allele.*

	Non-AMD controls (105)			Blood donor controls (350)		
LOC387715	OR (95% CI)			OR (95% CI)		
Case group (n)	(T versus G)	(TG versus GG)	(TT versus GG)	(T versus G)	(TG versus GG)	(TT versus GG)
Familial cases (181)	4.34 (2.93-6.53)	3.63 (2.12-6.33)	23.3 (7.90-104.2)	3.23 (2.47-4.22)	2.98 (1.96-4.58)	9.77 (5.48-18.0)
Sporadic cases (151)	3.14 (2.10-4.78)	3.11 (1.81-5.43)	12.1 (3.95-55.1)	2.34 (1.76-3.11)	2.55 (1.68-3.93)	5.08 (2.68-9.74)
All AMD cases (332)	2.98 (2.05-4.40)	3.37 (2.09-5.52)	17.7 (6.23-76.9)	2.79 (2.22-3.51)	2.77 (1.98-3.89)	7.39 (4.42-12.8)

5.2.2 Complement component 3 gene

The risk allele G of the R102G variant, corresponding to the electrophoretic protein variant C3F (fast), in exon 3 of *complement component 3* (*C3*) was associated with AMD in familial cases when compared to non-AMD controls (p=0.008, Chi-square test) or to blood donor controls (p=0.039), and in all AMD cases when compared to non-AMD controls (p=0.039) (Tables 10 and 11). The heterozygous risk genotype CG was also detected more often in familial cases than in non-AMD (p=0.013) or in blood donor controls (p=0.049). However, the difference in the frequency of the homozygous risk genotype GG between familial cases and controls did not to reach statistical significance since there were too few GG cases in our material, but the trend is clear: there seems to be higher OR for homozygotes (OR 2.41; non-AMD controls and OR;1.64, blood donor controls) than for heterozygous cases (OR 2.01; non-AMD controls and 1.48; blood donor controls).

Table 10. *Allele and genotype frequencies of the R102G polymorphism of the complement component 3 (C3) gene. G allele is the risk allele.*

	C3				
	Allele distribution		Genotype distribution		
Case group (n)	G (%)	C (%)	GG (%)	CG (%)	CC (%)
Familial cases (181)	24.9	75.1	5.5	38.7	55.8
Sporadic cases (151)	18.5	81.5	4.0	29.1	66.9
All AMD cases (332)	22.0	78.0	4.8	34.3	60.9
Non-AMD controls (105)	15.2	84.8	2.8	24.8	72.4
Blood donor controls (350)	19.3	80.7	4.0	30.6	65.4

Table 11. *Association between AMD and the R102G polymorphism of the complement component 3 gene. AMD cases are compared to non-AMD controls (n=105) and to blood donor controls (n=350). Shown are odds ratios (OR) with their 95% confidence intervals (95% CI) for case-control comparisons. G allele is the risk allele.*

	Non-AMD controls (105)			Blood donor controls (350)		
C3	OR (95% CI)			OR (95% CI)		
Case group (n)	(G versus C)	(GC versus CC)	(GG versus CC)	(G versus C)	(GC versus CC)	(GG versus CC)
Familial cases (181)	1.83 (1.18-2.90)	2.01 (1.18-3.51)	2.41 (0.70-11.62)	1.39 (1.02-1.88)	1.48 (1.01-2.17)	1.64 (0.68-3.82)
Sporadic cases (154)	1.26 (0.79-2.05)	1.27 (0.72-2.27)	1.47 (0.36-7.56)	0.96 (0.67-1.34)	0.93 (0.61-1.42)	0.99 (0.34-2.58)
All AMD cases (335)	1.56 (1.04-2.41)	1.64 (1.00-2.75)	1.92 (0.61-8.82)	1.18 (0.91-1.54)	1.21 (0.87-1.67)	1.30 (0.62-2.79)

5.2.3 Interaction analyses of the susceptibility factors

Two-locus joint ORs of the *CFH* Y402H and the *LOC387715* A69S polymorphisms as well as the three-locus joint risks of the *CFH* Y402H, the *LOC387715* A69S, and the *C3* R102G polymorphisms were also analysed. The risk of AMD for a carrier of both homozygous risk genotypes CC (*CFH*) and TT (*LOC387715*) was 27-fold ($p=1.66x10^{-12}$) when compared to a carrier of a non-risk genotype TTGG, with all the other joint OR:s ranging from 2 to 21 (Table 12). When individuals carrying at least one risk allele in each locus were compared to individuals with no risk alleles at any of the loci the risk of AMD was 18-fold (Figure 8). The joint OR for three loci and smoking was 74.3 (95% CI 10.81-2124, $p=1.54 \times 10^{-7}$).

Table 12. *Two-locus odds ratios (OR and 95% confidence intervals [95%CI]) for different genotypic combinations of the complement factor H gene rs1061170 (genotypes CC/CT/TT) and the LOC387715 rs10490924 (genotypes TT/TG/GG). Eight genotype combinations are compared to the non-risk genotype combination TTGG. Risk alleles are C (CFH) and T (LOC387715). All patients with AMD (n=332) are compared to blood donor controls (n=350).*

Genotypic combination of *CFH* and *LOC387715*	Number of risk alleles	OR	(95%CI)
TTGG	0	1.00	
CTGG	1	2.31	(1.09-5.26)
CCGG	2	8.83	(4.11-20.61)
TTTG	1	2.89	(1.20-7.31)
CTTG	2	8.48	(4.19-18.84)
CCTG	3	14.41	(6.67-33.85)
TTTT	2	21.38	(6.26-91.39)
CTTT	3	17.35	(7.03-46.92)
CCTT	4	26.57	(9.71-83.25)

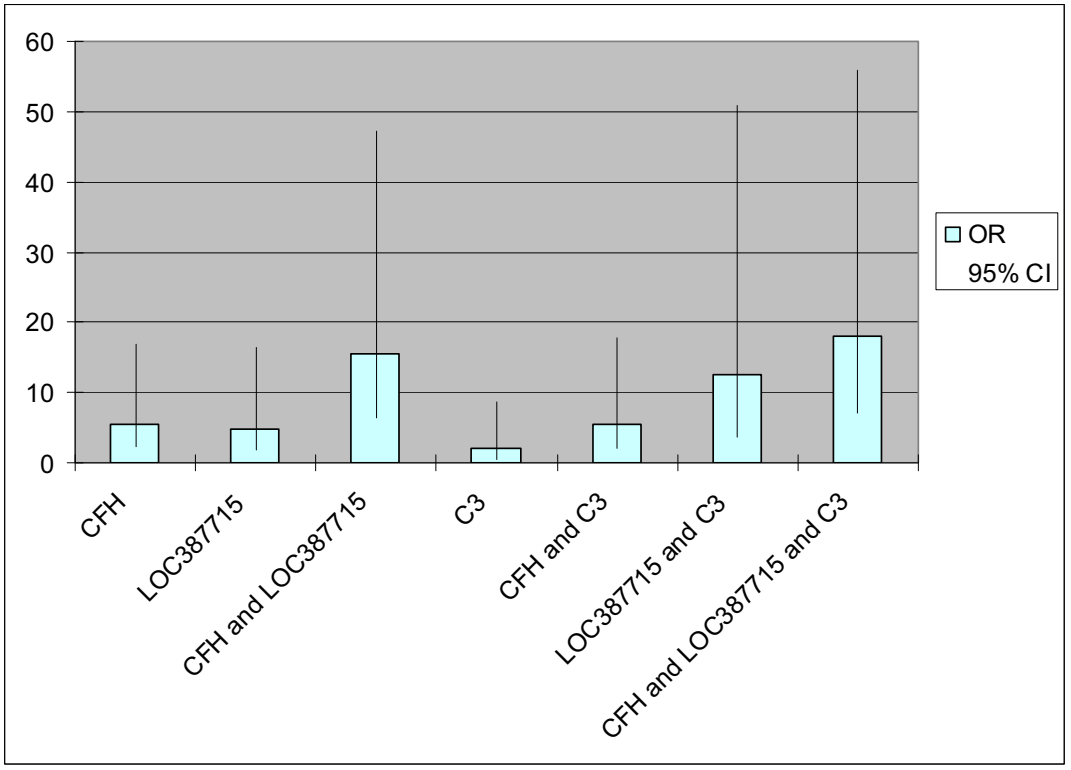


Figure 8. *Odds ratios (OR) with their 95% confidence intervals (95%CI) of three-locus risks for combinations of the risk allele carriers of the complement factor H (rs1061170), the LOC387715 (rs10490924) and the C3 (rs2230199) genes. Seven combinations are compared to individuals who carry no risk allele. Patients with AMD (n=332) are compared to blood donor controls (n=350).*

A highly significant ($p<0.001$) additive gene effect for the two main loci, *LOC387715* and *CFH*, was found in the logistic regression model. Their effects seem to be of the same size. The *C3* additive effect is slightly weaker with an effect size of 2.2, though it also is statistically siginificant. No dominance effect was seen for any of the loci (*CFH*, *LOC387715*, *C3*).

In logistic regression framework we did not detect an independent effect of smoking in our material. Neither could an interaction between *CFH* Y402H or *LOC387715* A69S and smoking be demonstrated. Instead, environment-environment (ExE) and gene-environment (GxE) type interactions were suggested for sex x smoking ($p=0.043$) and *C3* x smoking ($p=0.086$). Combined to a 3-way interaction, *C3* x sex x smoking ($p=0.016$), the 2-way interaction *C3* x smoking, disappeared. The sex x smoking interaction term, however, preserves its borderline significance (now, $p=0.065$). Explanations for this rather unexpected complexity showed up in further stratified analyses. In sex x smoking interaction the risk of AMD was statistically significantly higher in an

ever-smoker women ($p=3.60 \times 10^{-4}$) than in a never-smoker women, whereas in men the difference was borderline (p=0.054). Thus, the effect of smoking is more pronounced in women, which also explains the significance of the sex x smoking interaction, and at the same time explains why we did not get an independent main effect for smoking alone. In the *C3* x smoking interaction, smoking seems to have a significant effect in homozygous normal genotype CC ($p=1.30 \times 10^{-4}$), but not in G-carrying genotypes (p = non significant) (although the trend is the same). In the never-smokers, G allele predisposes for AMD whereas in ever-smokers the effect of the G allele is virtually indistinguishable. In *C3* x sex x smoking interaction we see highest OR for ever-smoker women with genotype CC ($p=1.93 \times 10^{-4}$), and non-significant effects in G-carrying genotypes (p=0.39) (though the trend is the same, that is, smoking predisposes to AMD in all genotype x sex classes, OR:s circa 2) (Table 13). Evidently, the final model explains the variation in the individuals' disease risks quite well; Nagelkerke R Square = 0.431.

Table 13. *Odds ratios (OR) with their 95% confidence intervals (95%CI) for the gene-environment interaction of the R102G polymorphism of the complement component 3 (C3) gene, sex and smoking. AMD cases (332) are compared to the non-AMD controls (n=105). G allele is the risk allele. Never/ever-smoker status is based on the information whether a study participant had smoked < one pack-year (never-smoker), or > one pack-year (ever-smoker) in his/her lifetime.*

	Comparison	OR (95%CI)
1	Ever-smoker female versus never-smoker female	4.68 (1.95-14.1)
2	Ever-smoker male vs. never-smoker male	2.57 (0.99-6.86)
3	*C3* CC-carrier+ever-smoker versus *C3* CC-carrier+never-smoker	4.18 (1.97-10.1)
4	*C3* G-carrier+ever-smoker versus *C3* G-carrier+never-smoker	1.94 (0.79-5.33)
5	*C3* G-carrier+never-smoker versus *C3* CC carrier+never-smoker	1.80 (1.03-3.24)
6	*C3* G-carrier+ever-smoker versus *C3* CC-carrier+never-smoker	3.50 (1.56-9.02)
7	*C3* CC-carrier+ever-smoker+female versus *C3* CC-carrier+never-smoker+female	8.55 (2.45-58.5)
8	*C3* G-carrier+ever-smoker+female versus *C3* G-carrier+never-smoker+female	1.89 (0.55-9.12)
9	*C3* CC-carrier+ever-smoker+male versus *C3* CC-carrier+never-smoker+male	2.81 (0.83-10.2)
10	*C3* G-carrier+ever-smoker+male versus *C3* G-carrier+never-smoker+male	2.05 (0.40-10.6)

Interestingly, an interaction between two major susceptibilty genes, *CFH* Y402H and *LOC387715* A69S, was found. The interaction was demonstrated in models assessing both the biological (departure-from-additivity) and the statistical (p=0.057) (logistic regression model) interaction. In the departure-from-additivity model (by Andersson et al 2005) the attributable proportion due to interaction of the loci was 70% (95%CI 51-89%) and S, the synergy index 3.79 (95%CI 1.82-7.89) (Figure 9).

The estimated population attributable risk (PAR) "reflects the prediction of how much of the disease would be eliminated from the case-control population if the high risk genotype were not present" (Gorin 2007). For a carrier of the risk alleles of *CFH* (rs1061170), *LOC387715* (rs10490924), and *C3* (rs2230199) the PARs were 58.2%, 51.4%, and 5.8%, respectively (using blood donor controls as reference group). The joint PAR for the three loci was 63%. The summary PAR is less than the sum of the three single PARs, since carrying a risk allele in one locus does not exclude carrying a risk allele also in the other locus (Schmidt et al. 2006). The PAR for smoking alone was 47.7%. Since non-AMD controls were used as a reference group this PAR cannot be interpreted as a true populationwise figure.

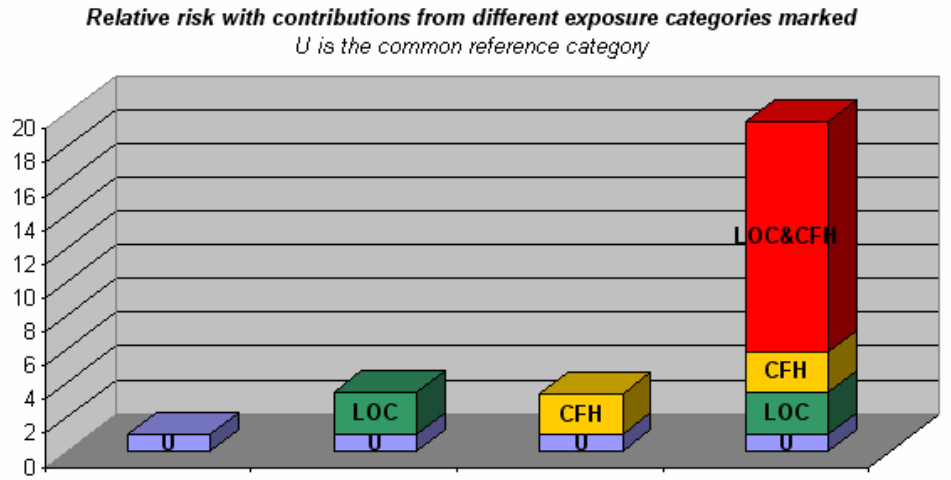


Figure 9. *Excel sheet from www.epinet.se, showing the results of biological interaction calculations (interaction between LOC387715 A69S [LOC] and CFH Y402H [CFH]) performed as Anderson et al. 2005.*

5.2.4 Elongation of very long chain fatty acids-like 4 (I) and hemicentin-1 genes (I)

In the *ELOVL4* gene (I) no association between AMD and the M299V polymorphism in exon 6 (rs3812153) was detected. Interestingly, none of familial cases (n=181), sporadic cases (n=154), or non-AMD controls (n=105) had the homozygous risk genotype (GG). No difference in the genotype or allele frequencies existed in familial cases compared to non-AMD controls (p=0.451;genotype and p=0.474; allele, Chi-square test) or to blood donor controls (n=350) (p=0.792;genotype and p=0.874;allele) or in sporadic cases compared to non-AMD controls (p=0.593;genotype and p=0.607;allele) or to blood donor controls (p=0.168;genotype and p=0.093;allele).

