Genetic studies of age-related macular degeneration
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Geographic Atrophy in Age-related Macular Degeneration and TLR3 (Letter)

Rando Allikmets, Arthur A. Bergen, Michael Dean, Robyn H. Guymer, Gregory S. Hageman, Caroline C. Klaver, Kari Stefansson, Bernhard H. Weber and the International AMD Genetics Consortium


Yang et al. report an association between the F412L (rs3775291) variant of TLR3 and protection against the development of geographic atrophy (GA), a phenotype of late-stage age-related macular degeneration (AMD). The International Age-related Macular Degeneration Genetics Consortium genotyped rs3775291 in eight well-known case–control studies involving data from a total of 1080 patients of European descent with GA and 2669 matched controls. Data from the eight studies are summarized in Table 1. The studies show, both individually and collectively, neither a significant association nor a trend toward an association between the TLR3 rs3775291 single nucleotide polymorphism (SNP) and protection against GA (P≥0.29 in all cohorts) (Table 1). The difference in the minor allele frequency (MAF) between patients with GA and controls did not exceed 4% in any study and, summarizing the data across studies, the MAF of the rs3775291 SNP was identical in the 2669 controls than in the 1080 patients with GA. Moreover, the MAF of rs3775291 did not differ significantly between any of the GA groups or between any of the control groups, making population stratification an unlikely explanation for the difference between our findings and those of Yang et al. Two other explanations remain. Random variability of this SNP in the general population can result in a chance finding in relatively small cohorts. Alternatively, the difference can be explained by experimental error. We and Yang et al. screened samples from the AREDS cohort [1] and obtained significantly different results. Since there were only 237 subjects with verified GA in the AREDS cohort (in the Coriell Cell Repositories), there should be substantial overlap and concordance between data generated in the two studies. Although the MAF in AREDS controls was very similar in our study and in the study by Yang et al. (0.30 and 0.31, respectively), in AREDS patients with GA, the MAF differed significantly between the two studies (0.28 and 0.21, respectively; P=0.02). The reasons underlying these differences could be resolved by a direct comparison of the genotypes obtained in the AREDS subjects in the two studies. We conclude that it is incorrect to describe TLR3 as being associated with dry AMD and therefore inappropriate to suggest revising therapeutic strategies on the basis of the available data.
Table 1. Genotyping and Association Analysis of the TLR3 Rs3775291 Variant in Eight Cohorts*

<table>
<thead>
<tr>
<th>Value</th>
<th>Columbia</th>
<th>Iowa</th>
<th>Amsterdam</th>
<th>Rotterdam</th>
<th>Germany</th>
<th>Iceland</th>
<th>ARDS</th>
<th>Australia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
<td>GA C</td>
</tr>
<tr>
<td>Total no. of subjects</td>
<td>211 365 102 295 99 264 64 843 184 366 210 169 163 204 57 163 1080 2669</td>
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<td></td>
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<tr>
<td>Genotype (no. of subjects)</td>
<td>CC 105 204 53 152 41 136 28 422 105 191 102 90 82 101 30 70 546 1366</td>
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<tr>
<td></td>
<td>CT</td>
<td>93</td>
<td>133 37 108 40 103 29 341 63 139 96 65 71 82 21 76 450 1047</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>TT</td>
<td>13</td>
<td>28 12 35 8 25 7 80 16 36 12 14 10 21 6 17 84 256</td>
<td></td>
<td></td>
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<tr>
<td>Minor allele (T allele) frequency</td>
<td>0.28 0.26 0.3 0.31 0.29 0.33 0.3 0.26 0.29 0.28 0.28 0.3 0.29 0.33 0.29 0.29</td>
<td></td>
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<tr>
<td>Hardy-Weinberg equilibrium</td>
<td>0.54 0.58 0.36 0.09 0.09 0.67 1 0.63 0.33 0.33 0.24 0.93 0.56 0.8 0.29 0.82</td>
<td></td>
<td></td>
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<tr>
<td>For difference in allele frequency</td>
<td>0.44 0.94 0.53 0.36 0.29 0.29 0.75 0.46 0.35 0.59</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Odds ratio (95% CI)**</td>
<td>1.12 (0.86-1.47)</td>
<td>0.99 (0.70-1.40)</td>
<td>1.12 (0.78-1.63)</td>
<td>1.20 (0.82-1.63)</td>
<td>0.86 (0.65-1.14)</td>
<td>0.89 (0.64-1.22)</td>
<td>1.05 (0.51-1.03)</td>
<td>0.89 (0.51-1.59)</td>
<td>1.03 (0.82-1.29)</td>
</tr>
</tbody>
</table>

*AREDS= Age-related Eye Disease Study; GA= geographic atrophy; CI= confidence interval; C= controls.

**Odds ratios are for the frequency of the TLR3 variant in the geographic atrophy group as compared with the control group.

Table 2. Disease Status, Gender and Age of Subjects in Eight Cohorts.

