A Variant of the HTRA1 Gene Increases Susceptibility to Age-Related Macular Degeneration

Zhenglin Yang, *et al.*

Science **314**, 992 (2006);
DOI: 10.1126/science.1133811

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005.

the AMD phenotype: *CFH* influences the drusen that characterize dry AMD, whereas *HTRA1* influences CNV, the hallmark of the wet disease type. These two processes can be combined, which leads to the composite phenotypes that are seen in some cases of AMD.

References and Notes

1. G. S. Hageman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7227 (2005).
2. R. J. Klein *et al.*, *Science* **308**, 385 (2005).
3. E. A. Postel *et al.*, *Ophthalmology* **113**, 1504 (2006).
4. J. Maller *et al.*, *Nat. Genet.* **38**, 1055 (2006).
5. S. Haddad, C. A. Chen, S. L. Santangelo, J. M. Seddon, *Surv. Ophthalmol.* **51**, 316 (2006).
6. M. Li *et al.*, *Nat. Genet.* **38**, 1049 (2006).
7. A. Thakkinstian *et al.*, *Hum. Mol. Genet.* **15**, 2784 (2006).
8. A. C. Bird, *Eye* **17**, 457 (2003).
9. R. Klein *et al.*, *Ophthalmology* **113**, 373 (2006).
10. T. S. Chang, D. Hay, P. Courtright, *Can. J. Ophthalmol.* **34**, 266 (1999).
11. M. A. Sandberg, A. Weiner, S. Miller, A. R. Gaudio, *Ophthalmology* **105**, 441 (1998).
12. M. Uyama *et al.*, *Br. J. Ophthalmol.* **84**, 1018 (2000).
13. M. Yuzawa, K. Hagita, T. Egawa, H. Minato, M. Matsui, *Jpn. J. Ophthalmol.* **35**, 87 (1991).
14. N. Gotoh *et al.*, *Hum. Genet.* **120**, 139 (2006).
15. H. Okamoto *et al.*, *Mol. Vis.* **12**, 156 (2006).
16. M. A. Grassi *et al.*, *Hum. Mutat.* **27**, 921 (2006).
17. L. Baum *et al.*, *Ophthalmologica* **217**, 111 (2003).
18. C. P. Pang *et al.*, *Ophthalmologica* **214**, 289 (2000).
19. Materials and methods are available online as supporting material on *Science* Online.
20. A. Rivera *et al.*, *Hum. Mol. Genet.* **14**, 3227 (2005).
21. J. Jakobsdottir *et al.*, *Am. J. Hum. Genet.* **77**, 389 (2005).
22. Z. Yang *et al.*, *Science* **314**, 992 (2006).
23. T. Clausen, C. Southan, M. Ehrmann, *Mol. Cell* **10**, 443 (2002).
24. J. Tocharus *et al.*, *Dev. Growth Differ.* **46**, 257 (2004).
25. D. H. Ly, D. J. Lockhart, R. A. Lerner, P. G. Schultz, *Science* **287**, 2486 (2000).

26. We express our greatest appreciation to the patients, the control subjects, and their families for participating in this study; and we thank S. Chiang and B. Zhang for technical and logistic assistance. Supported in part by the Lim Por Yen Eye Foundation (D.L., C.P.), the David Woods Kemper Memorial Foundation (C.B.), Macular Vision Research Foundation (C.B., J.H.), the Ellison Medical Foundation (J.H.), an institutional award from the Howard Hughes Medical Institute to the Yale School of Medicine (J.H.), and grants from the Claude D. Pepper Older Americans Independence Center at Yale University School of Medicine (J.H.), the NIH (M.S., C.B., J.H.), and the Verto Institute (J.H.). *HTRA1* sequencing data are available on http://variation.yale.edu/dataDownload.html.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1133807/DC1
Materials and Methods
Figs. S1 to S7
Tables S1 to S8
References and Notes

14 August 2006; accepted 5 October 2006
Published online 19 October 2006;
10.1126/science.1133807
Include this information when citing this paper.

A Variant of the *HTRA1* Gene Increases Susceptibility to Age-Related Macular Degeneration

Zhenglin Yang,[1,2,3]* Nicola J. Camp,[4]* Hui Sun,[5] Zongzhong Tong,[1,2] Daniel Gibbs,[1,2] D. Joshua Cameron,[1,2] Haoyu Chen,[1,2] Yu Zhao,[1,2] Erik Pearson,[1,2] Xi Li,[1,2] Jeremy Chien,[6] Andrew DeWan,[7] Jennifer Harmon,[1,2] Paul S. Bernstein,[1] Viji Shridhar,[6] Norman A. Zabriskie,[1] Josephine Hoh,[7] Kimberly Howes,[1] Kang Zhang[1,2]†