None of the patients or controls was carrying the Q5345R variant of exon 104 of the *hemicentin-1* gene (I).

6 DISCUSSION AND CONCLUSIONS

Significant advances have been made during the past few years in the field of molecular genetics of AMD although the pathogenesis is still poorly understood. The interplay between multiple genes and environmental risk factors in complex (multifactorial) disorders makes them a challenging research subject. AMD has often been presented as an example of a success story in terms of revealing the genetics of a complex disease (Amos 2007, Ropers 2007). Recently, a remarkable discussion has also been going on whether genome-wide linkage studies are the correct tools for detecting genes for complex diseases. Collecting patient material with a sufficient number of families for linkage studies is a slow and demanding task yet the material and methods still may be incapable of identifying genes involved in the pathogenesis of a common disease. However, in AMD, several genome-wide scans showed repeatedly linkage to the long arms of chromosomes 1 and 10 (Weeks et al. 2001, Majewski et al. 2003, Abecasis et al. 2004, Iyengar et al. 2004, Weeks et al. 2004, Fisher et al. 2005, Barral et al. 2006), which was the basis for later association studies that finally revealed the two major risk variants, Y402H polymorphism of the *CFH* gene on 1q32, and then the *LOC387715*/*HTRA1* locus on 10q26. Three out the four first studies reporting the *CFH* Y402H polymorphism (Edwards et al. 2005, Hageman et al. 2005, Haines et al. 2005) and the first study reporting the *LOC387715*/*HTRA1* locus (Jakobsdottir et al. 2005) exploited the prior results of genome-wide linkage analyses and performed a focused genotyping with SNPs flanking only these regions, 1q32 and 10q26. On the other hand, *CFH* Y402H polymorphism was also successfully found in a study using a whole-genome association approach in a relatively small patient sample. A total of 96 cases and 50 controls from AREDS study were genotyped with 16,204 SNPs of which only two had different frequencies between case and control populations. These two SNPs lay on the intron of the *complement factor H* gene, and on further sequencing of all the exons of *CFH* gene, an association between the *CFH* Y402H polymorphism and AMD was finally found (Klein et al. 2005). Thus, both genome-wide linkage analysis and the whole-genome association approach were useful tools in detecting susceptibility genes of AMD. The reasons contributing to the success have been accurate definition of the phenotype and the existence of major loci at 1q32 and 10q26. As mentioned above, this was a significant achievement not only in the genetics of AMD but also in the genetics of complex diseases in general. This forcefully guided research projects, including ours.

The population of Finland represents a genetic isolate. It is characterized, on one hand, by an enrichment of certain rare monogenic diseases, called the Finnish disease heritage and, on the other

hand, lacking many Mendelian disorders common in Caucasians (Norio 2003). We found that the *CFH* Y402H polymorphism is as strong risk factor for AMD in the Finnish as in several other Caucasian populations. Thus, the possible defect in CFH may contribute to pathogenesis of AMD similarly in the Finnish population as it is in less isolated Caucasian populations. The complement system is a critically important part of innate immunity and CFH plays a vital role in it by protecting host cells from the excessive attack of complement. Regarding this, it is understandable that CFH is universal in nature and its defects may be destructive irrespective of a population. On the other hand, the "common disease, common variant" hypothesis, which means that a relatively common allele predisposes to a common disease, appeares to be proved in AMD (Chakravarti 1999, Reich et al. 2001).

Drusen, the hallmarks of early AMD, have been known to contain components of the complement system, including C3, complement factor H, and membrane attack complex (MAC), for a relatively long time, but recently the complement system has been reported to be involved in the growth of experimental choroidal neovascularization (CNV), as well (Bora et al. 2005, Bora et al. 2006). Further, C3 and MAC were deposited in experimental murine laser induced CNV (Bora et al. 2005). The complement system was central for the expression of vascular endothelial growth factor (VEGF), transforming growth factor-β2 (TGF-β2), and β-fibroblast growth factor (β-FGF) in the experimental CNV lesions (Bora et al. 2005), and by inhibiting the alternative pathway of the complement system, the CNV formation could be abolished (Bora et al. 2006). Based on these data, we had generated a hypothesis that the *CFH* Y402H risk polymorphism would favour a more aggressive phenotype in neovascular AMD, but it turned out not to be the case in our studies. Genotype frequencies were similar in patients with predominantly classic, minimally classic, or occult CNV, and neither could an effect of *CFH* Y402H polymorphism on CNV lesion size be detected. Two independent groups have published contradictory results. They reported that a person with the risk allele had more often predominantly classic CNV lesions than other subtypes. (Brantley et al. 2007a, Wegscheider et al. 2007). The sizes of the patient materials in these studies were the same as in ours, and the angiograms were analysed at the same moment, namely at the initial presentation of active neovascularization. The criteria of the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) study were used for classification in two of the studies (criteria not stated in Brantley et al.) and the mean age of the patients was nearly the same. Thus, the reason we did not detect any difference in the CNV lesion types remains so far unclear, raising the question of whether other genetic factors, like variants in VEFG gene, may have an effect on CNV lesion characteristics (Churchill et al. 2006). However, similarly to us, Brantley et

al. could neither find any differences in CNV lesion sizes between the *CFH* Y402H genotypes, though they used greatest linear dimension (GLD) and we used area of lesion as our size criterion (Brantley et al. 2007a). When Brantley et al. further studied whether the Y402H polymorphism had an effect on the outcome of bevasizumab (Avastin®) therapy, they found that different genotype groups had similar frequencies of the predominantly classic lesions and, interestingly, they now found that the GLD was largest (p=0.02) in patients carrying the TT genotype (Brantley et al. 2007b).

The complement cascade has been shown to be a central element in the tissue response to experimental PDT in cancerous lesions. Complement is activated in experimental PDT, and the effect of PDT can be modified by complement activators or inhibitors (Korbelik 2006, Korbelik et al. 2007). Yet we failed to detect any overall difference in response to PDT in neovascular AMD lesions between different genotypes of the *CFH* Y402H polymorphism. These results can be interpreted by the belief that *CFH* Y402H polymorphism is an unlikely modulator of the response to acute tissue destruction (as caused by PDT) in the central choriocapillaris and the macula. This suggests that the increased risk for AMD is caused by a deficiency in a very specialized function of CFH unrelated to general damage control. So far, only one other group has reported *CFH* Y402H polymorphism and the effect of PDT (Goverdhan et al. 2007). In a study from United Kingdom, where 27 PDT-treated patients were evaluated, patients carrying the risk allele C seemed to need PDT more frequently (OR 2.16, 95% CI [1.19-3.93], p=0.011), and visual loss after PDT was more frequent in patients with the CC (n=13) when compared to TT (n=2) genotype (0.038) (Goverdhan et al. 2007). Considering this small data set, no conclusions can be made, about whether a difference between the genotypes of *CFH* Y402H in PDT-response really exists.

The role of complement in the pathogenesis of AMD has been suggested long before the major finding of the *CFH* Y402H polymorphism, based on the composition of drusen deposits (Mullins et al. 2000, Hageman et al. 2001). Complement factor H has an essential role in the regulation of the alternative pathway of the complement system. The Y402H risk variant is located in the short consensus repeat (SCR) 7 of CFH protein, which possesses a binding site for CRP. Study IV showed that the homozygous (CC) and heterozygous (CT) risk genotypes of *CFH* Y402H had a lower binding affinity to CRP than the TT variant, which has been later confirmed by Skerka et al. (Skerka et al. 2007). Serum CRP level is elevated in AMD and the protein is present in AMD lesions (Seddon et al. 2004, Seddon et al. 2005b). Deposition of CRP on the site of cellular damage and debris may, among other things, facilitate activation of the classical complement pathway,

resulting in C3 binding and all the consequences of complement activation. The binding of CFH to CRP might contribute to controlling such a process. Attachment of CFH to CRP would help in concentrating CFH at sites of CRP-related complement activation. The CRP-bound CFH would then contribute to inactivation of surface bound C3 to C3bi, preventing further steps in the complement cascade. C3bi is recognized by the complement receptor type 3 (CR3), CD11b/18, on phagocytes (Rothlein et al. 1985) and activation of CR3 leads to anti-inflammatory type cytokine response, and excessive inflammatory reaction and tissue damage is thus avoided (Wright et al. 1983, Yamamoto et al. 1984). However, at this point, there is insufficient evidence to confirm such a scenario and little experimental data on the interaction of CRP and CFH in retinal lesions exist. The observed difference in the binding affinity to CRP between genotypes was irrespective of case-control status, which indicates that other genetic/environmental risk factors or protective factors play a role in pathogenetic pathways of AMD.

As well as the *CFH* Y402H, the *LOC387715* A69S polymorphism almost certainly contributes to AMD pathogenesis.The function of LOC387715 is unknown, although just recently it was found on the mitochondrial outer membranes (Kanda et al. 2007). This may give hints of its function in the pathogenesis of AMD, since mitochondria have been shown to play an important role in aging processes (Harman 1981). In addition, already two decades ago it was noticed that mitochondria activate complement (Giclas et al. 1979). A risky, but attractive, hypothesis thus is that CFH and LOC388715 would be involved in a common pathogenetic pathway. In the Finnish population, the A69S *LOC387715* was seen as a strong risk factor for AMD as in other populations (Jakobsdottir et al. 2005, Rivera et al. 2005, Conley et al. 2006, Schmidt et al. 2006, Ross et al. 2007, Schaumberg et al. 2007, Tanimoto et al. 2007).

The significance of the *complement factor 3*-gene polymorphism is still to be resolved. Earlier, two independent groups found an association to AMD that was, however, weaker than that with the *CFH* and the *LOC387715* (Maller et al. 2007, Yates et al. 2007). In the *C3* locus, we detected an additive gene effect that was weaker than that of *LOC387715* or *CFH*.

No associations between AMD and polymorphisms in the *hemicentin-1* and the *elongation of very long chain fatty acids-like 4* (*ELOVL4*) gene were detected in our patient material. As the *hemicentin-1* gene codes a large extracellular member of the immunoglobulin superfamily (Schultz et al. 2003b) and variations in the *ELOVL4* gene have previously been identified in autosomal dominant macular dystrophy (Bernstein et al. 2001, Edwards et al. 2001, Zhang et al. 2001, Maugeri et al. 2004), an association would have been plausible. However, a risk variant of

hemicentin-1 gene has been found only in one large US family (Schultz et al. 2003b) and one positive association of *ELOVL4* with AMD (Conley et al. 2005) could not have been replicated in other populations either (Conley et al. 2006, DeAngelis et al. 2007). Possibly, variants of these three genes contribute only to a relatively small percentage of AMD cases.

Interestingly, to the best of our knowledge, for the first time (Jakobsdottir et al. 2005, Rivera et al. 2005, Schmidt et al. 2006), a statistical interaction between the *CFH* Y402H and the *LOC387715* A69S polymorphisms was detected in our study using both multiplicative (logistic regression) model and departure-from-additivity model. Smoking and these two variants seemed, however, to be independent risk factors, which, instead, was in line with the majority of earlier results (Conley et al. 2006, Sepp et al. 2006, DeAngelis et al. 2007, Ross et al. 2007, Scott et al. 2007, Shuler et al. 2007a, Wang et al. 2007b). The independent contribution of smoking and *CFH* was a little bit surprising, since smoking is a major environmental risk factor for AMD (Delcourt et al. 1998, Smith et al. 1996, Seddon et al. 2006,), and cigarette smoke has been shown to activate the complement system (Robbins et al. 1991). Smoking also increases serum CRP concentrations (Yanbaeva et al. 2007) and further, an impaired binding of CRP to the CC or CT variants of *CFH* (Y402H) was shown (Skerka et al. 2007).

Surprisingly, a *C3* x sex x smoking interaction was found with the highest OR for a women with the non-risk genotype of *C3* who has smoked over 1 pack-year. C3 plays a crucial role in all the three pathways of the complement system (Walport 2001), is a component of drusen deposits (Hageman et al. 2005), and activation of alternative pathway of complement system by cigarette smoke has been shown to be mediated by C3 (Kew et al. 1985, Kew et al. 1987). Hence, it is reasonable to think that a true *C3* x smoking interaction may exist. However, further studies in different populations are warranted to confirm both the association between *C3* R102G and AMD and the aforementioned gene-environment interaction.

The estimated population attributable risks (PAR) for a carrier of the risk allele of *CFH* Y402H, *LOC387715* A69S, and *C3* R102G were 58.2%, 51.4%, and 5.8%, respectively, and the joint attributable risk for the three loci was 63%. The risks are in agreement with previous studies with the PARs for *CFH* Y402H ranging from 43 to 68 % (Haines et al. 2005, Jakobsdottir et al. 2005, Baird et al. 2006, Conley et al. 2006, Schmidt et al. 2006) and for *LOC387715* A69S ranging from 36 to 57% (Jakobsdottir et al. 2005, Conley et al. 2006, Schmidt et al. 2006). Instead, the PAR for *C3* R102G in Yates et al. was higher (22%) (Yates et al. 2007) than that of ours (5.8%). It was

hypothesized that the effect of *C3* is diluted in our data by the stronger effect of CC genotype in ever-smoking women (*C3* x sex x smoking).

Our knowledge of the genetics of AMD has thus increased considerably in the past few years, but the benefits to clinical practice are still limited. Since no genotype-specific preventive therapy or AMD is available at the moment and it is unknown, whether the genetic profile had a true effect on therapy outcomes, the presymptomatic genetic testing gives no advantage for AMD patients yet. Despite this, it is evident that the risk of AMD of an individual carrying all the three risk alleles (*CFH* Y402H, *LOC387715* A69S and *C3* R102G) is substantial. Although these genetic observations do not unveil the exact mechanisms of AMD, they will certainly help in focusing future cell biological and clinical studies. Most likely, this knowledge will open up avenues for the development of novel therapies for AMD.

7 ACKNOWLEDGEMENTS

This research was carried out in the Department of Ophthalmology, Helsinki University Hospital, and in the Department of Medical Genetics, University of Helsinki.

I express my gratitude to Professor Tero Kivelä who has given me excellent research facilities to carry out this work and has also continuously encouraged me personally. Professor Päivi Peltomäki is also acknowledged for providing well-organized research facilities.

I am forever grateful to my supervisor Docent Ilkka Immonen. When I first met him six years ago I could feel nothing but surprise at his attitude towards me. I had been working only as a general practitioner for years but he did not show any hesitation when he promised to accept me to his research group. Since then, he has with his experienced, but humorous, way supervised me with devotion and by giving me plenty of time, though he has been extremely busy with his clinical work.

My warmest thanks go to another supervisor of mine, Docent Irma Järvelä. In addition to her wide knowledge of the field of molecular genetics she has a great manner of working: all troubles are welcomed and I think she sees them only as nice challenges to be conquered. In this respect she has been a real role model for me. Her feminine support has helped me considerably during these years. Irma has also played a crucial role in helping me to finalize my thesis.

I express special thanks to Professor Seppo Meri, my unofficial third supervisor. His extensive expertise in the field of immunology has been invaluable during these "complement-oriented" years. He has time after time amazed me by being so enthusiastic about the complement system and having always time for educative discussions.

