

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Allikmets R, Bergen AA, Dean M, et al. Geographic atrophy in age-related macular degeneration and TLR3. *N Engl J Med* 2009;360:2252-4.

## **Supplementary Appendix**

### **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<sup>1-3</sup>; University of Iowa, Iowa City, USA<sup>2, 3</sup>; AREDS cohort, NEI/NIH, USA<sup>4</sup>; Rotterdam Study, Erasmus University, Rotterdam, The Netherlands<sup>5, 6</sup>; The Netherlands Institute for Neurosciences (NIN), Amsterdam, The Netherlands<sup>1, 5</sup>; University Eye Clinic, Reykjavik, Iceland<sup>7</sup>; University of Würzburg, Würzburg, Germany<sup>8, 9</sup>; and the Center for Eye Research Australia, University of Melbourne, Royal Victorian Eye and Ear Hospital (RVEEH), Melbourne, Australia<sup>10, 11</sup>. The details of case-control series are provided in Table 1. 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<sup>12</sup> (Reykjavik, Melbourne and Würzburg sites), Rotterdam modification of the International classification<sup>6, 13</sup> (Columbia, Iowa, Amsterdam and Rotterdam sites), or according to the classification established by the AREDS study<sup>14</sup> (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 geographic atrophy (disease stage 4 according to all classifications) were defined as presenting a well-defined area of atrophy of the RPE and choriocapillaris with diameter  $\geq 175 \mu\text{m}$  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  $\sim 75$  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<sup>2, 3</sup>. 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.

## **International AMD Genetics Consortium members:**

Joanna E. Merriam, M.D., Ph.D.<sup>1</sup>, R. Theodore Smith, M.D., Ph.D.<sup>1</sup>, Gaetano R. Barile, M.D.<sup>1</sup>, Karen M. Gehrs, M.D.<sup>2</sup>, Lisa S. Hancox, B.A.<sup>2</sup>, Norma J. Miller, B.A.<sup>2</sup>, and Mary L. Howard, B.A.<sup>2</sup>, Paulus T. de Jong, M.D.<sup>3</sup>, Dominique Baas, Ph.D.<sup>4</sup>, Lintje Ho, M.D.<sup>5</sup>, Johannes R Vingerling, M.D., Ph.D.<sup>5</sup>, Andre G Uitterlinden, M.D., Ph.D.<sup>5</sup>, Lars G. Fritsche, M.Sc.<sup>6</sup>, Claudia N. Keilhauer, M.D.<sup>7</sup>, Paul N. Baird, Ph.D.<sup>8</sup>, Julie Sawitzke, M.A.<sup>9</sup>, Kristinn P. Magnusson, Ph.D.<sup>10</sup>, Gudmar Thorleifsson, Ph.D.<sup>11</sup>, Unnur Thorsteinsdottir, Ph.D.<sup>11</sup>

### **Affiliations for International AMD Genetics Consortium members:**

<sup>1</sup>Columbia University, New York, NY 10032, USA; ; <sup>2</sup>University of Iowa, Iowa City, IA 52240, USA; <sup>3</sup>Academic Medical Center (AMC), 1105 AZ Amsterdam, The Netherlands; <sup>4</sup>The Netherlands Institute for Neurosciences (NIN-KNAW), 1105 BA Amsterdam, The Netherlands; <sup>5</sup>Erasmus Medical Center, 3000 CA Rotterdam, The Netherlands; <sup>6</sup>University of Regensburg, D-93053 Regensburg, Germany; <sup>7</sup>University of Wuerzburg, D-97074 Wuerzburg, Germany; <sup>8</sup>Center for Eye Research Australia, Melbourne 3002, Australia; <sup>9</sup>National Cancer Institute, Frederick, MD 21702, USA; <sup>10</sup>University of Akureyri, IS-600 Akureyri, Iceland; <sup>11</sup>deCODE Genetics, IS-101 Reykjavik, Iceland.

