Genetic studies of age-related macular degeneration

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SUMMARY
Age-related macular degeneration (AMD) is a devastating disease of the retina and the most common cause of irreversible visual impairment in the developed world. This degenerative disease has a large impact on the quality of life of affected individuals. AMD has a multifactorial etiology and results from the interplay between numerous genetic susceptibility factors and environmental components. The studies conducted in this thesis explored (new) genes and molecular pathways of retinal aging and disease, based on previously implicated pathobiological processes in order to further unravel the genetic background of AMD.

**PART 1. ASPECTS OF OXIDATIVE STRESS**

In chapter 2 we investigated the role of oxidative stress in AMD. The retina, and especially the retinal pigment epithelium (RPE) are exposed to high levels of oxidative stress: The combination of high oxygen consumption, intense light exposure and the presence of photosensitizers such as lipofuscin, may locally lead to excessive oxidative damage, for example of the DNA. Therefore, the *ERCC6*/AMD manuscript of Tuo and coworkers (2006) caught our attention. This group reported that a single nucleotide polymorphism (SNP; rs3793784) in the promoter region of the *ERCC6* DNA repair gene was associated with AMD. *ERCC6* causes Cockayne syndrome, in which patients age rapidly and, among others, develop retinal degeneration. We assessed the potential genetic association of this gene with AMD. Additionally, we examined a possible functional relationship by determining both rs3793784 dependent as well as normal *ERCC6* mRNA expression levels in healthy and early AMD affected RPE isolated from human donor eyes. Our results showed no positive replication of the reported association between SNPs in *ERCC6* and AMD. Nevertheless, our findings on *ERCC6* expression in the RPE did suggest that *ERCC6* may be functionally involved in AMD. *ERCC6* expression in the human RPE did not depend on rs3793784 genotype, but, interestingly, on AMD status: Early AMD-affected donor eyes had a 50% lower *ERCC6* expression than healthy donor eyes (p=0.018). We hypothesized that the AMD-related reduced *ERCC6* expression may be caused by diverse, small and heterogeneous genetic and/or environmental determinants.

In chapter 3 we tested the role of another gene potentially involved in oxidative stress mediated AMD pathology. The sodium independent glucose transporter *SLC2A1* is the main glucose transporter in the retina. DNA sequence variations or altered expression levels in *SLC2A1* may influence glucose delivery to the retina and thereby profoundly affect local oxidative stress. Based on this hypothesis, we carried out a multicenter cohort association study. Three (rs3754219, rs4660687 and rs841853)
out of 22 SLC2A1 SNPs tested showed a significant allelic and genotypic association with AMD in the AMRO-NL discovery cohort. However, replication of these three SNPs in five independent cohorts yielded inconsistent association results. Meta-analysis revealed no overall association between SLC2A1 and AMD. For all three SNPs, we observed significant heterogeneity of effect and high inconsistency across the study populations. The heterogeneous findings on these three SLC2A1 SNPs provide further evidence for population-dependent genetic risk heterogeneity in AMD.

PART 2. ASPECTS OF THE IMMUNE SYSTEM

In chapter 4 we provide an extensive association analysis to discuss the role of complement component 5 (C5) gene variants in AMD susceptibility. The complement system is part of the innate immune system and plays an important role in the defense against microorganisms. Based on the crucial role of C5 in the complement pathway and the formation of the membrane attack complex, its role as chemoattractant regulating local inflammatory processes, and its localization in drusen, we hypothesized that genetic variants in C5 would mediate AMD susceptibility. We genotyped 15 C5 SNPs in our AMD discovery cohort (AMRO-NL). Significant allelic or genotypic associations between eight C5 SNPs and AMD were found in the AMRO-NL cohort. The strongest allelic associations were found for SNPs rs17611, rs7026551 and rs7037673. Although the complement pathway, including C5, plays a crucial role in AMD, replication testing of these three SNPs could not corroborate an association between these C5 SNPs and AMD. The negative outcome of our replication study may depend on genetic or clinical differences between the study populations.

In chapter 5 we discussed the association of a classical complement pathway member, SERPING1, with AMD. Previously, an association between SERPING1 SNPs, and especially, rs2511989, and AMD was found in 2 UK and USA populations. In view of possible population-based genetic risk heterogeneity in AMD, we retested this potential association, in seven additional large case-control studies involving individuals of European descent (4881 patients with AMD and 2842 matched controls). We found no evidence that this SERPING1 variant plays a role in AMD. Since there is no indication of a functional role for this SNP, it is unlikely that SERPING1 plays a significant role in the etiology of AMD across populations.

In chapter 6 we genotyped toll-like receptor 3 (TLR3) variant, rs3775291, in eight well-known case-control studies involving patients from European descent to investigate the alleged protective association of this gene with geographic atrophy,
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a phenotype of late-stage AMD. TLR3 is known to be involved in the innate immune system. Our studies did not find a significant association and it is therefore unlikely that this TLR3 variant, rs3775291, has a major effect on the risk of AMD.

PART 3. ASPECTS OF THE EXTRACELLULAR MATRIX

In chapter 7 we provided an extended review of the Bruch's membrane (BM). The BM is strategically located between the RPE and the choroidal blood vessels. The BM plays an essential role in maintaining the normal function of the outer retina and is implicated in many retinal disorders such as AMD. Accordingly, we reviewed the molecular, structural and functional properties of BM and attempted to correlate these to recently published mRNA expression profiles of the RPE and choroid. Moreover, we described the formation and composition of drusen and their relation to these gene expression profiles. We showed that during aging, many functional properties of BM change. This may influence not only the normal aging of RPE and photoreceptor cells, but also the onset and/or progression of retinal disorders like retinitis pigmentosa (RP) and AMD.

In chapter 8 we conducted the first European study describing the role of the fibulin 5 (FBLN5) gene in AMD. Fibulin 5 is a secreted extracellular matrix protein with a role in elastinogenesis through interactions with integrin. We determined the functional effects of missense mutations on fibulin 5 expression, correlated the FBLN5 genotype with the AMD phenotype and expanded the retinal phenotypes associated with FBLN5 mutations. Our study identified two novel heterozygous FBLN5 missense mutations and suggested that they lead to decreased fibulin 5 secretion with a possible corresponding reduction in elastinogenesis. Our study extends previous work identifying an association between rare FBLN5 variants and AMD.