My sincere gratitude are owed to Docent Iiris Sorri and Docent Markku Viander for their evaluation and constructive comments on the thesis.

I wish to thank all my research colleagues in the ophthalmic field. The articles of this thesis would never have been made without the contributions of Petri Tommila, MD, Päivi Ranta, MD, Docent Juha Holopainen, Professor Kai Kaarniranta, Jukka Moilanen, MD, and Tapani Palosaari, MD. With Ranaa Al Jamal, MD, Waldir Neira, MD, Päivi Toivonen, MD, and Seppo Tuomaala, MD, I have shared several unforgettable and educative moments discussing scientific and equally, definitely-not-scientific topics.

I further wish to thank the young geneticists of Irma Järvelä's group who have given me fresh ideas about genetics, and life in general. I would especially like to mention Susanna Lemmelä, MSc, who taught me all the secrets of lab, and thus brought me and genetics practically together. Hanna Komu and Milla Ihalainen are warmly thanked for their assistance in lab work.

Hanna Jarva, MD, and Matti Laine are thanked for their substantial role in the immunologic part of my thesis. They both have adopted the fine style of their supervisor Seppo Meri. Whenever I asked some trivial questions concerning immunology they had time and patience to answer.

Docent Päivi Onkamo and Antti Kotamies, MSc, are thanked for performing statistical analyses. Päivi offered statistical advice not only from a mathematical but also from a philosophical point of view.

Very special thanks go to Marja Ikonen who had the patience to recruit the patients for this study. During these years, we have discussed all topics on earth, from dogs to politics. Sometimes we have done it so loudly that we have been blamed, also for laughing too much.

I also wish to thank my friend Reetta Kivisaari who finished her excellent thesis recently. She has given me several useful tips on how to manage with those numerous details that one faces when finalizing a thesis. Most importantly, she has kindly supported me by listening to my monologues on various issues of life.

I want to thank my mother, Susanna Seitsonen, for her 40-year support of my life. While I have been all too busy during the last months she has helped in practical matters, especially, in preparing meals for the children. I am not sure if the children would have survived without granny.

My deepest gratitude goes to my nearest and dearest, to my children Pauli and Aini-Maija, and to my husband Topi. Pauli and Aini-Maija have experienced the most stressful months while I have been finalizing my thesis. It has definitely hurt my heart when they have asked me to come home, but only a promise of coming back in five hours could have been given. I hope that I can somehow compensate them for those lonely moments. The person who have encouraged me most through all these years, already at the time when I just planned the research work (and before that) is my husband Topi. He has given me total freedom to work, which, however, has not meant that he has taken care of the housework (nobody has done it in our home for the last six months). But as he has never complained about my long working hours he has given me the feeling that I was doing

something that he appreciated. Besides, he had contributed remarkably to my research work by helping non-stop with computers.

I wish to thank all the patients who participated in this study.

This study was financially supported by grants from the the Päivikki and Sakari Sohlberg Foundation, The Mary and Georg Ehrnrooth Foundation, The Eye and Tissue Bank Foundation, The Eye Foundation, The Evald and Hilda Nissi Foundation, The Oskar Öflunds Foundation, The Helsinki University Central Hospital Research Funds (TYH5117, TYH6233), and the National Institutes of Health (EY11515).

Helsinki, April 6th, 2008

Sanna Seitsonen

8 REFERENCES

Abecasis, G.R., Yashar, B.M., Zhao, Y., Ghiasvand, N.M., Zareparsi, S., Branham, K.E., Reddick, A.C., Trager, E.H., Yoshida, S., Bahling, J., Filippova, E., Elner, S., Johnson, M.W., Vine, A.K., Sieving, P.A., Jacobson, S.G., Richards, J.E. & Swaroop, A. 2004, "Age-related macular degeneration: a high-resolution genome scan for susceptibility loci in a population enriched for late-stage disease", *American Journal of Human Genetics,* vol. 74, no. 3, pp. 482-494.

Age-Related Eye Disease Study Research Group 2000, "Risk factors associated with age-related macular degeneration. A case-control study in the age-related eye disease study: Age-Related Eye Disease Study Report Number 3", *Ophthalmology,* vol. 107, no. 12, pp. 2224-2232.

Age-Related Eye Disease Study Research Group 2001, "A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8", *Archives of Ophthalmology,* vol. 119, no. 10, pp. 1417-1436.

Ajjan, R., Grant, P.J., Futers, T.S., Brown, J.M., Cymbalista, C.M., Boothby, M. & Carter, A.M. 2005, "Complement C3 and C-reactive protein levels in patients with stable coronary artery disease", *Thrombosis and haemostasis,* vol. 94, no. 5, pp. 1048-1053.

Allikmets, R., Shroyer, N.F., Singh, N., Seddon, J.M., Lewis, R.A., Bernstein, P.S., Peiffer, A., Zabriskie, N.A., Li, Y., Hutchinson, A., Dean, M., Lupski, J.R. & Leppert, M. 1997, "Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration", *Science (New York, N.Y.),* vol. 277, no. 5333, pp. 1805-1807.

Allikmets, R., Seddon, J.M., Bernstein, P.S., Hutchinson, A., Atkinson, A., Sharma, S., Gerrard, B., Li, W., Metzker, M.L., Wadelius, C., Caskey, C.T., Dean, M. & Petrukhin, K. 1999, "Evaluation of the Best disease gene in patients with age-related macular degeneration and other maculopathies", *Human genetics,* vol. 104, no. 6, pp. 449-453.

Allikmets, R. 2000, "Further evidence for an association of ABCR alleles with age-related macular degeneration. The International ABCR Screening Consortium", *American Journal of Human Genetics,* vol. 67, no. 2, pp. 487-491.

Ambati, J., Ambati, B.K., Yoo, S.H., Ianchulev, S. & Adamis, A.P. 2003, "Age-related macular degeneration: etiology, pathogenesis, and therapeutic strategies", *Survey of ophthalmology,* vol. 48, no. 3, pp. 257-293.

Amos, C.I. 2007, "Successful design and conduct of genome-wide association studies", *Human molecular genetics,* vol. 16 Spec No 2, pp. R220-5.

Anderson, D.H., Mullins, R.F., Hageman, G.S. & Johnson, L.V. 2002, "A role for local inflammation in the formation of drusen in the aging eye", *American Journal of Ophthalmology,* vol. 134, no. 3, pp. 411-431.

Andersson, T., Alfredsson, L., Kallberg, H., Zdravkovic, S. & Ahlbom, A. 2005, "Calculating measures of biological interaction", *European journal of epidemiology,* vol. 20, no. 7, pp. 575-579.

Arias, L., Garcia-Arumi, J., Ramon, J.M., Badia, M., Rubio, M. & Pujol, O. 2006, "Photodynamic therapy with intravitreal triamcinolone in predominantly classic choroidal neovascularization: one-year results of a randomized study", *Ophthalmology,* vol. 113, no. 12, pp. 2243-2250.

Ayyagari, R., Zhang, K., Hutchinson, A., Yu, Z., Swaroop, A., Kakuk, L.E., Seddon, J.M., Bernstein, P.S., Lewis, R.A., Tammur, J., Yang, Z., Li, Y., Zhang, H., Yashar, B.M., Liu, J., Petrukhin, K., Sieving, P.A. & Allikmets, R. 2001, "Evaluation of the ELOVL4 gene in patients with age-related macular degeneration", *Ophthalmic genetics,* vol. 22, no. 4, pp. 233-239.

Baird, P.N., Guida, E., Chu, D.T., Vu, H.T. & Guymer, R.H. 2004, "The epsilon2 and epsilon4 alleles of the apolipoprotein gene are associated with age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 45, no. 5, pp. 1311-1315.

Baird, P.N., Islam, F.M., Richardson, A.J., Cain, M., Hunt, N. & Guymer, R. 2006, "Analysis of the Y402H variant of the complement factor H gene in age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 47, no. 10, pp. 4194-4198.

Baldi, A., De Luca, A., Morini, M., Battista, T., Felsani, A., Baldi, F., Catricala, C., Amantea, A., Noonan, D.M., Albini, A., Natali, P.G., Lombardi, D. & Paggi, M.G. 2002, "The HtrA1 serine protease is down-regulated during human melanoma progression and represses growth of metastatic melanoma cells", *Oncogene,* vol. 21, no. 43, pp. 6684-6688.

Barlow, P.N., Steinkasserer, A., Norman, D.G., Kieffer, B., Wiles, A.P., Sim, R.B. & Campbell, I.D. 1993, "Solution structure of a pair of complement modules by nuclear magnetic resonance", *Journal of Molecular Biology,* vol. 232, no. 1, pp. 268-284.

Barral, S., Francis, P.J., Schultz, D.W., Schain, M.B., Haynes, C., Majewski, J., Ott, J., Acott, T., Weleber, R.G. & Klein, M.L. 2006, "Expanded genome scan in extended families with age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 47, no. 12, pp. 5453-5459.

Beatty, S., Koh, H., Phil, M., Henson, D. & Boulton, M. 2000, "The role of oxidative stress in the pathogenesis of age-related macular degeneration", *Survey of ophthalmology,* vol. 45, no. 2, pp. 115-134.

Bernstein, P.S., Tammur, J., Singh, N., Hutchinson, A., Dixon, M., Pappas, C.M., Zabriskie, N.A., Zhang, K., Petrukhin, K., Leppert, M. & Allikmets, R. 2001, "Diverse macular dystrophy phenotype caused by a novel complex mutation in the ELOVL4 gene", *Investigative ophthalmology & visual science,* vol. 42, no. 13, pp. 3331-3336.

Bird, A.C., Bressler, N.M., Bressler, S.B., Chisholm, I.H., Coscas, G., Davis, M.D., de Jong, P.T., Klaver, C.C., Klein, B.E. & Klein, R. 1995, "An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group", *Survey of ophthalmology,* vol. 39, no. 5, pp. 367-374.

Blackmore, T.K., Sadlon, T.A., Ward, H.M., Lublin, D.M. & Gordon, D.L. 1996, "Identification of a heparin binding domain in the seventh short consensus repeat of complement factor H", *Journal of immunology (Baltimore, Md.: 1950),* vol. 157, no. 12, pp. 5422-5427.

Blackmore, T.K., Fischetti, V.A., Sadlon, T.A., Ward, H.M. & Gordon, D.L. 1998, "M protein of the group A Streptococcus binds to the seventh short consensus repeat of human complement factor H", *Infection and immunity,* vol. 66, no. 4, pp. 1427-1431.

Blinder, K.J., Bradley, S., Bressler, N.M., Bressler, S.B., Donati, G., Hao, Y., Ma, C., Menchini, U., Miller, J., Potter, M.J., Pournaras, C., Reaves, A., Rosenfeld, P.J., Strong, H.A., Stur, M., Su, X.Y., Virgili, G., Treatment of Age-related Macular Degeneration with Photodynamic Therapy study group & Verteporfin in Photodynamic Therapy study group 2003, "Effect of lesion size, visual acuity, and lesion composition on visual acuity change with and without verteporfin therapy for choroidal neovascularization secondary to age-related macular degeneration: TAP and VIP report no. 1", *American Journal of Ophthalmology,* vol. 136, no. 3, pp. 407-418.

Bora, P.S., Sohn, J.H., Cruz, J.M., Jha, P., Nishihori, H., Wang, Y., Kaliappan, S., Kaplan, H.J. & Bora, N.S. 2005, "Role of complement and complement membrane attack complex in laser-induced choroidal neovascularization", *Journal of immunology (Baltimore, Md.: 1950),* vol. 174, no. 1, pp. 491-497.

Bora, N.S., Kaliappan, S., Jha, P., Xu, Q., Sohn, J.H., Dhaulakhandi, D.B., Kaplan, H.J. & Bora, P.S. 2006, "Complement activation via alternative pathway is critical in the development of laser-induced choroidal neovascularization: role of factor B and factor H", *Journal of immunology (Baltimore, Md.: 1950),* vol. 177, no. 3, pp. 1872-1878.

Brantley, M.A.,Jr, Edelstein, S.L., King, J.M., Apte, R.S., Kymes, S.M. & Shiels, A. 2007a, "Clinical phenotypes associated with the complement factor H Y402H variant in age-related macular degeneration", *American Journal of Ophthalmology,* vol. 144, no. 3, pp. 404-408.

Brantley, M.A.,Jr, Fang, A.M., King, J.M., Tewari, A., Kymes, S.M. & Shiels, A. 2007b, "Association of Complement Factor H and LOC387715 Genotypes with Response of Exudative Age-Related Macular Degeneration to Intravitreal Bevacizumab", *Ophthalmology,* vol. 114, no. 12, pp. 2168-2173.

Bressler, N.M. & Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group 2001, "Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2", *Archives of Ophthalmology,* vol. 119, no. 2, pp. 198-207.

Bressler, N.M., Bressler, S.B., Haynes, L.A., Hao, Y., Kaiser, P.K., Miller, J.W., Naor, J., Potter, M.J., Pournaras, C.J., Reaves, A., Rosenfeld, P.J., Schmidt-Erfurth, U., Slakter, J.S., Strong, A. & Vannier, S. 2005, "Verteporfin therapy for subfoveal choroidal neovascularization in age-related macular degeneration: four-year results of an open-label extension of 2 randomized clinical trials: TAP Report No. 7", *Archives of Ophthalmology,* vol. 123, no. 9, pp. 1283-1285.

Brown, D.M., Kaiser, P.K., Michels, M., Soubrane, G., Heier, J.S., Kim, R.Y., Sy, J.P., Schneider, S. & ANCHOR Study Group 2006, "Ranibizumab versus verteporfin for neovascular age-related macular degeneration", *The New England journal of medicine,* vol. 355, no. 14, pp. 1432-1444.

Cameron, D.J., Yang, Z., Gibbs, D., Chen, H., Kaminoh, Y., Jorgensen, A., Zeng, J., Luo, L., Brinton, E., Brinton, G., Brand, J.M., Bernstein, P.S., Zabriskie, N.A., Tang, S., Constantine, R., Tong, Z. & Zhang, K. 2007, "HTRA1 variant confers similar risks to geographic atrophy and neovascular age-related macular degeneration", *Cell cycle (Georgetown, Tex.),* vol. 6, no. 9, pp. 1122-1125.

Chakravarthy, U., Augood, C., Bentham, G.C., de Jong, P.T., Rahu, M., Seland, J., Soubrane, G., Tomazzoli, L., Topouzis, F., Vingerling, J.R., Vioque, J., Young, I.S. & Fletcher, A.E. 2007, "Cigarette smoking and age-related macular degeneration in the EUREYE Study", *Ophthalmology,* vol. 114, no. 6, pp. 1157-1163.

Chakravarti, A. 1999, "Population genetics--making sense out of sequence", *Nature genetics,* vol. 21, no. 1 Suppl, pp. 56-60.

Chen, L.J., Liu, D.T., Tam, P.O., Chan, W.M., Liu, K., Chong, K.K., Lam, D.S. & Pang, C.P. 2006, "Association of complement factor H polymorphisms with exudative age-related macular degeneration", *Molecular vision,* vol. 12, pp. 1536-1542.

Chiu, C.J., Milton, R.C., Gensler, G. & Taylor, A. 2007a, "Association between dietary glycemic index and age-related macular degeneration in nondiabetic participants in the Age-Related Eye Disease Study", *The American Journal of Clinical Nutrition,* vol. 86, no. 1, pp. 180-188.

Chiu, C.J., Milton, R.C., Klein, R., Gensler, G. & Taylor, A. 2007b, "Dietary carbohydrate and the progression of age-related macular degeneration: a prospective study from the Age-Related Eye Disease Study", *The American Journal of Clinical Nutrition,* vol. 86, no. 4, pp. 1210-1218.