Age-related macular degeneration (AMD) is the most common cause of irreversible vision loss in the developed world and has a strong genetic predisposition. A locus at human chromosome 10q26 affects the risk of AMD, but the precise gene(s) have not been identified. We genotyped 581 AMD cases and 309 normal controls in a Caucasian cohort in Utah. We demonstrate that a single-nucleotide polymorphism, rs11200638, in the promoter region of *HTRA1* is the most likely causal variant for AMD at 10q26 and is estimated to confer a population attributable risk of 49.3%. The *HTRA1* gene encodes a secreted serine protease. Preliminary analysis of lymphocytes and retinal pigment epithelium from four AMD patients revealed that the risk allele was associated with elevated expression levels of *HTRA1* mRNA and protein. We also found that drusen in the eyes of AMD patients were strongly immunolabeled with HTRA1 antibody. Together, these findings support a key role for HTRA1 in AMD susceptibility and identify a potential new pathway for AMD pathogenesis.

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in developed countries, affecting 10 million people worldwide (*1*, *2*). Early AMD is characterized by the presence of soft drusen in the macula without vision loss. Advanced AMD is associated with vision loss due to either geographic atrophy of retinal pigment epithelium (RPE) and photoreceptors (GA or dry AMD) or neovascular choriocapillary invasion across Bruch's membrane into RPE and photoreceptor layers (wet AMD). The prevalence of AMD is rising as a result of increasing life expectancy, but the etiology remains poorly understood. Both genetic predisposition (*3*) and environmental factors such as smoking (*4*) play an important role in AMD pathogenesis.

Previous work has shown that allelic variants of genes encoding members of the alternative complement pathway, including complement factor H (CFH) (*5*–*9*) and factor B/C2, affect an individual's risk of developing AMD (*10*). In particular, variants of the *CFH* gene on chromosome 1q31 confer a major risk for AMD (*5*–*9*, *11*, *12*). Several independent association studies have implicated a second major locus for AMD at chromosome 10q26 (*12*–*16*). To identify the critical gene at this locus, we genotyped 442 AMD cases and 309 controls in a Caucasian cohort in Utah, using a panel of 15 single-nucleotide polymorphisms (SNPs) centered around the highest risk associated SNP, rs10490924. In agreement with previous reports, rs10490924 was found to have a significant association signal [$P = 8.1 \times 10^{-8}$ for an additive allele-dosage model, $OR_{het} = 1.35$ (0.99, 1.86), $OR_{hom} = 6.09$ (3.27, 11.34), T allele: 39.7% in cases versus 24.7% in controls]. However, of the 15 SNPs analyzed, rs11200638 was the most significantly associated variant [$P = 1 \times 10^{-9}$, $OR_{het} = 1.86$ (1.35, 2.56), $OR_{hom} = 6.56$ (3.23, 13.31), A allele: 40.3% in cases versus 25.2% in controls] (Fig. 1A and table S2). In terms of the significance of the association, the TA haplotype across rs10490924 and rs11200638 was superior to rs10490924 ($P = 2.2 \times 10^{-9}$), but inferior to rs11200638.

To investigate these associations further, we genotyped an additional 139 AMD patients for these two variants. The results for both SNPs increased in significance (rs10490924, $P = 1.2 \times 10^{-8}$; rs11200638, $P = 1.6 \times 10^{-11}$), with variant rs11200638 remaining the best single variant explaining the association [$OR_{het} = 1.90$ (1.40, 2.58), $OR_{hom} = 7.51$ (3.75, 15.04)]. We next considered association analyses based on genotypes at both rs11200638 and the CFH rs1061170 (Y402H) variant at chromosome 1q31. In a global two-locus analysis enumerating all nine two-locus genotype combinations, the association with AMD was significant ($\chi^2 = 56.56$, 8 df; $P = 2.2 \times 10^{-9}$). Table 1 shows the risk estimates for each two-locus genotype combination compared with the baseline of no risk genotypes (TT at CFHY402H and GG at