### **Acknowledgements**

The authors thank Sarah Schlotterbeck, Kendal Lemon, Melinda Cain, Andrea Richardson, Pascal Arp, Mila Jhamai, Dr Fernando Rivadeneira, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for technical assistance and Haraldur Sigurdsson and Fridbert Jonasson for phenotyping. Supported in part by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research and SAIC-Frederick under contract #NO1-CO-12400 and with grants from the National Eye Institute EY13435 (RA) and EY017404 (RA, GSH); the Macula Vision Research Foundation (RA); Wallach Foundation (RA); Elyachar Foundation (RA); Kaplen Foundation (RA); Wigdeon 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 Commission (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

1. Despriet DD, Bergen AA, Merriam JE, et al. Comprehensive analysis of the candidate genes CCL2, CCR2, and TLR4 in age-related macular degeneration. *Investigative ophthalmology & visual science* 2008;49(1):364-71.
2. Gold B, Merriam JE, Zernant J, et al. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nature genetics* 2006;38(4):458-62.
3. Hageman GS, Anderson DH, Johnson LV, et al. 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* 2005;102(20):7227-32.
4. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. *Archives of ophthalmology* 2001;119(10):1439-52.
5. Despriet DD, Klaver CC, Witteman JC, et al. Complement factor H polymorphism, complement activators, and risk of age-related macular degeneration. *Jama* 2006;296(3):301-9.
6. van Leeuwen R, Klaver CC, Vingerling JR, Hofman A, de Jong PT. The risk and natural course of age-related maculopathy: follow-up at 6 1/2 years in the Rotterdam study. *Archives of ophthalmology* 2003;121(4):519-26.

7. Magnusson KP, Duan S, Sigurdsson H, et al. CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS medicine* 2006;3(1):e5.
8. Fritsche LG, Loenhardt T, Janssen A, et al. Age-related macular degeneration is associated with an unstable ARMS2 (LOC387715) mRNA. *Nature genetics* 2008;40(7):892-6.
9. Rivera A, Fisher SA, Fritsche LG, et al. 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* 2005;14(21):3227-36.
10. Baird PN, Islam FM, Richardson AJ, Cain M, Hunt N, Guymer R. Analysis of the Y402H variant of the complement factor H gene in age-related macular degeneration. *Investigative ophthalmology & visual science* 2006;47(10):4194-8.
11. Richardson AJ, Islam FA, Guymer RH, Baird PN. Analysis of rare variants in the complement component 2 (C2) and Factor B (BF) genes refine association for age-related macular degeneration (AMD). *Investigative ophthalmology & visual science* 2008.
12. Bird AC, Bressler NM, Bressler SB, et al. An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Survey of ophthalmology* 1995;39(5):367-74.
13. Klaver CC, Assink JJ, van Leeuwen R, et al. Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Investigative ophthalmology & visual science* 2001;42(10):2237-41.
14. The Age-Related Eye Disease Study (AREDS): design implications. AREDS report no. 1. *Controlled clinical trials* 1999;20(6):573-600.

Table 1. Disease status, gender and age of subjects in eight cohorts.

Case-control series	Columbia		Iowa		Amsterdam		Rotterdam		Germany		Iceland		AREDS		Australia	
	GA	C	GA	C	GA	C	GA	C	GA	C	GA	C	GA	C	GA	C
<b>N</b>	209	365	102	295	89	264	64	843	192	384	210	169	163	204	54	163
<b>Gender (%)</b>																
<b>Female</b>	139 (66.5)	201 (55.2)	64 (62.7)	187 (63.4)	54 (61.0)	144 (54.5)	35 (54.7)	314 (37.2)	127 (66.1)	240 (62.5)	69 (32.9)	96 (56.8)	93 (57.1)	108 (52.9)	35 (64.8)	95 (52.3)
<b>Male</b>	70 (33.5)	164 (44.8)	38 (37.3)	108 (36.6)	35 (39.0)	120 (45.5)	29 (45.3)	529 (62.8)	65 (33.9)	144 (37.5)	141 (67.1)	73 (43.2)	70 (42.9)	96 (47.1)	19 (35.2)	68 (41.7)
<b>Average Age (s.d.)</b>	79.6 (8.6)	74.6 (7.1)	76.8 (7.7)	80.9 (8.6)	78.7 (7.6)	74.8 (6.7)	76.4 (8.3)	77.0 (5.5)	78.5 (6.2)	78.8 (5.4)	84.4 (7.4)	92.6 (2.0)	79.5 (5.3)	76.9 (4.6)	70.5 (9.8)	70.9 (9.8)

N, number of study subjects; GA, geographic atrophy; C, controls. In AREDS cohort the average age is given from the last documented photograph, the enrolment ages are younger for about a decade.