Cho, E., Hankinson, S.E., Willett, W.C., Stampfer, M.J., Spiegelman, D., Speizer, F.E., Rimm, E.B. & Seddon, J.M. 2000, "Prospective study of alcohol consumption and the risk of age-related macular degeneration", *Archives of Ophthalmology,* vol. 118, no. 5, pp. 681-688.

Cho, E., Hung, S., Willett, W.C., Spiegelman, D., Rimm, E.B., Seddon, J.M., Colditz, G.A. & Hankinson, S.E. 2001, "Prospective study of dietary fat and the risk of age-related macular degeneration", *The American Journal of Clinical Nutrition,* vol. 73, no. 2, pp. 209-218.

Churchill, A.J., Carter, J.G., Lovell, H.C., Ramsden, C., Turner, S.J., Yeung, A., Escardo, J. & Atan, D. 2006, "VEGF polymorphisms are associated with neovascular age-related macular degeneration", *Human molecular genetics,* vol. 15, no. 19, pp. 2955-2961.

Clemons, T.E., Milton, R.C., Klein, R., Seddon, J.M., Ferris, F.L.,3rd & Age-Related Eye Disease Study Research Group 2005, "Risk factors for the incidence of Advanced Age-Related Macular Degeneration in the Age-Related Eye Disease Study (AREDS) AREDS report no. 19", *Ophthalmology,* vol. 112, no. 4, pp. 533-539.

Cleveland, D.W., Fischer, S.G., Kirschner, M.W. & Laemmli, U.K. 1977, "Peptide mapping by limited proteolysis in sodium dodecyl sulfate and analysis by gel electrophoresis", *The Journal of biological chemistry,* vol. 252, no. 3, pp. 1102-1106.

Coffey, P.J., Gias, C., McDermott, C.J., Lundh, P., Pickering, M.C., Sethi, C., Bird, A., Fitzke, F.W., Maass, A., Chen, L.L., Holder, G.E., Luthert, P.J., Salt, T.E., Moss, S.E. & Greenwood, J. 2007, "Complement factor H deficiency in aged mice causes retinal abnormalities and visual dysfunction", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 104, no. 42, pp. 16651-16656.

Collins, F.S., Guyer, M.S. & Charkravarti, A. 1997, "Variations on a theme: cataloging human DNA sequence variation", *Science (New York, N.Y.),* vol. 278, no. 5343, pp. 1580-1581.

Conley, Y.P., Thalamuthu, A., Jakobsdottir, J., Weeks, D.E., Mah, T., Ferrell, R.E. & Gorin, M.B. 2005, "Candidate gene analysis suggests a role for fatty acid biosynthesis and regulation of the complement system in the etiology of age-related maculopathy", *Human molecular genetics,* vol. 14, no. 14, pp. 1991-2002.

Conley, Y.P., Jakobsdottir, J., Mah, T., Weeks, D.E., Klein, R., Kuller, L., Ferrell, R.E. & Gorin, M.B. 2006, "CFH, ELOVL4, PLEKHA1 and LOC387715 genes and susceptibility to age-related maculopathy: AREDS and CHS cohorts and meta-analyses", *Human molecular genetics,* vol. 15, no. 21, pp. 3206-3218.

Cordell, H.J. 2002, "Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans", *Human molecular genetics,* vol. 11, no. 20, pp. 2463-2468.

Darzins, P., Mitchell, P. & Heller, R.F. 1997, "Sun exposure and age-related macular degeneration. An Australian case-control study", *Ophthalmology,* vol. 104, no. 5, pp. 770-776.

De La Paz, M.A., Pericak-Vance, M.A., Lennon, F., Haines, J.L. & Seddon, J.M. 1997, "Exclusion of TIMP3 as a candidate locus in age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 38, no. 6, pp. 1060-1065.

DeAngelis, M.M., Ji, F., Kim, I.K., Adams, S., Capone, A.,Jr, Ott, J., Miller, J.W. & Dryja, T.P. 2007, "Cigarette smoking, CFH, APOE, ELOVL4, and risk of neovascular age-related macular degeneration", *Archives of Ophthalmology,* vol. 125, no. 1, pp. 49-54.

Delcourt, C., Diaz, J.L., Ponton-Sanchez, A. & Papoz, L. 1998, "Smoking and age-related macular degeneration. The POLA Study. Pathologies Oculaires Liees a l'Age", *Archives of Ophthalmology,* vol. 116, no. 8, pp. 1031-1035.

Delcourt, C., Carriere, I., Ponton-Sanchez, A., Fourrey, S., Lacroux, A., Papoz, L. & POLA Study Group 2001, "Light exposure and the risk of age-related macular degeneration: the Pathologies Oculaires Liees a l'Age (POLA) study", *Archives of Ophthalmology,* vol. 119, no. 10, pp. 1463-1468.

Despriet, D.D., Klaver, C.C., Witteman, J.C., Bergen, A.A., Kardys, I., de Maat, M.P., Boekhoorn, S.S., Vingerling, J.R., Hofman, A., Oostra, B.A., Uitterlinden, A.G., Stijnen, T., van Duijn, C.M. & de Jong, P.T. 2006, "Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration", *JAMA : the journal of the American Medical Association,* vol. 296, no. 3, pp. 301-309.

Dewan, A., Liu, M., Hartman, S., Zhang, S.S., Liu, D.T., Zhao, C., Tam, P.O., Chan, W.M., Lam, D.S., Snyder, M., Barnstable, C., Pang, C.P. & Hoh, J. 2006, "HTRA1 promoter polymorphism in wet age-related macular degeneration", *Science (New York, N.Y.),* vol. 314, no. 5801, pp. 989-992.

Donoso, L.A., Kim, D., Frost, A., Callahan, A. & Hageman, G. 2006, "The role of inflammation in the pathogenesis of age-related macular degeneration", *Survey of ophthalmology,* vol. 51, no. 2, pp. 137-152.

Duan, Y., Mo, J., Klein, R., Scott, I.U., Lin, H.M., Caulfield, J., Patel, M. & Liao, D. 2007, "Age-related macular degeneration is associated with incident myocardial infarction among elderly Americans", *Ophthalmology,* vol. 114, no. 4, pp. 732-737.

Edwards, A.O., Donoso, L.A. & Ritter, R.,3rd 2001, "A novel gene for autosomal dominant Stargardt-like macular dystrophy with homology to the SUR4 protein family", *Investigative ophthalmology & visual science,* vol. 42, no. 11, pp. 2652-2663.

Edwards, A.O., Ritter, R.,3rd, Abel, K.J., Manning, A., Panhuysen, C. & Farrer, L.A. 2005, "Complement factor H polymorphism and age-related macular degeneration", *Science (New York, N.Y.),* vol. 308, no. 5720, pp. 421-424.

Engstrom, G., Hedblad, B., Eriksson, K.F., Janzon, L. & Lindgarde, F. 2005, "Complement C3 is a risk factor for the development of diabetes: a population-based cohort study", *Diabetes,* vol. 54, no. 2, pp. 570-575.

Esparza-Gordillo, J., Soria, J.M., Buil, A., Almasy, L., Blangero, J., Fontcuberta, J. & Rodriguez de Cordoba, S. 2004, "Genetic and environmental factors influencing the human factor H plasma levels", *Immunogenetics,* vol. 56, no. 2, pp. 77-82.

Evans, J.R. 2001, "Risk factors for age-related macular degeneration", *Progress in retinal and eye research,* vol. 20, no. 2, pp. 227-253.

Ferris, F.L.,3rd, Fine, S.L. & Hyman, L. 1984, "Age-related macular degeneration and blindness due to neovascular maculopathy", *Archives of Ophthalmology,* vol. 102, no. 11, pp. 1640-1642.

Fisher, S.A., Abecasis, G.R., Yashar, B.M., Zareparsi, S., Swaroop, A., Iyengar, S.K., Klein, B.E., Klein, R., Lee, K.E., Majewski, J., Schultz, D.W., Klein, M.L., Seddon, J.M., Santangelo, S.L., Weeks, D.E., Conley, Y.P., Mah, T.S., Schmidt, S., Haines, J.L., Pericak-Vance, M.A., Gorin, M.B., Schulz, H.L., Pardi, F., Lewis, C.M. & Weber, B.H. 2005, "Meta-analysis of genome scans of age-related macular degeneration", *Human molecular genetics,* vol. 14, no. 15, pp. 2257-2264.

Francis, P.J., Schultz, D.W., Hamon, S., Ott, J., Weleber, R.G. & Klein, M.L. 2007, "Haplotypes in the Complement Factor H (CFH) Gene: Associations with Drusen and Advanced Age-Related Macular Degeneration", *PLoS ONE,* vol. 2, no. 11, pp. e1197.

Friedman, D.S., Katz, J., Bressler, N.M., Rahmani, B. & Tielsch, J.M. 1999, "Racial differences in the prevalence of age-related macular degeneration: the Baltimore Eye Survey", *Ophthalmology,* vol. 106, no. 6, pp. 1049-1055.

Friedman, D.S., O'Colmain, B.J., Munoz, B., Tomany, S.C., McCarty, C., de Jong, P.T., Nemesure, B., Mitchell, P., Kempen, J. & Eye Diseases Prevalence Research Group 2004, "Prevalence of age-related macular degeneration in the United States", *Archives of Ophthalmology,* vol. 122, no. 4, pp. 564-572.

Fuse, N., Miyazawa, A., Mengkegale, M., Yoshida, M., Wakusawa, R., Abe, T. & Tamai, M. 2006, "Polymorphisms in Complement Factor H and Hemicentin-1 genes in a Japanese population with dry-type age-related macular degeneration", *American Journal of Ophthalmology,* vol. 142, no. 6, pp. 1074-1076.

Gale, C.R., Hall, N.F., Phillips, D.I. & Martyn, C.N. 2003, "Lutein and zeaxanthin status and risk of age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 44, no. 6, pp. 2461-2465.

Gass, J.D. 1973, "Drusen and disciform macular detachment and degeneration", *Archives of Ophthalmology,* vol. 90, no. 3, pp. 206-217.

Gehrs, K.M., Anderson, D.H., Johnson, L.V. & Hageman, G.S. 2006, "Age-related macular degeneration--emerging pathogenetic and therapeutic concepts", *Annals of Medicine,* vol. 38, no. 7, pp. 450-471.

Giclas, P.C., Pinckard, R.N. & Olson, M.S. 1979, "In vitro activation of complement by isolated human heart subcellular membranes", *Journal of immunology (Baltimore, Md.: 1950),* vol. 122, no. 1, pp. 146-151.

Gilicze, A., Kohalmi, B., Pocza, P., Keszei, M., Jaeger, J., Gorbe, E., Papp, Z., Toth, S., Falus, A. & Wiener, Z. 2007, "HtrA1 is a novel mast cell serine protease of mice and men", *Molecular immunology,* vol. 44, no. 11, pp. 2961-2968.

Gold, B., Merriam, J.E., Zernant, J., Hancox, L.S., Taiber, A.J., Gehrs, K., Cramer, K., Neel, J., Bergeron, J., Barile, G.R., Smith, R.T., AMD Genetics Clinical Study Group, Hageman, G.S., Dean, M. & Allikmets, R. 2006, "Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration", *Nature genetics,* vol. 38, no. 4, pp. 458-462.

Gorin, M.B. 2007, "A clinician's view of the molecular genetics of age-related maculopathy", *Archives of Ophthalmology,* vol. 125, no. 1, pp. 21-29.

Gotoh, N., Yamada, R., Hiratani, H., Renault, V., Kuroiwa, S., Monet, M., Toyoda, S., Chida, S., Mandai, M., Otani, A., Yoshimura, N. & Matsuda, F. 2006, "No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese", *Human genetics,* vol. 120, no. 1, pp. 139-143.

Goverdhan, S.V., Howell, M.W., Mullins, R.F., Osmond, C., Hodgkins, P.R., Self, J., Avery, K. & Lotery, A.J. 2005, "Association of HLA class I and class II polymorphisms with age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 46, no. 5, pp. 1726-1734.

Goverdhan, S.V., Lotery, A.J., Cree, A.J. & Ye, S. 2006, "Complement factor H Y402H gene polymorphism in coronary artery disease and atherosclerosis", *Atherosclerosis,* vol. 188, no. 1, pp. 213-214.

Goverdhan, S.V., Hannan, S., Newsom, R.B., Luff, A.J., Griffiths, H. & Lotery, A.J. 2007, "An analysis of the CFH Y402H genotype in AMD patients and controls from the UK, and response to PDT treatment", *Eye (London, England),* 2007 Apr 27 [Epub ahead of print].

Grau, S., Richards, P.J., Kerr, B., Hughes, C., Caterson, B., Williams, A.S., Junker, U., Jones, S.A., Clausen, T. & Ehrmann, M. 2006, "The role of human HtrA1 in arthritic disease", *The Journal of biological chemistry,* vol. 281, no. 10, pp. 6124-6129.

Grizzard, S.W., Arnett, D. & Haag, S.L. 2003, "Twin study of age-related macular degeneration", *Ophthalmic epidemiology,* vol. 10, no. 5, pp. 315-322.

Guymer, R.H., Heon, E., Lotery, A.J., Munier, F.L., Schorderet, D.F., Baird, P.N., McNeil, R.J., Haines, H., Sheffield, V.C. & Stone, E.M. 2001, "Variation of codons 1961 and 2177 of the Stargardt disease gene is not associated with age-related macular degeneration", *Archives of Ophthalmology,* vol. 119, no. 5, pp. 745-751.

Haddad, S., Chen, C.A., Santangelo, S.L. & Seddon, J.M. 2006, "The genetics of age-related macular degeneration: a review of progress to date", *Survey of ophthalmology,* vol. 51, no. 4, pp. 316-363.

Hageman, G.S., Luthert, P.J., Victor Chong, N.H., Johnson, L.V., Anderson, D.H. & Mullins, R.F. 2001, "An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration", *Progress in retinal and eye research,* vol. 20, no. 6, pp. 705-732.

Hageman, G.S., Anderson, D.H., Johnson, L.V., Hancox, L.S., Taiber, A.J., Hardisty, L.I., Hageman, J.L., Stockman, H.A., Borchardt, J.D., Gehrs, K.M., Smith, R.J., Silvestri, G., Russell, S.R., Klaver, C.C., Barbazetto, I., Chang, S., Yannuzzi, L.A., Barile, G.R., Merriam, J.C., Smith, R.T., Olsh, A.K., Bergeron, J., Zernant, J., Merriam, J.E., Gold, B., Dean, M. & Allikmets, R. 2005, "A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, no. 20, pp. 7227-7232.

Hageman, G.S., Hancox, L.S., Taiber, A.J., Gehrs, K.M., Anderson, D.H., Johnson, L.V., Radeke, M.J., Kavanagh, D., Richards, A., Atkinson, J., Meri, S., Bergeron, J., Zernant, J., Merriam, J., Gold, B., Allikmets, R., Dean, M. & AMD Clinical Study Group 2006, "Extended haplotypes in the complement factor H (CFH) and CFH-related (CFHR) family of genes protect against age-related macular degeneration: characterization, ethnic distribution and evolutionary implications", *Annals of Medicine,* vol. 38, no. 8, pp. 592-604.

Haines, J.L., Hauser, M.A., Schmidt, S., Scott, W.K., Olson, L.M., Gallins, P., Spencer, K.L., Kwan, S.Y., Noureddine, M., Gilbert, J.R., Schnetz-Boutaud, N., Agarwal, A., Postel, E.A. & Pericak-Vance, M.A. 2005, "Complement factor H variant increases the risk of age-related macular degeneration", *Science (New York, N.Y.),* vol. 308, no. 5720, pp. 419-421.