[1]Department of Ophthalmology and Visual Sciences, Moran Eye Center, University of Utah School of Medicine, Salt Lake City, UT 84132, USA. [2]Program in Human Molecular Biology and Genetics, Eccles Institute of Human Genetics, University of Utah School of Medicine, Salt Lake City, UT 84132, USA. [3]Sichuan Medical Science Academy and Sichuan Provincial People's Hospital, Sichuan 610071, China. [4]Division of Genetic Epidemiology, Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, UT 84108, USA. [5]Department of Physiology and Jules Stein Eye Institute, School of Medicine at UCLA, Los Angeles, CA 90095, USA. [6]Department of Laboratory Medicine and Experimental Pathology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA. [7]Department of Epidemiology and Public Health, Yale University, New Haven, CT 06520, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: kzhang@hmbg.utah.edu

rs11200638). The association of rs11200638 to AMD was significant when analyzed conditional on the presence of the CFH C risk allele ($P = 5.9 \times 10^{-8}$). In particular, this conditional analysis indicates an allele-dosage effect such that homozygotes for the A risk allele of rs11200638 are at an increased risk [$OR_{hom} = 7.29$ (3.18, 16.74)] over that of heterozygotes [$OR_{het} = 1.83$ (1.25, 2.68)] in all AMD cases, even when compared with a baseline that includes individuals who carry the risk genotypes at *CFH*. With an allele-dosage model, the estimated population attributable risk (PAR) for rs11200638 is 49.3%. Consistent with an additive effect, the estimated PAR from a joint model with CFH Y402H (that is, for a risk allele at either locus) is 71.4%.

The SNP rs11200638 is located 512 base pairs (bp) upstream of the transcription start site of the *HTRA1* gene (also known as *PRSS11, NM_002775*). Using MatInspector (Genomatix Software, GmbH, München, Germany), to scan putative transcription factor binding sites within this region, we identified a conserved AP2/SRF binding element that is altered by the A risk allele. To investigate the functional significance of the SNP, we used real-time reverse transcription polymerase chain reaction (RT-PCR) to study the expression levels of *HTRA1* mRNA in lymphocytes of four AMD patients carrying the risk allele AA and three normal controls carrying the normal allele GG (Fig. 1B and fig. S1A). The *HTRA1* mRNA levels in lymphocytes from AMD patients with the AA genotype were higher by a factor of ~2.7 than those in normal controls with the GG genotype (Fig. 1B). The mean HTRA1 protein level in RPE of four AMD donor eyes with a homozygous AA risk allele was higher by a factor of 1.7 than that of six normal controls with a homozygous GG allele (fig. S2). Unfortunately, our analysis of human eye tissue was limited because we were able to obtain only four AMD donor eyes with an AA genotype out of the 60 donors for this study; therefore, these data thus far show a trend toward higher expression with the risk AA allele. Immunohistochemistry experiments revealed that HTRA1 immunolabeling is present in the drusen of three AMD patients (Fig. 1C and fig. S1, B and C).

The *HTRA1* gene encodes a member of a family of serine proteases expressed in the mouse retina and RPE (*17*). HTRA1 appears to regulate the degradation of extracellular matrix proteoglycans. This activity is thought to facilitate access of other degradative matrix enzymes, such as collagenases and matrix metalloproteinases, to their substrates (*18*). Conceivably, overexpression of HTRA1 may alter the integrity of Bruch's membrane, favoring the invasion of choroid capillaries across the extracellular matrix, as occurs in wet AMD. HTRA1 also binds and inhibits transforming growth factor-β (TGF-β), an important regulator of extracellular matrix deposition and angiogenesis (*17*). DeWan *et al.* (*19*) report that the same *HTRA1* SNP is associated with a wet AMD phenotype in a Chinese population. Together, these findings support a key role for HTRA1 in AMD susceptibility and identify a potential new pathway for AMD pathogenesis.

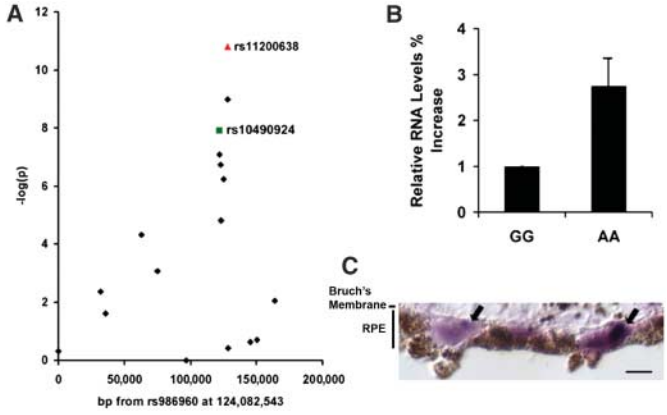

Fig. 1. Genetic association (**A**) and preliminary analysis of HTRA1 mRNA and protein expression in AMD (**B** and **C**). (A) Graphed log *P*-values (*y* axis) from association analyses for the 15 SNPs at the 10q AMD region using 442 AMD cases and 309 controls (see also table S2). For the rs10490924 (green square) and rs11200638 (red triangle) SNPs, associations were derived from a larger sample size (581 AMD cases). (B) Real-time RT-PCR semi-quantitative analysis of *HTRA1* RNA levels in blood lymphocytes from three AMD patients with the AA genotype and three normal controls with the GG genotype. The statistical significance of the differences in expression level was examined using an independent samples *t* test (SPSS version 13.0): AA:GG ($P = 0.02$). Each RT-PCR reaction was run twice, and the error bars indicate the 95.0% confidence interval of the mean. (C) Drusen is immunolabeled with HTRA1 antibody in an eye from a patient with wet AMD. The staining (arrows) occurs between RPE and Bruch's membrane. Similar results were obtained in another two AMD eyes (fig. S1, B and C). Scale bar = 20 μm.

