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

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Purpose: A strong association of a Tyr402His polymorphism in the complement factor H (CFH) gene and a Met299Val polymorphism in the elongation of very long chain fatty acids-like 4 (ELOVL4) gene with age-related macular degeneration (AMD) has been identified in Caucasian populations in the United States. Earlier a Gln534Arg variant in the hemicentin 1 (HMCN1) gene was reported in a large AMD family in the United States. We wanted to investigate whether the polymorphisms of the CFH and the ELOVL4 genes or the mutation of the HMCN1 gene are associated with AMD in patients originating from the Finnish population with characteristics of a genetic isolate.

Methods: The material consisted of familial (n=181) and sporadic cases (n=154) with AMD, a control group with no AMD (non-AMD controls, n=105), and a control group of anonymous blood donors (blood donor controls, n=350). The DNA of the subjects was sequenced to analyze the variants of the three genes.

Results: We detected a strong association between the C/C-genotype compared to the T/T-genotype of Tyr402His polymorphism (first base of the Tyr-codon changes) of the CFH gene and AMD in the AMD cases compared to the non-AMD (p=8.86x10-12) or to blood donor controls (p=2.02x10-08). The frequency of the C/C genotype was significantly increased in both familial cases compared to non-AMD controls with non-adjusted odds ratio (OR) 10.1 (confidence intervals [CI] 95% 4.64-22.2) or compared to blood donor controls with non-adjusted OR 5.50 (CI 95% 3.17-9.55) and in sporadic cases with non-adjusted OR 9.33 (CI 95% 4.10-21.3; non-AMD-controls), OR 5.06 (CI 95% 2.75-9.28; blood donor controls). Frequency of C allele differed significantly between cases and controls (p=1.32x10-11; non-AMD-controls and p=3.94x10-14; blood donor controls). No association with AMD was detected with Met299Val polymorphism in the ELOVL4 gene in the familial or sporadic cases compared to non-AMD or blood donor controls. None of our subjects (258 AMD cases, 72 non-AMD controls) had the Gln534Arg variant in the HMCN1 gene.

Conclusions: The CFH gene polymorphism seems to be an important etiologic factor for AMD also in the isolated Finnish population.

In the developed world, the leading cause of irreversible visual loss in the elderly is age-related macular degeneration (AMD). It is a disorder of the macular area of the retina characterized by small deposits called drusen under the retina and changes in the retinal pigment epithelium in the early stage. The late stages are central geographic atrophy and exudative macular degeneration, also called wet AMD [1].

Although the disease mechanism is still largely unknown, both genetic and lifestyle factors appear to raise the risk for AMD. Smoking and age are generally accepted risk factors but inflammation, hypertension, and dietary fats have also been shown to associate with increased risk for AMD [2-5].

The genetic background of AMD was suggested by twin studies and family aggregation analysis [6-9]. In several age studies the two chromosomal regions, 1q and 10q, repeatedly showed evidence for linkage, the 1q31 region being most often replicated [10-17]. On chromosome 1q31 in the hemicentin 1 (HMCN1) gene a sequence variant (16263A>G transition in exon 104 producing Gln534Arg change) was identified in a large family with AMD [18], but replication has never again been successful [15,17,19]. In a recent meta-analysis, 10q26 showed the strongest evidence for linkage, while chromosomes 1q, 2p, 3p, and 16 also contained linked loci [20]. Allele 2 of the apolipoprotein E (APOE) gene at chromosome 19q may be associated with an increased risk and allele 4 with a decreased risk for AMD [21-23]. The contribution of the ABCA4 gene to AMD has been controversial but in a small number of patients it may play a role in the pathogenesis of AMD [24].