Haines, J.L., Schnetz-Boutaud, N., Schmidt, S., Scott, W.K., Agarwal, A., Postel, E.A., Olson, L., Kenealy, S.J., Hauser, M., Gilbert, J.R. & Pericak-Vance, M.A. 2006, "Functional candidate genes in age-related macular degeneration: significant association with VEGF, VLDLR, and LRP6", *Investigative ophthalmology & visual science,* vol. 47, no. 1, pp. 329-335.

Hammond, C.J., Webster, A.R., Snieder, H., Bird, A.C., Gilbert, C.E. & Spector, T.D. 2002, "Genetic influence on early age-related maculopathy: a twin study", *Ophthalmology,* vol. 109, no. 4, pp. 730-736.

Harman, D. 1981, "The aging process", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 78, no. 11, pp. 7124-7128.

Hayashi, M., Merriam, J.E., Klaver, C.C., Zernant, J., Bergen, A.A., Smith, R.T., Chang, S., Merriam, J.C. & Allikmets, R. 2004, "Evaluation of the ARMD1 locus on 1q25-31 in patients with age-related maculopathy: genetic variation in laminin genes and in exon 104 of HEMICENTIN-1", *Ophthalmic genetics,* vol. 25, no. 2, pp. 111-119.

Hellwage, J., Jokiranta, T.S., Friese, M.A., Wolk, T.U., Kampen, E., Zipfel, P.F. & Meri, S. 2002, "Complement C3b/C3d and cell surface polyanions are recognized by overlapping binding sites on the most carboxyl-terminal domain of complement factor H", *Journal of immunology (Baltimore, Md.: 1950),* vol. 169, no. 12, pp. 6935-6944.

Heuberger, R.A., Mares-Perlman, J.A., Klein, R., Klein, B.E., Millen, A.E. & Palta, M. 2001, "Relationship of dietary fat to age-related maculopathy in the Third National Health and Nutrition Examination Survey", *Archives of Ophthalmology,* vol. 119, no. 12, pp. 1833-1838.

Hirvelä, H., Luukinen, H., Laara, E., Sc, L. & Laatikainen, L. 1996, "Risk factors of age-related maculopathy in a population 70 years of age or older", *Ophthalmology,* vol. 103, no. 6, pp. 871-877.

Hogg, R.E., Woodside, J.V., Gilchrist, S.E., Graydon, R., Fletcher, A.E., Chan, W., Knox, A., Cartmill, B. & Chakravarthy, U. 2007, "Cardiovascular Disease and Hypertension Are Strong Risk Factors for Choroidal Neovascularization", *Ophthalmology,* 2007 Oct 20 [Epub ahead of print].

Horstmann, R.D., Sievertsen, H.J., Knobloch, J. & Fischetti, V.A. 1988, "Antiphagocytic activity of streptococcal M protein: selective binding of complement control protein factor H", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 85, no. 5, pp. 1657-1661.

Hughes, A.E., Orr, N., Esfandiary, H., Diaz-Torres, M., Goodship, T. & Chakravarthy, U. 2006, "A common CFH haplotype, with deletion of CFHR1 and CFHR3, is associated with lower risk of age-related macular degeneration", *Nature genetics,* vol. 38, no. 10, pp. 1173-1177.

Hughes, A.E., Orr, N., Patterson, C., Esfandiary, H., Hogg, R., McConnell, V., Silvestri, G. & Chakravarthy, U. 2007, "Neovascular age-related macular degeneration risk based on CFH, LOC387715/HTRA1, and smoking", *PLoS medicine,* vol. 4, no. 12, pp. e355.

Husain, D., Kramer, M., Kenny, A.G., Michaud, N., Flotte, T.J., Gragoudas, E.S. & Miller, J.W. 1999, "Effects of photodynamic therapy using verteporfin on experimental choroidal neovascularization and normal retina and choroid up to 7 weeks after treatment", *Investigative ophthalmology & visual science,* vol. 40, no. 10, pp. 2322-2331.

Hyman, L., Schachat, A.P., He, Q. & Leske, M.C. 2000, "Hypertension, cardiovascular disease, and age-related macular degeneration. Age-Related Macular Degeneration Risk Factors Study Group", *Archives of Ophthalmology,* vol. 118, no. 3, pp. 351-358.

International HapMap Consortium, Frazer, K.A., Ballinger, D.G., Cox, D.R., Hinds, D.A., Stuve, L.L., Gibbs, R.A., Belmont, J.W., Boudreau, A., Hardenbol, P., Leal, S.M., Pasternak, S., Wheeler, D.A., Willis, T.D., Yu, F., Yang, H., Zeng, C., Gao, Y., Hu, H., Hu, W., Li, C., Lin, W., Liu, S., Pan, H., Tang, X., Wang, J., Wang, W., Yu, J., Zhang, B., Zhang, Q., Zhao, H., Zhao, H., Zhou, J., Gabriel, S.B., Barry, R., Blumenstiel, B., Camargo, A., Defelice, M., Faggart, M., Goyette, M., Gupta, S., Moore, J., Nguyen, H., Onofrio, R.C., Parkin, M., Roy, J., Stahl, E., Winchester, E., Ziaugra, L., Altshuler, D., Shen, Y., Yao, Z., Huang, W., Chu, X., He, Y., Jin, L., Liu, Y., Shen, Y., Sun, W., Wang, H., Wang, Y., Wang, Y., Xiong, X., Xu, L., Waye, M.M., Tsui, S.K., Xue, H., Wong, J.T., Galver, L.M., Fan, J.B., Gunderson, K., Murray, S.S., Oliphant, A.R., Chee, M.S., Montpetit, A., Chagnon, F., Ferretti, V., Leboeuf, M., Olivier, J.F., Phillips, M.S., Roumy, S., Sallee, C., Verner, A., Hudson, T.J., Kwok, P.Y., Cai, D., Koboldt, D.C., Miller, R.D., Pawlikowska, L., Taillon-Miller, P., Xiao, M., Tsui, L.C., Mak, W., Song, Y.Q., Tam, P.K., Nakamura, Y., Kawaguchi, T., Kitamoto, T., Morizono, T., Nagashima, A., Ohnishi, Y., Sekine, A., Tanaka, T., Tsunoda, T., Deloukas, P., Bird, C.P., Delgado, M., Dermitzakis, E.T., Gwilliam, R., Hunt, S., Morrison, J., Powell, D., Stranger, B.E., Whittaker, P., Bentley, D.R., Daly, M.J., de Bakker, P.I., Barrett, J., Chretien, Y.R., Maller, J., McCarroll, S., Patterson, N., Pe'er, I., Price, A., Purcell, S., Richter, D.J., Sabeti, P., Saxena, R., Schaffner, S.F., Sham, P.C., Varilly, P., Altshuler, D., Stein, L.D., Krishnan, L., Smith, A.V., Tello-Ruiz, M.K., Thorisson, G.A., Chakravarti, A., Chen, P.E., Cutler, D.J., Kashuk, C.S., Lin, S., Abecasis, G.R., Guan, W., Li, Y., Munro, H.M., Qin, Z.S., Thomas, D.J., McVean, G., Auton, A., Bottolo, L.,

Cardin, N., Eyheramendy, S., Freeman, C., Marchini, J., Myers, S., Spencer, C., Stephens, M., Donnelly, P., Cardon, L.R., Clarke, G., Evans, D.M., Morris, A.P., Weir, B.S., Tsunoda, T., Mullikin, J.C., Sherry, S.T., Feolo, M., Skol, A., Zhang, H., Zeng, C., Zhao, H., Matsuda, I., Fukushima, Y., Macer, D.R., Suda, E., Rotimi, C.N., Adebamowo, C.A., Ajayi, I., Aniagwu, T., Marshall, P.A., Nkwodimmah, C., Royal, C.D., Leppert, M.F., Dixon, M., Peiffer, A., Qiu, R., Kent, A., Kato, K., Niikawa, N., Adewole, I.F., Knoppers, B.M., Foster, M.W., Clayton, E.W., Watkin, J., Gibbs, R.A., Belmont, J.W., Muzny, D., Nazareth, L., Sodergren, E., Weinstock, G.M., Wheeler, D.A., Yakub, I., Gabriel, S.B., Onofrio, R.C., Richter, D.J., Ziaugra, L., Birren, B.W., Daly, M.J., Altshuler, D., Wilson, R.K., Fulton, L.L., Rogers, J., Burton, J., Carter, N.P., Clee, C.M., Griffiths, M., Jones, M.C., McLay, K., Plumb, R.W., Ross, M.T., Sims, S.K., Willey, D.L., Chen, Z., Han, H., Kang, L., Godbout, M., Wallenburg, J.C., L'Archeveque, P., Bellemare, G., Saeki, K., Wang, H., An, D., Fu, H., Li, Q., Wang, Z., Wang, R., Holden, A.L., Brooks, L.D., McEwen, J.E., Guyer, M.S., Wang, V.O., Peterson, J.L., Shi, M., Spiegel, J., Sung, L.M., Zacharia, L.F., Collins, F.S., Kennedy, K., Jamieson, R. & Stewart, J. 2007, "A second generation human haplotype map of over 3.1 million SNPs", *Nature,* vol. 449, no. 7164, pp. 851-861.

Iyengar, S.K., Song, D., Klein, B.E., Klein, R., Schick, J.H., Humphrey, J., Millard, C., Liptak, R., Russo, K., Jun, G., Lee, K.E., Fijal, B. & Elston, R.C. 2004, "Dissection of genomewide-scan data in extended families reveals a major locus and oligogenic susceptibility for age-related macular degeneration", *American Journal of Human Genetics,* vol. 74, no. 1, pp. 20-39.

Jakobsdottir, J., Conley, Y.P., Weeks, D.E., Mah, T.S., Ferrell, R.E. & Gorin, M.B. 2005, "Susceptibility genes for age-related maculopathy on chromosome 10q26", *American Journal of Human Genetics,* vol. 77, no. 3, pp. 389-407.

Jarva, H., Jokiranta, T.S., Hellwage, J., Zipfel, P.F. & Meri, S. 1999, "Regulation of complement activation by C-reactive protein: targeting the complement inhibitory activity of factor H by an interaction with short consensus repeat domains 7 and 8-11", *Journal of immunology (Baltimore, Md.: 1950),* vol. 163, no. 7, pp. 3957-3962.

Johnson, P.T., Betts, K.E., Radeke, M.J., Hageman, G.S., Anderson, D.H. & Johnson, L.V. 2006, "Individuals homozygous for the age-related macular degeneration risk-conferring variant of complement factor H have elevated levels of CRP in the choroid", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 103, no. 46, pp. 17456-17461.

Jokiranta, T.S., Zipfel, P.F., Hakulinen, J., Kuhn, S., Pangburn, M.K., Tamerius, J.D. & Meri, S. 1996, "Analysis of the recognition mechanism of the alternative pathway of complement by monoclonal anti-factor H antibodies: evidence for multiple interactions between H and surface bound C3b", *FEBS letters,* vol. 393, no. 2-3, pp. 297-302.

Jokiranta, T.S., Jaakola, V.P., Lehtinen, M.J., Parepalo, M., Meri, S. & Goldman, A. 2006, "Structure of complement factor H carboxyl-terminus reveals molecular basis of atypical haemolytic uremic syndrome", *The EMBO journal,* vol. 25, no. 8, pp. 1784-1794.

Jozsi, M., Richter, H., Loschmann, I., Skerka, C., Buck, F., Beisiegel, U., Erdei, A. & Zipfel, P.F. 2005, "FHR-4A: a new factor H-related protein is encoded by the human FHR-4 gene", *European journal of human genetics : EJHG,* vol. 13, no. 3, pp. 321-329.

Jozsi, M., Oppermann, M., Lambris, J.D. & Zipfel, P.F. 2007, "The C-terminus of complement factor H is essential for host cell protection", *Molecular immunology,* vol. 44, no. 10, pp. 2697-2706.

Kaare, M, Seitsonen, S, Järvelä, I, Meri, S & Laivuori, H 2007, “Complement factor H variant Y402H is not a risk factor for pre-eclampsia in the Finnish population”, *Hypertension in Pregnancy*, in press.

Kanda, A., Chen, W., Othman, M., Branham, K.E., Brooks, M., Khanna, R., He, S., Lyons, R., Abecasis, G.R. & Swaroop, A. 2007, "A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 104, no. 41, pp. 16227-16232.

Kardys, I., Klaver, C.C., Despriet, D.D., Bergen, A.A., Uitterlinden, A.G., Hofman, A., Oostra, B.A., Van Duijn, C.M., de Jong, P.T. & Witteman, J.C. 2006, "A common polymorphism in the complement factor H gene is associated with increased risk of myocardial infarction: the Rotterdam Study", *Journal of the American College of Cardiology,* vol. 47, no. 8, pp. 1568-1575.

Kaur, I., Hussain, A., Hussain, N., Das, T., Pathangay, A., Mathai, A., Hussain, A., Nutheti, R., Nirmalan, P.K. & Chakrabarti, S. 2006, "Analysis of CFH, TLR4, and APOE polymorphism in India suggests the Tyr402His variant of CFH to be a global marker for age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 47, no. 9, pp. 3729-3735.

Kenealy, S.J., Schmidt, S., Agarwal, A., Postel, E.A., De La Paz, M.A., Pericak-Vance, M.A. & Haines, J.L. 2004, "Linkage analysis for age-related macular degeneration supports a gene on chromosome 10q26", *Molecular vision,* vol. 10, pp. 57-61.

Kew, R.R., Ghebrehiwet, B. & Janoff, A. 1985, "Cigarette smoke can activate the alternative pathway of complement in vitro by modifying the third component of complement", *The Journal of clinical investigation,* vol. 75, no. 3, pp. 1000-1007.

Kew, R.R., Ghebrehiwet, B. & Janoff, A. 1987, "Characterization of the third component of complement (C3) after activation by cigarette smoke", *Clinical immunology and immunopathology,* vol. 44, no. 2, pp. 248-258.

Khan, J.C., Shahid, H., Thurlby, D.A., Bradley, M., Clayton, D.G., Moore, A.T., Bird, A.C., Yates, J.R. & Genetic Factors in AMD Study 2006, "Age related macular degeneration and sun exposure, iris colour, and skin sensitivity to sunlight", *The British journal of ophthalmology,* vol. 90, no. 1, pp. 29-32.

Klaver, C.C., Kliffen, M., van Duijn, C.M., Hofman, A., Cruts, M., Grobbee, D.E., van Broeckhoven, C. & de Jong, P.T. 1998a, "Genetic association of apolipoprotein E with age-related macular degeneration", *American Journal of Human Genetics,* vol. 63, no. 1, pp. 200-206.

Klaver, C.C., Wolfs, R.C., Assink, J.J., van Duijn, C.M., Hofman, A. & de Jong, P.T. 1998b, "Genetic risk of age-related maculopathy. Population-based familial aggregation study", *Archives of Ophthalmology,* vol. 116, no. 12, pp. 1646-1651.

Klein, R., Klein, B.E. & Linton, K.L. 1992, "Prevalence of age-related maculopathy. The Beaver Dam Eye Study", *Ophthalmology,* vol. 99, no. 6, pp. 933-943.

Klein, R., Klein, B.E. & Franke, T. 1993, "The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study", *Ophthalmology,* vol. 100, no. 3, pp. 406-414.

