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Geographic Atrophy in Age-Related Macular Degeneration and *TLR3*

To the Editor

Yang et al. (Oct. 2 issue)[1] describe the association between a variant of the toll-like receptor 3 gene (*TLR3*) and protection from geographic atrophy, a major cause of blindness. They also show that the *TLR3* genotype affects the sensitivity of human retinal pigment epithelial cells and the retinal pigment epithelium of mice to the proapoptotic effects of long double-stranded RNA (dsRNA), a recognized ligand of TLR3. The dsRNA that they used in these experiments is roughly 100 times the length of small interfering RNA (siRNA) molecules, and yet they conclude that the "results suggest a role of viral dsRNA in the development of geographic atrophy and point to the potential toxic effects of short-interfering-RNA therapies in the eye." I am not persuaded that this conclusion is supported by their data.

Those of us developing small dsRNA molecules for therapy are aware of their potential to activate components of the innate immune system. That said, the remark by Yang et al.[1] about the risks of RNA-based therapeutic agents may be misleading.

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References

1. Yang Z, Stratton C, Francis PJ, et al. Toll-like receptor 3 and geographic atrophy in age-related macular degeneration. N Engl J Med. 2008; 359:1456–1463. [Erratum, N Engl J Med 2008;359: 1859.]. [PubMed: 18753640]

To the Editor

Given the strong relationship between geographic atrophy and age,[1,2] the difference in age between cases and controls in the studies that Yang et al. describe is a concern. The mean age of the patients with geographic atrophy in the primary study (the Utah case–control series) and the first replication study was 84 years, as compared with a mean age of 77 years among the controls. In the Blue Mountains Eye Study,[1] a 7-year difference in age was associated with a prevalence of geographic atrophy that was increased by a factor of three (unpublished data), whereas a meta-analysis[2] showed that the risk differed by a factor of five between these age groups. These data, together with reports that age-related macular degeneration is associated with the rate of death from cardiovascular causes,[3,4] raise the possibility of confounding by age or selective mortality. Although this limitation does not apply to the second replication study, which used age-matched data from the Age-Related

Eye Disease Study (AREDS) (ClinicalTrials.gov number, NCT00000145), it is unclear how the 184 patients with geographic atrophy and 134 controls were selected from among the more than 3600 AREDS subjects. Finally, the mean age of persons with choroidal neovascularization in the Han Chinese sample was approximately 10 years younger than the mean age of persons in the other samples, suggesting disease processes other than age-related macular degeneration (e.g., myopic choroidal neovascularization or polypoidal choroidal vasculopathy). Thus, adjustment for age would strengthen the validity of these important findings.

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References

1. Mitchell P, Smith W, Attebo K, Wang JJ. Prevalence of age-related maculopathy in Australia: the Blue Mountains Eye Study. Ophthalmology. 1995; 102:1450–1460. [PubMed: 9097791]
2. Friedman DS, O'Colmain BJ, Muñoz B, et al. Prevalence of age-related macular degeneration in the United States. Arch Ophthalmol. 2004; 122:564–572. [PubMed: 15078675]
3. Wong TY, Klein R, Sun C, et al. Age-related macular degeneration and risk for stroke. Ann Intern Med. 2006; 145:98–106. [PubMed: 16847292]
4. Tan JS, Wang JJ, Liew G, Rochtchina E, Mitchell P. Age-related macular degeneration and mortality from cardiovascular disease or stroke. Br J Ophthalmol. 2008; 92:509–512. [PubMed: 18310310]

To the Editor

Yang et al. report an association between the F412L (rs3775291) variant of *TLR3* and protection against the development of geographic atrophy, a phenotype of late-stage age-related macular degeneration. 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 geographic atrophy and 2669 matched controls (for consortium members and methods, see the Supplementary Appendix, available with the full text of this letter at NEJM.org).

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 geographic atrophy (P≥0.29 in all cohorts) (Table 1). The difference in the

minor-allele frequency between patients with geographic atrophy and controls did not exceed 4% in any study and, summarizing the data across studies, the minor-allele frequency of the rs3775291 SNP was identical in the 2669 controls than in the 1080 patients with geographic atrophy. Moreover, the minor-allele frequency of rs3775291 did not differ significantly between any of the geographic-atrophy 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 geographic atrophy 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 minor-allele frequency 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 geographic atrophy, the minor-allele frequency 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 age-related macular degeneration and therefore inappropriate to suggest revising therapeutic strategies on the basis of the available data.

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Table 1

Genotyping and Association Analysis of the *TLR3* rs3775291 Variant in Eight Cohorts.*

Value	Columbia Series		Iowa Series		Amsterdam Series		Rotterdam Series		Germany Series		Iceland Series		AREDS Series		Australia Series		Total	
	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls	Patients with Geographic Atrophy	Controls
Total no. of subjects	211	365	102	295	89	264	64	843	184	366	210	169	163	204	57	163	1080	2669
Genotype (no. of subjects)																		
CC	105	204	53	152	41	136	28	422	105	191	102	90	82	101	30	70	546	1366
CT	93	133	37	108	40	103	29	341	63	139	96	65	71	82	21	76	450	1047
TT	13	28	12	35	8	25	7	80	16	36	12	14	10	21	6	17	84	256
Minor-allele (T allele) frequency	0.28	0.26	0.30	0.30	0.31	0.29	0.33	0.30	0.26	0.29	0.29	0.28	0.28	0.30	0.29	0.33	0.29	0.29
P value																		
Hardy–Weinberg equilibrium	0.54	0.58	0.36	0.09	0.89	0.67	1.00	0.63	0.33	0.33	0.24	0.93	0.56	0.80	0.82	0.82		
For difference in allele frequency	0.44		0.94		0.53		0.36		0.29		0.75		0.46		0.35		0.59	
Odds ratio (95% CI)†	1.12 (0.86–1.47)		0.99 (0.70–1.40)		1.12 (0.78–1.63)		1.20 (0.82–1.75)		0.86 (0.65–1.14)		1.05 (0.77–1.45)		0.89 (0.64–1.22)		0.8 (0.5–1.27)		1.03 (0.92–1.15)	

*AREDS denotes Age-Related Eye Disease Study, and CI confidence interval.