Three groups have identified the first notable AMD candidate gene, the complement factor H gene (CFH) at 1q32 [25-27]. CFH is one of the complement genes, which constitute the Regulation of Complement Activation (RCA) gene cluster on chromosome 1q32 [28]. In the CFH gene a
The type of the most advanced AMD lesion in the eyes of a study participant

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age mean (range)</th>
<th>Large drusen</th>
<th>Geographic atrophy involving the fovea</th>
<th>Acute exudative or a disciform lesion</th>
<th>Bilateral late AMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporadic AMD</td>
<td>154</td>
<td>75.4 (52.4–88.5)</td>
<td>13.0%</td>
<td>0%</td>
<td>87.0%</td>
<td>33.8%</td>
</tr>
<tr>
<td>Familial AMD</td>
<td>106</td>
<td>76.1 (56.3–92.7)</td>
<td>16.0%</td>
<td>12.3%</td>
<td>71.7%</td>
<td>42.8%</td>
</tr>
<tr>
<td>index patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Familial AMD</td>
<td>75</td>
<td>77.7 (52.9–91.5)</td>
<td>17.3%</td>
<td>13.3%</td>
<td>69.4%</td>
<td>48.6%</td>
</tr>
<tr>
<td>relatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non AMD controls</td>
<td>105</td>
<td>76.9 (66.8–87.7)</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

For the type of the most advanced AMD lesion, the lesions were graded in the order of increasing severity: drusen, 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.
rest of the subjects (index cases, sporadic cases and non-AMD controls) the AMD stage was verified from fundus photographs or angiograms in 341 patients (93.4%), from an examination by a retinal specialist belonging to the study group in four (1.1%), and from medical records of 20 patients (5.5%) of the subjects.

Blood samples for genotyping were obtained from all 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.

*Polymerase chain reaction-sequencing:* DNA was extracted from 10 ml of peripheral blood using a phenol-chlorophorm method. The DNA of the study subjects was amplified by the polymerase chain reaction (PCR) and sequenced (primers available on request). Sequencing was performed using cycle sequencing with the Big Dye Terminator kit (version 3.1) supplied by Applied Biosystems (ABI, Foster City, CA), and reactions were run on an ABI 3730 capillary sequencer according to the manufacturer’s instructions.

**Statistical analysis:** All data analysis was carried out according to a previously established analysis plan, and statistical analyses were conducted using Tixel (version 8.1), which

The genotype and allele frequencies are shown for cases and controls (A). Familial and sporadic age-related macular degeneration (AMD) cases are compared to non-AMD controls (B) and blood donor controls (C) for the Tyr402His polymorphism in the complement factor H (CFH) gene. The p values, odds ratios and confidence intervals (CI) are for the comparison of familial, sporadic, and all (combined analysis of familial and sporadic) AMD cases to non-AMD and blood donor controls within each group. No significant difference exists in the allele or the genotype frequencies of Tyr402His polymorphism between familial and sporadic cases.

The genotype and allele frequencies are shown for cases and controls (A). Familial and sporadic age-related macular degeneration (AMD) cases are compared to non-AMD controls (B) and blood donor controls (C) for the Met299Val polymorphism in the elongation of very long chain fatty acids-like 4 gene. The p values, odds ratios (ORs), and confidence intervals (CI) are for the comparison of familial and sporadic AMD cases to non-AMD and blood donor controls within each group.
RESULTS

We found that the frequency of the C allele of the nonsynonymous SNP 1277T>C (Tyr402His) in exon 9 of the CFH gene was overrepresented in AMD patients in the familial cases compared both to the non-AMD (p=1.18x10^{-10}) or to the blood donor control groups (p=1.85x10^{-11}) and in the sporadic cases compared both to the non-AMD (p=3.01x10^{-9}) or to the blood donor control groups (p=4.21x10^{-8}). No difference in the C allele frequency was detected between familial and sporadic cases (p=0.419). Both familial and sporadic AMD patients were more often homozygous for the C/C-genotype than for the T/T-genotype compared to the non-AMD controls (p=4.83x10^{-10}; familial and p=1.85x10^{-9}; sporadic) or to blood donor controls (p=2.64x10^{-10}; familial and p=4.52x10^{-8}; sporadic) but between familial cases and sporadic cases there was no difference (p=0.814). For homozygotes, combined analysis of familial and sporadic AMD cases showed relative risk of 5.31 CI 95% (3.35-8.40) for AMD compared to blood donor controls and of 9.79 CI 95% (4.76-20.1) compared to non-AMD controls. For heterozygotes the risk was 2.34 CI 95% (1.54-3.57) compared to blood donor controls and of 9.79 CI 95% (4.76-20.1) compared to non-AMD controls (Table 2).