Klein, M.L., Mauldin, W.M. & Stoumbos, V.D. 1994, "Heredity and age-related macular degeneration. Observations in monozygotic twins", *Archives of Ophthalmology,* vol. 112, no. 7, pp. 932-937.

Klein, R., Klein, B.E., Jensen, S.C. & Meuer, S.M. 1997, "The five-year incidence and progression of age-related maculopathy: the Beaver Dam Eye Study", *Ophthalmology,* vol. 104, no. 1, pp. 7-21.

Klein, M.L., Schultz, D.W., Edwards, A., Matise, T.C., Rust, K., Berselli, C.B., Trzupek, K., Weleber, R.G., Ott, J., Wirtz, M.K. & Acott, T.S. 1998, "Age-related macular degeneration. Clinical features in a large family and linkage to chromosome 1q", *Archives of Ophthalmology,* vol. 116, no. 8, pp. 1082-1088.

Klein, R., Klein, B.E. & Cruickshanks, K.J. 1999a, "The prevalence of age-related maculopathy by geographic region and ethnicity", *Progress in retinal and eye research,* vol. 18, no. 3, pp. 371-389.

Klein, R., Clegg, L., Cooper, L.S., Hubbard, L.D., Klein, B.E., King, W.N. & Folsom, A.R. 1999b, "Prevalence of age-related maculopathy in the Atherosclerosis Risk in Communities Study", *Archives of Ophthalmology,* vol. 117, no. 9, pp. 1203-1210.

Klein, R., Klein, B.E., Tomany, S.C., Meuer, S.M. & Huang, G.H. 2002, "Ten-year incidence and progression of age-related maculopathy: The Beaver Dam eye study", *Ophthalmology,* vol. 109, no. 10, pp. 1767-1779.

Klein, R., Klein, B.E., Tomany, S.C. & Cruickshanks, K.J. 2003, "The association of cardiovascular disease with the long-term incidence of age-related maculopathy: the Beaver Dam Eye Study", *Ophthalmology,* vol. 110, no. 6, pp. 1273-1280.

Klein, R.J., Zeiss, C., Chew, E.Y., Tsai, J.Y., Sackler, R.S., Haynes, C., Henning, A.K., SanGiovanni, J.P., Mane, S.M., Mayne, S.T., Bracken, M.B., Ferris, F.L., Ott, J., Barnstable, C. & Hoh, J. 2005, "Complement factor H polymorphism in age-related macular degeneration", *Science (New York, N.Y.),* vol. 308, no. 5720, pp. 385-389.

Klein, R., Klein, B.E., Knudtson, M.D., Meuer, S.M., Swift, M. & Gangnon, R.E. 2007, "Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study", *Ophthalmology,* vol. 114, no. 2, pp. 253-262.

Knudtson, M.D., Klein, R. & Klein, B.E. 2007, "Alcohol consumption and the 15-year cumulative incidence of age-related macular degeneration", *American Journal of Ophthalmology,* vol. 143, no. 6, pp. 1026-1029.

Korbelik, M. 2006, "PDT-associated host response and its role in the therapy outcome", *Lasers in surgery and medicine,* vol. 38, no. 5, pp. 500-508.

Korbelik, M. & Cooper, P.D. 2007, "Potentiation of photodynamic therapy of cancer by complement: the effect of gamma-inulin", *British journal of cancer,* vol. 96, no. 1, pp. 67-72.

Kramer, M., Miller, J.W., Michaud, N., Moulton, R.S., Hasan, T., Flotte, T.J. & Gragoudas, E.S. 1996, "Liposomal benzoporphyrin derivative verteporfin photodynamic therapy. Selective treatment of choroidal neovascularization in monkeys", *Ophthalmology,* vol. 103, no. 3, pp. 427-438.

Laatikainen, L. & Hirvelä, H. 1995, "Prevalence and visual consequences of macular changes in a population aged 70 years and older", *Acta Ophthalmologica Scandinavica,* vol. 73, no. 2, pp. 105-110.

Lander, E. & Kruglyak, L. 1995, "Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results", *Nature genetics,* vol. 11, no. 3, pp. 241-247.

Lau, L.I., Chen, S.J., Cheng, C.Y., Yen, M.Y., Lee, F.L., Lin, M.W., Hsu, W.M. & Wei, Y.H. 2006, "Association of the Y402H polymorphism in complement factor H gene and neovascular age-related macular degeneration in Chinese patients", *Investigative ophthalmology & visual science,* vol. 47, no. 8, pp. 3242-3246.

Li, M., Atmaca-Sonmez, P., Othman, M., Branham, K.E., Khanna, R., Wade, M.S., Li, Y., Liang, L., Zareparsi, S., Swaroop, A. & Abecasis, G.R. 2006, "CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration", *Nature genetics,* vol. 38, no. 9, pp. 1049-1054.

Lu, F., Hu, J., Zhao, P., Lin, Y., Yang, Y., Liu, X., Fan, Y., Chen, B., Liao, S., Du, Q., Lei, C., Cameron, D.J., Zhang, K. & Yang, Z. 2007, "HTRA1 variant increases risk to neovascular age-related macular degeneration in Chinese population", *Vision research,* vol. 47, no. 24, pp. 3120-3123.

Macular Photocoagulation Study Group. 1991a, "Argon laser photocoagulation for neovascular maculopathy. Five-year results from randomized clinical trials. ", *Archives of Ophthalmology,* vol. 109, no. 8, pp. 1109-1114.

Macular Photocoagulation Study Group. 1991b, "Subfoveal neovascular lesions in age-related macular degeneration. Guidelines for evaluation and treatment in the macular photocoagulation study.", *Archives of Ophthalmology,* vol. 109, no. 9, pp. 1242-1257.

Magnusson, K.P., Duan, S., Sigurdsson, H., Petursson, H., Yang, Z., Zhao, Y., Bernstein, P.S., Ge, J., Jonasson, F., Stefansson, E., Helgadottir, G., Zabriskie, N.A., Jonsson, T., Bjornsson, A., Thorlacius, T., Jonsson, P.V., Thorleifsson, G., Kong, A., Stefansson, H., Zhang, K., Stefansson, K. & Gulcher, J.R. 2006, "CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD", *PLoS medicine,* vol. 3, no. 1, pp. e5.

Majewski, J., Schultz, D.W., Weleber, R.G., Schain, M.B., Edwards, A.O., Matise, T.C., Acott, T.S., Ott, J. & Klein, M.L. 2003, "Age-related macular degeneration--a genome scan in extended families", *American Journal of Human Genetics,* vol. 73, no. 3, pp. 540-550.

Maller, J., George, S., Purcell, S., Fagerness, J., Altshuler, D., Daly, M.J. & Seddon, J.M. 2006, "Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration", *Nature genetics,* vol. 38, no. 9, pp. 1055-1059.

Maller, J.B., Fagerness, J.A., Reynolds, R.C., Neale, B.M., Daly, M.J. & Seddon, J.M. 2007, "Variation in complement factor 3 is associated with risk of age-related macular degeneration", *Nature genetics,* vol. 39, no. 10, pp. 1200-1201.

Mandal, M.N. & Ayyagari, R. 2006, "Complement factor H: spatial and temporal expression and localization in the eye", *Investigative ophthalmology & visual science,* vol. 47, no. 9, pp. 4091-4097.

Maugeri, A., Meire, F., Hoyng, C.B., Vink, C., Van Regemorter, N., Karan, G., Yang, Z., Cremers, F.P. & Zhang, K. 2004, "A novel mutation in the ELOVL4 gene causes autosomal dominant Stargardt-like macular dystrophy", *Investigative ophthalmology & visual science,* vol. 45, no. 12, pp. 4263-4267.

McGwin, G., Hall, T.A., Xie, A. & Owsley, C. 2005, "The relation between C reactive protein and age related macular degeneration in the Cardiovascular Health Study", *The British journal of ophthalmology,* vol. 89, no. 9, pp. 1166-1170.

Melrose, M.A., Magargal, L.E. & Lucier, A.C. 1985, "Identical twins with subretinal neovascularization complicating senile macular degeneration", *Ophthalmic surgery,* vol. 16, no. 10, pp. 648-651.

Meri, S. & Pangburn, M.K. 1990, "Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H", *Proceedings of the National Academy of Sciences of the United States of America,* vol. 87, no. 10, pp. 3982-3986.

Meri, S. 2007, "Loss of self-control in the complement system and innate autoreactivity", *Annals of the New York Academy of Sciences,* vol. 1109, pp. 93-105.

Meyers, S.M. & Zachary, A.A. 1988, "Monozygotic twins with age-related macular degeneration", *Archives of Ophthalmology,* vol. 106, no. 5, pp. 651-653.

Meyers, S.M., Greene, T. & Gutman, F.A. 1995, "A twin study of age-related macular degeneration", *American Journal of Ophthalmology,* vol. 120, no. 6, pp. 757-766.

Mitchell, P., Smith, W., Attebo, K. & Wang, J.J. 1995, "Prevalence of age-related maculopathy in Australia. The Blue Mountains Eye Study", *Ophthalmology,* vol. 102, no. 10, pp. 1450-1460.

Mitchell, P., Wang, J.J., Foran, S. & Smith, W. 2002a, "Five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study", *Ophthalmology,* vol. 109, no. 6, pp. 1092-1097.

Mitchell, P., Wang, J.J., Smith, W. & Leeder, S.R. 2002b, "Smoking and the 5-year incidence of age-related maculopathy: the Blue Mountains Eye Study", *Archives of Ophthalmology,* vol. 120, no. 10, pp. 1357-1363.

Mori, K., Gehlbach, P.L., Kabasawa, S., Kawasaki, I., Oosaki, M., Iizuka, H., Katayama, S., Awata, T. & Yoneya, S. 2007a, "Coding and noncoding variants in the CFH gene and cigarette smoking influence the risk of age-related macular degeneration in a Japanese population", *Investigative ophthalmology & visual science,* vol. 48, no. 11, pp. 5315-5319.

Mori, K., Horie-Inoue, K., Kohda, M., Kawasaki, I., Gehlbach, P.L., Awata, T., Yoneya, S., Okazaki, Y. & Inoue, S. 2007b, "Association of the HTRA1 gene variant with age-related macular degeneration in the Japanese population", *Journal of human genetics,* vol. 52, no. 7, pp. 636-641.

Mullins, R.F., Russell, S.R., Anderson, D.H. & Hageman, G.S. 2000, "Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease", *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology,* vol. 14, no. 7, pp. 835-846.

Mullins, R.F., Aptsiauri, N. & Hageman, G.S. 2001, "Structure and composition of drusen associated with glomerulonephritis: implications for the role of complement activation in drusen biogenesis", *Eye (London, England),* vol. 15, no. Pt 3, pp. 390-395.

Narayanan, R., Butani, V., Boyer, D.S., Atilano, S.R., Resende, G.P., Kim, D.S., Chakrabarti, S., Kuppermann, B.D., Khatibi, N., Chwa, M., Nesburn, A.B. & Kenney, M.C. 2007, "Complement factor H polymorphism in age-related macular degeneration", *Ophthalmology,* vol. 114, no. 7, pp. 1327-1331.

Nicaud, V., Francomme, C., Ruidavets, J.B., Luc, G., Arveiler, D., Kee, F., Evans, A., Morrison, C., Blankenberg, S., Cambien, F. & Tiret, L. 2007, "Lack of association between complement factor H polymorphisms and coronary artery disease or myocardial infarction", *Journal of Molecular Medicine (Berlin, Germany),* vol. 85, no. 7, pp. 771-775.

Norio, R. 2003, "Finnish Disease Heritage I: characteristics, causes, background", *Human genetics,* vol. 112, no. 5-6, pp. 441-456.

North, B.V., Curtis, D. & Sham, P.C. 2005, "Application of logistic regression to case-control association studies involving two causative loci", *Human heredity,* vol. 59, no. 2, pp. 79-87.

Obisesan, T.O., Hirsch, R., Kosoko, O., Carlson, L. & Parrott, M. 1998, "Moderate wine consumption is associated with decreased odds of developing age-related macular degeneration in NHANES-1", *Journal of the American Geriatrics Society,* vol. 46, no. 1, pp. 1-7.

Odgen TE, Schachat AP, Ryan SJ. Retina, 2nd ed. St Louis, Mosby-Year Book, Inc, 1994, pp 5-94.

Oka, C., Tsujimoto, R., Kajikawa, M., Koshiba-Takeuchi, K., Ina, J., Yano, M., Tsuchiya, A., Ueta, Y., Soma, A., Kanda, H., Matsumoto, M. & Kawaichi, M. 2004, "HtrA1 serine protease inhibits signaling mediated by Tgfbeta family proteins", *Development (Cambridge, England),* vol. 131, no. 5, pp. 1041-1053.

Okamoto, H., Umeda, S., Obazawa, M., Minami, M., Noda, T., Mizota, A., Honda, M., Tanaka, M., Koyama, R., Takagi, I., Sakamoto, Y., Saito, Y., Miyake, Y. & Iwata, T. 2006, "Complement factor H polymorphisms in Japanese population with age-related macular degeneration", *Molecular vision,* vol. 12, pp. 156-158.

Oksjoki, R., Jarva, H., Kovanen, P.T., Laine, P., Meri, S. & Pentikainen, M.O. 2003, "Association between complement factor H and proteoglycans in early human coronary atherosclerotic lesions: implications for local regulation of complement activation", *Arteriosclerosis, Thrombosis, and Vascular Biology,* vol. 23, no. 4, pp. 630-636.

Pai, J.K., Manson, J.E., Rexrode, K.M., Albert, C.M., Hunter, D.J. & Rimm, E.B. 2007, "Complement factor H (Y402H) polymorphism and risk of coronary heart disease in US men and women", *European heart journal,* vol. 28, no. 11, pp. 1297-1303.

Pieramici, D.J., Bressler, N.M., Bressler, S.B. & Schachat, A.P. 1994, "Choroidal neovascularization in black patients", *Archives of Ophthalmology,* vol. 112, no. 8, pp. 1043-1046.

Postel, E.A., Agarwal, A., Caldwell, J., Gallins, P., Toth, C., Schmidt, S., Scott, W.K., Hauser, M.A., Haines, J.L. & Pericak-Vance, M.A. 2006, "Complement factor H increases risk for atrophic age-related macular degeneration", *Ophthalmology,* vol. 113, no. 9, pp. 1504-1507.

Reich, D.E. & Lander, E.S. 2001, "On the allelic spectrum of human disease", *Trends in genetics : TIG,* vol. 17, no. 9, pp. 502-510.

Rivera, A., White, K., Stohr, H., Steiner, K., Hemmrich, N., Grimm, T., Jurklies, B., Lorenz, B., Scholl, H.P., Apfelstedt-Sylla, E. & Weber, B.H. 2000, "A comprehensive survey of sequence variation in the ABCA4 (ABCR) gene in Stargardt disease and age-related macular degeneration", *American Journal of Human Genetics,* vol. 67, no. 4, pp. 800-813.

Rivera, A., Fisher, S.A., Fritsche, L.G., Keilhauer, C.N., Lichtner, P., Meitinger, T. & Weber, B.H. 2005, "Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk", *Human molecular genetics,* vol. 14, no. 21, pp. 3227-3236.

Robbins, R.A., Nelson, K.J., Gossman, G.L., Koyama, S. & Rennard, S.I. 1991, "Complement activation by cigarette smoke", *The American Journal of Physiology,* vol. 260, no. 4 Pt 1, pp. L254-9.