Table 1. Two-locus odds ratios for *HTRA1* rs11200638 and *CFH* rs1061170. Odds ratios with 95% confidence intervals in parentheses were calculated to compare each genotypic combination to the baseline of homozygosity for the common allele at both loci (TT/GG).

SNP		*HTRA1* rs11200638		
		GG	AG	AA
***CFH* rs1061170 (Y402H)**	**TT**	1.00	1.80 (0.93,3.49)	3.43 (0.62,19.00)
	CT	1.07 (0.59,1.94)	2.31 (1.28,4.17)	7.31 (2.68,19.93)
	CC	3.07 (1.50,6.27)	3.97 (1.93,8.15)	31.52 (4.01,247.96)

References and Notes

1. National Advisory Eye Council, *Report of the Retinal Diseases Panel: Vision Research: A National Plan, 1994–1998* (U.S. Department of Health and Human Services, Publication NIH 93-3186, Bethesda, MD, 1993).
2. K. Attebo, P. Mitchell, W. Smith, *Ophthalmology* **103**, 357 (1996).
3. C. C. Klaver *et al.*, *Arch. Ophthalmol.* **116**, 1646 (1998).
4. J. M. Seddon, W. C. Willett, F. E. Speizer, S. E. Hankinson, *JAMA* **276**, 1141 (1996).
5. R. J. Klein *et al.*, *Science* **308**, 385 (2005).
6. A. O. Edwards *et al.*, *Science* **308**, 421 (2005).
7. J. L. Haines *et al.*, *Science* **308**, 419 (2005).
8. G. S. Hageman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 7227 (2005).
9. K. P. Magnusson *et al.*, *PLoS Med.* **3**, e5 (2006).
10. B. Gold *et al.*, *Nat. Genet.* **38**, 458 (2006).
11. M. Li *et al.*, *Nat. Genet.* **38**, 1049 (2006).
12. J. Maller *et al.*, *Nat. Genet.* **38**, 1055 (2006).
13. A. Rivera *et al.*, *Hum. Mol. Genet.* **14**, 3227 (2005).
14. J. Jakobsdottir *et al.*, *Am. J. Hum. Genet.* **77**, 389 (2005).
15. S. A. Fisher *et al.*, *Hum. Mol. Genet.* **14**, 2257 (2005).
16. S. Schmidt *et al.*, *Am. J. Hum. Genet.* **78**, 852 (2006).
17. C. Oka *et al.*, *Development* **131**, 1041 (2004).
18. S. Grau *et al.*, *J. Biol. Chem.* **281**, 6124 (2006).
19. A. DeWan *et al.*, *Science* **314**, 989. Published online 19 October 2006. 10.1126/science.1133807 (2006).

20. We thank the participating AMD patients and their families. We thank G. Brinton, J. Carver, A. Crandall, M. Teske, A. Jorgensen, E. Brinton, and R. MacArthur for assistance in obtaining blood samples; W. Baehr, R. Marc, and N. Perrimon for critical reading of the manuscript; D. Lim and E. Smith for editorial assistance; and the Utah Lions Eye Bank and P. Bhosale for assistance in obtaining eye donor tissues. We acknowledge the following grant support to K.Z.: NIH (R01EY14428, R01EY14448, P30EY014800, and GCRC M01-RR00064), Foundation Fighting Blindness, Ruth and Milton Steinbach Fund, Ronald McDonald House Charities, Macular Vision Research Foundation, Research to Prevent Blindness, Val and Edith Green Foundation, and the Simmons Foundation; to H.S.: Ruth and Milton Steinbach Fund; to Z.Y.: Knights Templar Eye Research Foundation; to N.C.: K07 (NCI CA98364); to J.H.: NIH (R01EY15771), Ellison Medical Foundation, Macular Vision Research Foundation, an institutional award from Howard Hughes Medical Institute to Yale School of Medicine, and grants from the Claude D. Pepper Older Americans Independence Center at Yale University School of Medicine, and the Verto Institute; to V.S.: Mayo Clinic and Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1133811/DC1
Materials and Methods
Figs. S1 and S2
Tables S1 and S2
References

14 August 2006; accepted 6 October 2006
Published online 19 October 2006;
10.1126/science.1133811
Include this information when citing this paper.