No association of Met299Val polymorphism with AMD was detected in the ELOVL4 gene. No difference in genotype distribution existed in familial cases compared to non-AMD controls (p=0.451) or to blood donor controls (p=0.792) or in sporadic cases compared to non-AMD controls (p=0.593) or to blood donor controls (p=0.168). In allele frequencies no association was found either in familial cases compared to non-AMD controls (p=0.474) or to blood donor controls (p=0.874) or in sporadic cases compared to non-AMD controls (p=0.607) or to blood donor controls (p=0.093; Table 3).

No patient or control carried the Gln5345Arg variant of the HMCN1 gene.

DISCUSSION

Significant association of the Tyr402His variant of the CFH protein was demonstrated in both familial and sporadic AMD patients originating from the isolated Finnish population. The strength of the association for both familial and sporadic cases was of the same range as previously found in the United States population [25]. The high incidence of the C allele of Tyr402His also in the sporadic cases is in agreement with previous studies and further strengthens the role of the Tyr402His change of CFH in the disease pathogenesis [25,32]. It might indicate that in some cases the penetrance of this allele is reduced if not modified by other genetic or environmental factors, since the sporadic cases were not aware of any AMD in their relatives.

In contrast, Met299Val polymorphism in the ELOVL4 gene was not associated with AMD in the Finnish patient material. The studies on the role of the ELOVL4 gene in AMD have been controversial [29,38]. No association was observed between the Met299Val variant and AMD in a sporadic sample (n=513) with a greater proportion of patients with atrophic AMD [38], but a strong association was identified in a study, which was comprised of both sporadic (n=196) and familial (n=796) Caucasian samples with an overrepresentation of exudative AMD [29]. It was assumed that the difference could be explained by the different patient material (the former study with only sporadic cases and overrepresentation of atrophic form of AMD, the latter with both sporadic and familial cases and overrepresentation of exudative form of AMD) [29]. We, however, investigated both familial cases (n=181) and sporadic cases (n=154), who mainly had exudative AMD (Table 1), but still no association was found. This may suggest that the Met299Val polymorphism does not carry a risk in Finnish patients with AMD. On the other hand Conley et al. [29] had a sample size larger than ours. It is possible our patient material was too small to be capable of detecting weaker associations.

Since the Gln5345Arg variant of the HMCN1 gene has been reported in a large American family with AMD [18], we wanted to evaluate it in our AMD patients, but none of them had the variant, which is in agreement with some of the later studies [15,17,19].

Over 30 autosomal recessive disorders have been identified in the 5 million Finnish population that are almost nonexistent in non-Finnish populations [39]. The mutation spectrum underlying monogenic disorders differs significantly from that found in other populations [40]. This has raised the question whether different spectrum of risk alleles would underlie complex diseases like AMD in the Finnish patient material. The AMD-associated CFH risk allele seems, however, to be as prevalent in the Finnish population as in Caucasians populations in the United States. The AMD family group and the sporadic group members had the risk allele C markedly more often than the control groups, as in previous studies [25-27,29,31].

The elderly population is continuing to grow in number, so it is increasingly important to identify AMD’s risk factors in order to learn how to prevent the disease and to discover an effective treatment. CFH as an essential regulator of the complement system, which protects the body against infection and dysplastic cells may have a key role in the AMD pathogenesis. The Tyr402His polymorphism in the CFH protein is in the region that binds heparin [41,42], C-reactive protein (CRP) [43], and group A streptococcal M-protein [44,45]. AMD has been associated with inflammatory factors such as CRP, levels of which are elevated in AMD patients [4,5].

The fact that CFH has a binding site for CRP and regulates the complement system suggests that inflammation plays an important role in the pathogenesis of AMD. CFH protects host cells and tissues from damage by complement activation [46]. This action may be restricted if the polymorphism in the CFH gene changes the protein product and an uncontrolled action of complement cascade is able to damage the cells of
the retina. The tools we now have for a diagnostic test to detect a patient carrying a risk allele in the CFH gene may help future clinicians establish an AMD diagnosis.

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REFERENCES