Rodriguez de Cordoba, S., Esparza-Gordillo, J., Goicoechea de Jorge, E., Lopez-Trascasa, M. & Sanchez-Corral, P. 2004, "The human complement factor H: functional roles, genetic variations and disease associations", *Molecular immunology,* vol. 41, no. 4, pp. 355-367.

Ropers, H.H. 2007, "New perspectives for the elucidation of genetic disorders", *American Journal of Human Genetics,* vol. 81, no. 2, pp. 199-207.

Rosenfeld, P.J., Brown, D.M., Heier, J.S., Boyer, D.S., Kaiser, P.K., Chung, C.Y., Kim, R.Y. & MARINA Study Group 2006, "Ranibizumab for neovascular age-related macular degeneration", *The New England journal of medicine,* vol. 355, no. 14, pp. 1419-1431.

Ross, R.J., Bojanowski, C.M., Wang, J.J., Chew, E.Y., Rochtchina, E., Ferris, F.L.,3rd, Mitchell, P., Chan, C.C. & Tuo, J. 2007, "The LOC387715 polymorphism and age-related macular degeneration: replication in three case-control samples", *Investigative ophthalmology & visual science,* vol. 48, no. 3, pp. 1128-1132.

Rothlein, R. & Springer, T.A. 1985, "Complement receptor type three-dependent degradation of opsonized erythrocytes by mouse macrophages", *Journal of immunology (Baltimore, Md.: 1950),* vol. 135, no. 4, pp. 2668-2672.

SanGiovanni, J.P., Chew, E.Y., Clemons, T.E., Davis, M.D., Ferris, F.L.,3rd, Gensler, G.R., Kurinij, N., Lindblad, A.S., Milton, R.C., Seddon, J.M., Sperduto, R.D. & Age-Related Eye Disease Study Research Group 2007, "The relationship of dietary lipid intake and age-related macular degeneration in a case-control study: AREDS Report No. 20", *Archives of Ophthalmology,* vol. 125, no. 5, pp. 671-679.

Santangelo, S.L., Yen, C.H., Haddad, S., Fagerness, J., Huang, C. & Seddon, J.M. 2005, "A discordant sib-pair linkage analysis of age-related macular degeneration", *Ophthalmic genetics,* vol. 26, no. 2, pp. 61-67.

Schachat, A.P., Hyman, L., Leske, M.C., Connell, A.M. & Wu, S.Y. 1995, "Features of age-related macular degeneration in a black population. The Barbados Eye Study Group", *Archives of Ophthalmology,* vol. 113, no. 6, pp. 728-735.

Schaumberg, D.A., Hankinson, S.E., Guo, Q., Rimm, E. & Hunter, D.J. 2007, "A prospective study of 2 major age-related macular degeneration susceptibility alleles and interactions with modifiable risk factors", *Archives of Ophthalmology,* vol. 125, no. 1, pp. 55-62.

Schick, J.H., Iyengar, S.K., Klein, B.E., Klein, R., Reading, K., Liptak, R., Millard, C., Lee, K.E., Tomany, S.C., Moore, E.L., Fijal, B.A. & Elston, R.C. 2003, "A whole-genome screen of a quantitative trait of age-related maculopathy in sibships from the Beaver Dam Eye Study", *American Journal of Human Genetics,* vol. 72, no. 6, pp. 1412-1424.

Schmidt, S., Klaver, C., Saunders, A., Postel, E., De La Paz, M., Agarwal, A., Small, K., Udar, N., Ong, J., Chalukya, M., Nesburn, A., Kenney, C., Domurath, R., Hogan, M., Mah, T., Conley, Y., Ferrell, R., Weeks, D., de Jong, P.T., van Duijn, C., Haines, J., Pericak-Vance, M. & Gorin, M. 2002, "A pooled case-control study of the apolipoprotein E (APOE) gene in age-related maculopathy", *Ophthalmic genetics,* vol. 23, no. 4, pp. 209-223.

Schmidt, S., Postel, E.A., Agarwal, A., Allen, I.C.,Jr, Walters, S.N., De la Paz, M.A., Scott, W.K., Haines, J.L., Pericak-Vance, M.A. & Gilbert, J.R. 2003, "Detailed analysis of allelic variation in the ABCA4 gene in age-related maculopathy", *Investigative ophthalmology & visual science,* vol. 44, no. 7, pp. 2868-2875.

Schmidt, S., Haines, J.L., Postel, E.A., Agarwal, A., Kwan, S.Y., Gilbert, J.R., Pericak-Vance, M.A. & Scott, W.K. 2005, "Joint effects of smoking history and APOE genotypes in age-related macular degeneration", *Molecular vision,* vol. 11, pp. 941-949.

Schmidt, S., Hauser, M.A., Scott, W.K., Postel, E.A., Agarwal, A., Gallins, P., Wong, F., Chen, Y.S., Spencer, K., Schnetz-Boutaud, N., Haines, J.L. & Pericak-Vance, M.A. 2006, "Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration", *American Journal of Human Genetics,* vol. 78, no. 5, pp. 852-864.

Schmidt-Erfurth, U., Hasan, T., Gragoudas, E., Michaud, N., Flotte, T.J. & Birngruber, R. 1994, "Vascular targeting in photodynamic occlusion of subretinal vessels", *Ophthalmology,* vol. 101, no. 12, pp. 1953-1961.

Schmidt-Erfurth, U. & Hasan, T. 2000, "Mechanisms of action of photodynamic therapy with verteporfin for the treatment of age-related macular degeneration", *Survey of ophthalmology,* vol. 45, no. 3, pp. 195-214.

Schmidt-Erfurth, U., Michels, S., Barbazetto, I. & Laqua, H. 2002, "Photodynamic effects on choroidal neovascularization and physiological choroid", *Investigative ophthalmology & visual science,* vol. 43, no. 3, pp. 830-841.

Schmidt-Erfurth, U.M. & Pruente, C. 2007, "Management of neovascular age-related macular degeneration", *Progress in retinal and eye research,* vol. 26, no. 4, pp. 437-451.

Schultz, D.W., Klein, M.L., Humpert, A., Majewski, J., Schain, M., Weleber, R.G., Ott, J. & Acott, T.S. 2003a, "Lack of an association of apolipoprotein E gene polymorphisms with familial age-related macular degeneration", *Archives of Ophthalmology,* vol. 121, no. 5, pp. 679-683.

Schultz, D.W., Klein, M.L., Humpert, A.J., Luzier, C.W., Persun, V., Schain, M., Mahan, A., Runckel, C., Cassera, M., Vittal, V., Doyle, T.M., Martin, T.M., Weleber, R.G., Francis, P.J. & Acott, T.S. 2003b, "Analysis of the ARMD1 locus: evidence that a mutation in HEMICENTIN-1 is associated with age-related macular degeneration in a large family", *Human molecular genetics,* vol. 12, no. 24, pp. 3315-3323.

Scott, W.K., Schmidt, S., Hauser, M.A., Gallins, P., Schnetz-Boutaud, N., Spencer, K.L., Gilbert, J.R., Agarwal, A., Postel, E.A., Haines, J.L. & Pericak-Vance, M.A. 2007, "Independent effects of complement factor H Y402H polymorphism and cigarette smoking on risk of age-related macular degeneration", *Ophthalmology,* vol. 114, no. 6, pp. 1151-1156.

Seddon, J.M., Ajani, U.A. & Mitchell, B.D. 1997, "Familial aggregation of age-related maculopathy", *American Journal of Ophthalmology,* vol. 123, no. 2, pp. 199-206.

Seddon, J.M., Rosner, B., Sperduto, R.D., Yannuzzi, L., Haller, J.A., Blair, N.P. & Willett, W. 2001, "Dietary fat and risk for advanced age-related macular degeneration", *Archives of Ophthalmology,* vol. 119, no. 8, pp. 1191-1199.

Seddon, J.M., Santangelo, S.L., Book, K., Chong, S. & Cote, J. 2003a, "A genomewide scan for age-related macular degeneration provides evidence for linkage to several chromosomal regions", *American Journal of Human Genetics,* vol. 73, no. 4, pp. 780-790.

Seddon, J.M., Cote, J., Davis, N. & Rosner, B. 2003b, "Progression of age-related macular degeneration: association with body mass index, waist circumference, and waist-hip ratio", *Archives of Ophthalmology,* vol. 121, no. 6, pp. 785-792.

Seddon, J.M., Cote, J. & Rosner, B. 2003c, "Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts, and fish intake", *Archives of Ophthalmology,* vol. 121, no. 12, pp. 1728-1737.

Seddon, J.M., Gensler, G., Milton, R.C., Klein, M.L. & Rifai, N. 2004, "Association between C-reactive protein and age-related macular degeneration", *JAMA : the journal of the American Medical Association,* vol. 291, no. 6, pp. 704-710.

Seddon, J.M., Cote, J., Page, W.F., Aggen, S.H. & Neale, M.C. 2005a, "The US twin study of age-related macular degeneration: relative roles of genetic and environmental influences", *Archives of Ophthalmology,* vol. 123, no. 3, pp. 321-327.

Seddon, J.M., George, S., Rosner, B. & Rifai, N. 2005b, "Progression of age-related macular degeneration: prospective assessment of C-reactive protein, interleukin 6, and other cardiovascular biomarkers", *Archives of Ophthalmology,* vol. 123, no. 6, pp. 774-782.

Seddon, J.M., George, S. & Rosner, B. 2006, "Cigarette smoking, fish consumption, omega-3 fatty acid intake, and associations with age-related macular degeneration: the US Twin Study of Age-Related Macular Degeneration", *Archives of Ophthalmology,* vol. 124, no. 7, pp. 995-1001.

Sepp, T., Khan, J.C., Thurlby, D.A., Shahid, H., Clayton, D.G., Moore, A.T., Bird, A.C. & Yates, J.R. 2006, "Complement factor H variant Y402H is a major risk determinant for geographic atrophy and choroidal neovascularization in smokers and nonsmokers", *Investigative ophthalmology & visual science,* vol. 47, no. 2, pp. 536-540.

Shridhar, V., Sen, A., Chien, J., Staub, J., Avula, R., Kovats, S., Lee, J., Lillie, J. & Smith, D.I. 2002, "Identification of underexpressed genes in early- and late-stage primary ovarian tumors by suppression subtraction hybridization", *Cancer research,* vol. 62, no. 1, pp. 262-270.

Shrive, A.K., Cheetham, G.M., Holden, D., Myles, D.A., Turnell, W.G., Volanakis, J.E., Pepys, M.B., Bloomer, A.C. & Greenhough, T.J. 1996, "Three dimensional structure of human C-reactive protein", *Nature structural biology,* vol. 3, no. 4, pp. 346-354.

Shroyer, N.F., Lewis, R.A., Yatsenko, A.N., Wensel, T.G. & Lupski, J.R. 2001, "Cosegregation and functional analysis of mutant ABCR (ABCA4) alleles in families that manifest both Stargardt disease and age-related macular degeneration", *Human molecular genetics,* vol. 10, no. 23, pp. 2671-2678.

Shuler, R.K.,Jr, Hauser, M.A., Caldwell, J., Gallins, P., Schmidt, S., Scott, W.K., Agarwal, A., Haines, J.L., Pericak-Vance, M.A. & Postel, E.A. 2007a, "Neovascular age-related macular degeneration and its association with LOC387715 and complement factor H polymorphism", *Archives of Ophthalmology,* vol. 125, no. 1, pp. 63-67.

Shuler, R.K.,Jr, Schmidt, S., Gallins, P., Hauser, M.A., Scott, W.K., Caldwell, J., Agarwal, A., Haines, J.L., Pericak-Vance, M.A. & Postel, E.A. 2007b, "Phenotype Analysis of Patients With the Risk Variant LOC387715 (A69S) in Age-related Macular Degeneration", *American Journal of Ophthalmology,* 2008, vol. 145, no. 2, pp. 303-307. Epub 2007 Dec 3.

Simonelli, F., Frisso, G., Testa, F., di Fiore, R., Vitale, D.F., Manitto, M.P., Brancato, R., Rinaldi, E. & Sacchetti, L. 2006, "Polymorphism p.402Y>H in the complement factor H protein is a risk factor for age related macular degeneration in an Italian population", *The British journal of ophthalmology,* vol. 90, no. 9, pp. 1142-1145.

Skerka, C., Lauer, N., Weinberger, A.A., Keilhauer, C.N., Suhnel, J., Smith, R., Schlotzer-Schrehardt, U., Fritsche, L., Heinen, S., Hartmann, A., Weber, B.H. & Zipfel, P.F. 2007, "Defective complement control of factor H (Y402H) and FHL-1 in age-related macular degeneration", *Molecular immunology,* vol. 44, no. 13, pp. 3398-3406.

Smith, W., Mitchell, P. & Leeder, S.R. 1996, "Smoking and age-related maculopathy. The Blue Mountains Eye Study", *Archives of Ophthalmology,* vol. 114, no. 12, pp. 1518-1523.

Smith, W., Mitchell, P. & Leeder, S.R. 2000, "Dietary fat and fish intake and age-related maculopathy", *Archives of Ophthalmology,* vol. 118, no. 3, pp. 401-404.

Smith, W., Assink, J., Klein, R., Mitchell, P., Klaver, C.C., Klein, B.E., Hofman, A., Jensen, S., Wang, J.J. & de Jong, P.T. 2001, "Risk factors for age-related macular degeneration: Pooled findings from three continents", *Ophthalmology,* vol. 108, no. 4, pp. 697-704.

Souied, E.H., Benlian, P., Amouyel, P., Feingold, J., Lagarde, J.P., Munnich, A., Kaplan, J., Coscas, G. & Soubrane, G. 1998, "The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration", *American Journal of Ophthalmology,* vol. 125, no. 3, pp. 353-359.

Souied, E.H., Leveziel, N., Richard, F., Dragon-Durey, M.A., Coscas, G., Soubrane, G., Benlian, P. & Fremeaux-Bacchi, V. 2005, "Y402H complement factor H polymorphism associated with exudative age-related macular degeneration in the French population", *Molecular vision,* vol. 11, pp. 1135-1140.

Spencer, K.L., Hauser, M.A., Olson, L.M., Schmidt, S., Scott, W.K., Gallins, P., Agarwal, A., Postel, E.A., Pericak-Vance, M.A. & Haines, J.L. 2007a, "Protective effect of complement factor B and complement component 2 variants in age-related macular degeneration", *Human molecular genetics,* vol. 16, no. 16, pp. 1986-1992.

Spencer, K.L., Hauser, M.A., Olson, L.M., Schnetz-Boutaud, N., Scott, W.K., Schmidt, S., Gallins, P., Agarwal, A., Postel, E.A., Pericak-Vance, M.A. & Haines, J.L. 2007b, "Haplotypes spanning the complement factor H gene are protective against age-related macular degeneration", *Investigative ophthalmology & visual science,* vol. 48, no. 9, pp. 4277-4283.

Stark, K., Neureuther, K., Sedlacek, K., Hengstenberg, W., Fischer, M., Baessler, A., Wiedmann, S., Jeron, A., Holmer, S., Erdmann, J., Schunkert, H. & Hengstenberg, C. 2007, "The common Y402H variant in complement factor H gene is not associated with susceptibility to myocardial infarction and its related risk factors", *Clinical science (London, England : 1979),* vol. 113, no. 4, pp. 213-218.

Stone, E.M., Braun, T.A., Russell, S.R., Kuehn, M.H., Lotery, A.J., Moore, P.A., Eastman, C.G., Casavant, T.L. & Sheffield, V.C. 2004, "Missense variations in the fibulin 5 gene and age-related macular degeneration", *The New England journal of medicine,* vol. 351, no. 4, pp. 346-353.