<table>
<thead>
<tr>
<th>Case-control series</th>
<th>Columbia</th>
<th>Iowa</th>
<th>Amsterdam</th>
<th>Rotterdam</th>
<th>Germany</th>
<th>Iceland</th>
<th>ARDS</th>
<th>Australia</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>209</td>
<td>365</td>
<td>102</td>
<td>295</td>
<td>89</td>
<td>264</td>
<td>64</td>
<td>843</td>
<td>184</td>
</tr>
<tr>
<td>Gender (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>129 (66.5)</td>
<td>201 (55.2)</td>
<td>64 (62.7)</td>
<td>187 (63.4)</td>
<td>54 (61.0)</td>
<td>144 (54.5)</td>
<td>35 (54.7)</td>
<td>314 (57.2)</td>
<td>127 (66.1)</td>
</tr>
<tr>
<td>Male</td>
<td>70 (33.5)</td>
<td>164 (44.8)</td>
<td>38 (37.3)</td>
<td>108 (36.6)</td>
<td>35 (39.0)</td>
<td>120 (45.5)</td>
<td>29 (45.3)</td>
<td>529 (42.8)</td>
<td>65 (33.9)</td>
</tr>
<tr>
<td>Average age (s.d.)</td>
<td>79.6 (8.6)</td>
<td>74.6 (7.1)</td>
<td>76.8 (7.7)</td>
<td>80.9 (8.6)</td>
<td>78.7 (7.6)</td>
<td>748 (6.7)</td>
<td>76.4 (8.3)</td>
<td>77.0 (5.5)</td>
<td>78.5 (6.2)</td>
</tr>
</tbody>
</table>

N= number of study subjects; GA= geographic atrophy; C= controls.
Chapter 6

MATERIALS AND METHODS

Patients

Study cohorts

The consortium included eight international cohorts of patients with GA and matched by age and ethnicity controls, all of which have been extensively characterized and described in previous genetic and epidemiological studies of AMD as follows: from Columbia University, New York, USA [2-4]; University of Iowa, Iowa City, USA [3,4]; AREDS cohort, NEI/NIH, USA [5]; Rotterdam Study, Erasmus University, Rotterdam, The Netherlands [6,7]; The Netherlands Institute for Neurosciences (NIN), Amsterdam, The Netherlands [2,6]; University Eye Clinic, Reykjavik, Iceland [8]; University of Würzburg, Würzburg, Germany [9,10]; and the Center for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital (RVEEH), Melbourne, Australia [11,12]. The details of case-control series are provided in Table 2. All study subjects provided written informed consent before participating. The study was conducted at all sites in strict adherence to the tenets of the Declaration of Helsinki and was approved by the respective Institutional Review Boards at Columbia University and University of Iowa, by the Age-Related Eye Disease Study (AREDS) Access Committee, Data Protection Authorities and National and Institutional Ethics Committees (at Amsterdam, Rotterdam, Reykjavik and Würzburg sites) and according to the National Health and Medical Research Council of Australia’s statement on ethical conduct in research involving humans (revised in 1999) at the Melbourne site.

Grading

All study subjects underwent clinical examination and stereoscopic color fundus photography after pharmacologic mydriasis, and were graded according to the International Classification and Grading System for AMD [13] (Reykjavik, Melbourne and Würzburg sites), Rotterdam modification of the International classification [7,14] (Columbia, Iowa, Amsterdam and Rotterdam sites), or according to the classification established by the AREDS study [1] (for the AREDS cohort). The detailed description of each cohort has been provided in earlier publications, as described in the previous section. In summary, cases with GA (disease stage 4 according to all classifications) were defined as presenting a well-defined area of atrophy of the retinal pigment epithelium (RPE) and choriocapillaris with diameter >= 175 μm in one (more severely affected) eye and early stage (soft drusen and/or pigmentary abnormalities) AMD in the other eye, or with distinct bilateral GA. Patients with atrophy due to other causes, such as RPE scarring and post choroidal...
neovascularization (CNV)-associated degeneration were excluded from the GA category, as were patients with mixed (GA and CNV) phenotype in the same eye or in contralateral eyes. The average age of subjects with GA was ~75 years in all cohorts. Subjects were graded as ‘unaffected controls’ only when fundus photographs showed absolutely no signs of AMD; i.e., they had clear fundi at >60 years of age or <5 small hard drusen (grade 1 by AREDS classification or grade 0 by International/Rotterdam classification). In some cohorts the selection criteria for controls were made even more stringent by either raising the age threshold (>70 in the Rotterdam Study and >90 in the Iceland cohort), or by extending the follow-up period to several years (Rotterdam Study).

Genotyping
The rs3775291 SNP was genotyped by the Applied Biosystems (Foster City, CA) Taqman® 5’ nucleotidase assay (Columbia, AREDS, Würzburg and Amsterdam cohorts), by the Illumina HumanHap arrays (Iceland and Rotterdam Study cohorts) or, in Iowa and Melbourne cohorts, by a combination of SSCP, direct sequencing and Molecular Inversion Probe Genotyping (ParAllele Biosciences/Affymetrix).

Statistical analysis
Genotypes were tabulated in Microsoft Excel and presented to SPSS for contingency table analysis as described previously [3,4]. Compliance to Hardy-Weinberg equilibrium was checked using SAS/Genetics (SAS Institute), and all cohorts in both cases and controls survived a cutoff of P>0.05. For the combined analysis of all studies we calculated the weighted Mantel-Haenszel estimate for the odds ratio, and obtained similarly non-significant values.

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Point Charitable Foundation (RA); an unrestricted grant to the Department of Ophthalmology, Columbia University, from Research to Prevent Blindness; German Research Foundation (DFG) WE1259/18-1 and WE1259/19-1, the Ruth and Milton Steinbach Foundation (BHW, RA), the Alcon Research Institute (BHW, RA), the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO; nr. 050-060-810); NWO Investments (nr. 175.010.2005.011, 911-03 012); Research Institute for Diseases in the Elderly (RIDE2; 014-93-015); Netherlands Organization for the Health Research and Development (ZonMw); Ministry of Education, Culture and Science, Ministry for Health, Welfare and Sports, European Commision (DG XII), Municipality of Rotterdam; the Netherlands Macula Society, Netherlands National Foundation for the Blind (LSBS), the Netherlands National Society for Prevention of Blindness (ANVVB), National Health and Medical Research Council, Australia, Career development fellowship (RHG), J A COM Foundation (PNB).
REFERENCES