Tan, J.S., Mitchell, P., Smith, W. & Wang, J.J. 2007a, "Cardiovascular risk factors and the long-term incidence of age-related macular degeneration: the Blue Mountains Eye Study", *Ophthalmology,* vol. 114, no. 6, pp. 1143-1150.

Tan, J.S., Wang, J.J., Flood, V., Rochtchina, E., Smith, W. & Mitchell, P. 2007b, "Dietary Antioxidants and the Long-term Incidence of Age-Related Macular Degeneration The Blue Mountains Eye Study", *Ophthalmology,* 2008 vol. 115, no. 2, pp. 334-341. Epub 2007 Jul 30.

Tanimoto, S., Tamura, H., Ue, T., Yamane, K., Maruyama, H., Kawakami, H. & Kiuchi, Y. 2007, "A polymorphism of LOC387715 gene is associated with age-related macular degeneration in the Japanese population", *Neuroscience letters,* vol. 414, no. 1, pp. 71-74.

Tedeschi-Blok, N., Buckley, J., Varma, R., Triche, T.J. & Hinton, D.R. 2007, "Population-based study of early age-related macular degeneration: role of the complement factor H Y402H polymorphism in bilateral but not unilateral disease", *Ophthalmology,* vol. 114, no. 1, pp. 99-103.

Thornton, J., Edwards, R., Mitchell, P., Harrison, R.A., Buchan, I. & Kelly, S.P. 2005, "Smoking and age-related macular degeneration: a review of association", *Eye (London, England),* vol. 19, no. 9, pp. 935-944.

Tocharus, J., Tsuchiya, A., Kajikawa, M., Ueta, Y., Oka, C. & Kawaichi, M. 2004, "Developmentally regulated expression of mouse HtrA3 and its role as an inhibitor of TGF-beta signaling", *Development, growth & differentiation,* vol. 46, no. 3, pp. 257-274.

Tomany, S.C., Klein, R. & Klein, B.E. 2001, "The relation of coffee and caffeine to the 5-year incidence of early age-related maculopathy: the Beaver Dam Eye Study", *American Journal of Ophthalmology,* vol. 132, no. 2, pp. 271-273.

Tomany, S.C., Cruickshanks, K.J., Klein, R., Klein, B.E. & Knudtson, M.D. 2004a, "Sunlight and the 10-year incidence of age-related maculopathy: the Beaver Dam Eye Study", *Archives of Ophthalmology,* vol. 122, no. 5, pp. 750-757.

Tomany, S.C., Wang, J.J., Van Leeuwen, R., Klein, R., Mitchell, P., Vingerling, J.R., Klein, B.E., Smith, W. & De Jong, P.T. 2004b, "Risk factors for incident age-related macular degeneration: pooled findings from 3 continents", *Ophthalmology,* vol. 111, no. 7, pp. 1280-1287.

Treatment of age-related macular degeneration with photodynamic therapy (TAP) Study Group, 1999, “Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: one-year results of two randomized clinical trials--TAP Report 1”, *Archives of Ophthalmology,* vol. 117, no. 10, pp. 1329-1345.

Uka, J., Tamura, H., Kobayashi, T., Yamane, K., Kawakami, H., Minamoto, A. & Mishima, H.K. 2006, "No association of complement factor H gene polymorphism and age-related macular degeneration in the Japanese population", *Retina (Philadelphia, Pa.),* vol. 26, no. 9, pp. 985-987.

van Leeuwen, R., Ikram, M.K., Vingerling, J.R., Witteman, J.C., Hofman, A. & de Jong, P.T. 2003a, "Blood pressure, atherosclerosis, and the incidence of age-related maculopathy: the Rotterdam Study", *Investigative ophthalmology & visual science,* vol. 44, no. 9, pp. 3771-3777.

van Leeuwen, R., Klaver, C.C., Vingerling, J.R., Hofman, A. & de Jong, P.T. 2003b, "The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study", *Archives of Ophthalmology,* vol. 121, no. 4, pp. 519-526.

van Leeuwen, R., Boekhoorn, S., Vingerling, J.R., Witteman, J.C., Klaver, C.C., Hofman, A. & de Jong, P.T. 2005, "Dietary intake of antioxidants and risk of age-related macular degeneration", *JAMA : the journal of the American Medical Association,* vol. 294, no. 24, pp. 3101-3107.

van Oostrom, A.J., Alipour, A., Plokker, T.W., Sniderman, A.D. & Cabezas, M.C. 2007, "The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers", *Atherosclerosis,* vol. 190, no. 1, pp. 167-173.

VanNewkirk, M.R., Nanjan, M.B., Wang, J.J., Mitchell, P., Taylor, H.R. & McCarty, C.A. 2000, "The prevalence of age-related maculopathy: the visual impairment project", *Ophthalmology,* vol. 107, no. 8, pp. 1593-1600.

VEGF Inhibition Study in Ocular Neovascularization (V.I.S.I.O.N.) Clinical Trial Group, Chakravarthy, U., Adamis, A.P., Cunningham, E.T.,Jr, Goldbaum, M., Guyer, D.R., Katz, B. & Patel, M. 2006, "Year 2 efficacy results of 2 randomized controlled clinical trials of pegaptanib for neovascular age-related macular degeneration", *Ophthalmology,* vol. 113, no. 9, pp. 1508.e1-1508.25.

Verteporfin In Photodynamic Therapy Study Group 2001, "Verteporfin therapy of subfoveal choroidal neovascularization in age-related macular degeneration: two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularization--verteporfin in photodynamic therapy report 2", *American Journal of Ophthalmology,* vol. 131, no. 5, pp. 541-560.

Vingerling, J.R., Dielemans, I., Hofman, A., Grobbee, D.E., Hijmering, M., Kramer, C.F. & de Jong, P.T. 1995, "The prevalence of age-related maculopathy in the Rotterdam Study", *Ophthalmology,* vol. 102, no. 2, pp. 205-210.

Vingerling, J.R., Hofman, A., Grobbee, D.E. & de Jong, P.T. 1996, "Age-related macular degeneration and smoking. The Rotterdam Study", *Archives of Ophthalmology,* vol. 114, no. 10, pp. 1193-1196.

Walport, M.J. 2001, "Complement. First of two parts", *The New England journal of medicine,* vol. 344, no. 14, pp. 1058-1066.

Wang, J.J., Rochtchina, E., Lee, A.J., Chia, E.M., Smith, W., Cumming, R.G. & Mitchell, P. 2007a, "Ten-year incidence and progression of age-related maculopathy: the blue Mountains Eye Study", *Ophthalmology,* vol. 114, no. 1, pp. 92-98.

Wang, J.J., Ross, R.J., Tuo, J., Burlutsky, G., Tan, A.G., Chan, C.C., Favaloro, E.J., Williams, A. & Mitchell, P. 2007b, "The LOC387715 Polymorphism, Inflammatory Markers, Smoking, and Age-Related Macular Degeneration A Population-Based Case-Control Study", *Ophthalmology,* 2008, vol. 115, no. 4, pp. 693-699. Epub 2007 Aug 2.

Webster, A.R., Heon, E., Lotery, A.J., Vandenburgh, K., Casavant, T.L., Oh, K.T., Beck, G., Fishman, G.A., Lam, B.L., Levin, A., Heckenlively, J.R., Jacobson, S.G., Weleber, R.G., Sheffield, V.C. & Stone, E.M. 2001, "An analysis of allelic variation in the ABCA4 gene", *Investigative ophthalmology & visual science,* vol. 42, no. 6, pp. 1179-1189.

Weeks, D.E., Conley, Y.P., Mah, T.S., Paul, T.O., Morse, L., Ngo-Chang, J., Dailey, J.P., Ferrell, R.E. & Gorin, M.B. 2000, "A full genome scan for age-related maculopathy", *Human molecular genetics,* vol. 9, no. 9, pp. 1329-1349.

Weeks, D.E., Conley, Y.P., Tsai, H.J., Mah, T.S., Rosenfeld, P.J., Paul, T.O., Eller, A.W., Morse, L.S., Dailey, J.P., Ferrell, R.E. & Gorin, M.B. 2001, "Age-related maculopathy: an expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions", *American Journal of Ophthalmology,* vol. 132, no. 5, pp. 682-692.

Weeks, D.E., Conley, Y.P., Tsai, H.J., Mah, T.S., Schmidt, S., Postel, E.A., Agarwal, A., Haines, J.L., Pericak-Vance, M.A., Rosenfeld, P.J., Paul, T.O., Eller, A.W., Morse, L.S., Dailey, J.P., Ferrell, R.E. & Gorin, M.B. 2004, "Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions", *American Journal of Human Genetics,* vol. 75, no. 2, pp. 174-189.

Weger, M., Renner, W., Steinbrugger, I., Kofer, K., Wedrich, A., Groselj-Strele, A., El-Shabrawi, Y., Schmut, O. & Haas, A. 2007, "Association of the HTRA1 -625G>A promoter gene polymorphism with exudative age-related macular degeneration in a Central European population", *Molecular vision,* vol. 13, pp. 1274-1279.

Wegscheider, B.J., Weger, M., Renner, W., Steinbrugger, I., Marz, W., Mossbock, G., Temmel, W., El-Shabrawi, Y., Schmut, O., Jahrbacher, R. & Haas, A. 2007, "Association of complement factor H Y402H gene polymorphism with different subtypes of exudative age-related macular degeneration", *Ophthalmology,* vol. 114, no. 4, pp. 738-742.

West, S.K., Rosenthal, F.S., Bressler, N.M., Bressler, S.B., Munoz, B., Fine, S.L. & Taylor, H.R. 1989, "Exposure to sunlight and other risk factors for age-related macular degeneration", *Archives of Ophthalmology,* vol. 107, no. 6, pp. 875-879.

West, S.K., Munoz, B., Rubin, G.S., Schein, O.D., Bandeen-Roche, K., Zeger, S., German, S. & Fried, L.P. 1997, "Function and visual impairment in a population-based study of older adults. The SEE project. Salisbury Eye Evaluation", *Investigative ophthalmology & visual science,* vol. 38, no. 1, pp. 72-82.

Wong, T.Y., Klein, R., Sun, C., Mitchell, P., Couper, D.J., Lai, H., Hubbard, L.D., Sharrett, A.R. & Atherosclerosis Risk in Communities Study 2006a, "Age-related macular degeneration and risk for stroke", *Annals of Internal Medicine,* vol. 145, no. 2, pp. 98-106.

Wong, T.Y., Shankar, A., Klein, R., Bray, M.S., Couper, D.J., Klein, B.E., Sharrett, A.R. & Folsom, A.R. 2006b, "Apolipoprotein E gene and early age-related maculopathy: the Atherosclerosis Risk in Communities Study", *Ophthalmology,* vol. 113, no. 2, pp. 255-259.

Wong, T.Y., Tikellis, G., Sun, C., Klein, R., Couper, D.J. & Sharrett, A.R. 2007, "Age-related macular degeneration and risk of coronary heart disease: the Atherosclerosis Risk in Communities Study", *Ophthalmology,* vol. 114, no. 1, pp. 86-91.

Wright, S.D. & Silverstein, S.C. 1983, "Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes", *The Journal of experimental medicine,* vol. 158, no. 6, pp. 2016-2023.

Yamamoto, K. & Johnston, R.B.,Jr 1984, "Dissociation of phagocytosis from stimulation of the oxidative metabolic burst in macrophages", *The Journal of experimental medicine,* vol. 159, no. 2, pp. 405-416.

Yanbaeva, D.G., Dentener, M.A., Creutzberg, E.C., Wesseling, G. & Wouters, E.F. 2007, "Systemic effects of smoking", *Chest,* vol. 131, no. 5, pp. 1557-1566.

Yang, Z., Camp, N.J., Sun, H., Tong, Z., Gibbs, D., Cameron, D.J., Chen, H., Zhao, Y., Pearson, E., Li, X., Chien, J., Dewan, A., Harmon, J., Bernstein, P.S., Shridhar, V., Zabriskie, N.A., Hoh, J., Howes, K. & Zhang, K. 2006, "A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration", *Science (New York, N.Y.),* vol. 314, no. 5801, pp. 992-993.

Yates, J.R., Sepp, T., Matharu, B.K., Khan, J.C., Thurlby, D.A., Shahid, H., Clayton, D.G., Hayward, C., Morgan, J., Wright, A.F., Armbrecht, A.M., Dhillon, B., Deary, I.J., Redmond, E., Bird, A.C., Moore, A.T. & Genetic Factors in AMD Study Group 2007, "Complement C3 variant and the risk of age-related macular degeneration", *The New England journal of medicine,* vol. 357, no. 6, pp. 553-561.

Yoshida, T., DeWan, A., Zhang, H., Sakamoto, R., Okamoto, H., Minami, M., Obazawa, M., Mizota, A., Tanaka, M., Saito, Y., Takagi, I., Hoh, J. & Iwata, T. 2007, "HTRA1 promoter polymorphism predisposes Japanese to age-related macular degeneration", *Molecular vision,* vol. 13, pp. 545-548.

Zareparsi, S., Reddick, A.C., Branham, K.E., Moore, K.B., Jessup, L., Thoms, S., Smith-Wheelock, M., Yashar, B.M. & Swaroop, A. 2004, "Association of apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center", *Investigative ophthalmology & visual science,* vol. 45, no. 5, pp. 1306-1310.

Zareparsi, S., Branham, K.E., Li, M., Shah, S., Klein, R.J., Ott, J., Hoh, J., Abecasis, G.R. & Swaroop, A. 2005a, "Strong association of the Y402H variant in complement factor H at 1q32 with susceptibility to age-related macular degeneration", *American Journal of Human Genetics,* vol. 77, no. 1, pp. 149-153.

Zareparsi, S., Buraczynska, M., Branham, K.E., Shah, S., Eng, D., Li, M., Pawar, H., Yashar, B.M., Moroi, S.E., Lichter, P.R., Petty, H.R., Richards, J.E., Abecasis, G.R., Elner, V.M. & Swaroop, A. 2005b, "Toll-like receptor 4 variant D299G is associated with susceptibility to age-related macular degeneration", *Human molecular genetics,* vol. 14, no. 11, pp. 1449-1455.

Zarkadis, I.K., Mastellos, D. & Lambris, J.D. 2001, "Phylogenetic aspects of the complement system", *Developmental and comparative immunology,* vol. 25, no. 8-9, pp. 745-762.

Zee, R.Y., Diehl, K.A. & Ridker, P.M. 2006, "Complement factor H Y402H gene polymorphism, C-reactive protein, and risk of incident myocardial infarction, ischaemic stroke, and venous thromboembolism: a nested case-control study", *Atherosclerosis,* vol. 187, no. 2, pp. 332-335.

Zhang, K., Kniazeva, M., Han, M., Li, W., Yu, Z., Yang, Z., Li, Y., Metzker, M.L., Allikmets, R., Zack, D.J., Kakuk, L.E., Lagali, P.S., Wong, P.W., MacDonald, I.M., Sieving, P.A., Figueroa, D.J., Austin, C.P., Gould, R.J., Ayyagari, R. & Petrukhin, K. 2001, "A 5-bp deletion in ELOVL4 is associated with two related forms of autosomal dominant macular dystrophy", *Nature genetics,* vol. 27, no. 1, pp. 89-93.

Zipfel, P.F., Skerka, C., Hellwage, J., Jokiranta, S.T., Meri, S., Brade, V., Kraiczy, P., Noris, M. & Remuzzi, G. 2002, "Factor H family proteins: on complement, microbes and human diseases", *Biochemical Society transactions,* vol. 30, no. Pt 6, pp. 971-978.