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

References

1. Age-Related Eye Disease Study Research Group. The Age-Related Eye Disease Study (AREDS): design implications: AREDS report no. 1. Control Clin Trials. 1999; 20:573–600. [PubMed: 10588299]

To the Editor

Age-related macular degeneration is a leading cause of visual impairment in older persons and is a late-onset complex trait influenced by heredity and modifiable risk factors, including cigarette smoking. The accumulation of inflammatory deposits called drusen under the retina heralds the clinical onset of early age-related macular degeneration. The extent of these changes is a primary predictor of which patients with early age-related macular degeneration will have the advanced complications of age-related macular degeneration: atrophy of the retina (geographic atrophy) and abnormal angiogenesis ("wet" age-related macular degeneration).[1,2] Genetic variants consistently associated with age-related macular degeneration (complement factor H [*CFH*], complement component 2–complement factor B [*C2–CFB*], complement component 3 [*C3*], and age-related maculopathy susceptibility 2 gene–HtrA serine peptidase 1 [*ARMS2–HTRA1*]) increase the risk of early and advanced age-related macular degeneration.

Yang et al. report that in their study, geographic atrophy — but not early or neovascular age-related macular degeneration — was associated with a variant of *TLR3* (a SNP at rs3775291) that results in the substitution of phenylalanine for leucine at amino acid 412. We have previously reported that there is no consistent association between this SNP and early or advanced age-related macular degeneration.[3]

Table 1 shows distributions of alleles and genotypes for rs3775291 among four independent samples composed of case patients and controls of European descent. We did not detect a consistent effect of Leu412Phe on the risk of age-related macular degeneration. In contrast to the study by Yang et al., showing a protective effect of the minor allele, a meta-analysis involving our 880 case patients with geographic atrophy and 1189 controls showed a higher minor-allele frequency among the case patients (odds ratio with the use of the Mantel–Haenszel test, 1.05; 95% confidence interval, 0.91 to 1.21) and no evidence of the reported protective effect on disease (P = 0.75).

The four samples were genotyped with the use of different techniques with call rates that were higher than 97%. We sequenced the region around rs3775291 in subgroups of subjects to verify the results of genotyping. Genotyping of SNPs associated with risk at other loci, such as *CFH,* has yielded results that are consistent with those of other genetic studies of age-related macular degeneration. We are therefore confident that our failure to detect an association between *TLR3* and age-related macular degeneration is not explained by genotyping errors or misclassification of cases and controls.

The prevalence of genotypes among the AREDS subjects, with genotyping performed by the Center for Inherited Disease Research with the use of the Human-1 platform (Illumina) (Table 1) differs from that among the AREDS subjects in the study by Yang et al.

We conclude that the Leu412Phe variant in *TLR3* is unlikely to have a major effect on the risk of age-related macular degeneration.

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Table 1

Association between Phenotypes of Age-Related Macular Degeneration and the *TLR3* Variant rs3775291 (Leu412Phe).*

Value	Edwards Series			Swaroop Series			Seddon Series			AREDS Series†		
	Patients with Geographic Atrophy	Patients with Exudative AMD	Controls	Patients with Geographic Atrophy	Patients with Exudative AMD	Controls	Patients with Geographic Atrophy	Patients with Exudative AMD	Controls	Patients with Geographic Atrophy	Patients with Exudative AMD	Controls
No. of participants	89	178	222	279	377	317	218	646	479	184	247	171
Genotype — no. (%)												
CC	42 (47)	73 (41)	124 (56)	135 (48)	171 (45)	158 (50)	113 (52)	305 (47)	235 (49)	86 (47)	117 (47)	81 (47)
CT	41 (46)	88 (49)	81 (36)	120 (43)	171 (45)	124 (39)	78 (36)	304 (47)	210 (44)	82 (45)	108 (44)	78 (46)
TT	6 (7)	17 (10)	17 (8)	24 (9)	35 (9)	35 (11)	27 (12)	37 (6)	34 (7)	16 (9)	22 (9)	12 (7)
Frequency of minor T allele	0.30	0.34	0.26	0.30	0.32	0.31	0.30	0.29	0.29	0.31	0.31	0.30
P value												
Allele	0.33	0.01		0.86	0.58		0.68	0.94		0.74	0.76	
Additive genotype	0.33	0.01		0.86	0.59		0.63	0.90		0.73	0.77	

*The TaqMan genotyping platform was used for the Edwards and Swaroop series, the Affymetrix 6.0 genotyping platform for the Seddon series, and the Illumina Human-1 genotyping platform for the Age-Related Eye Disease Study (AREDS). AMD denotes age-related macular degeneration. P values are for comparisons with controls with the use of an additive model of genotype effect on the risk of AMD.

†Data are from the National Institutes of Health Genotype and Phenotype database (dbGaP) (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000001.v2.p1; accession number, phs000001.v2.p1).

References

1. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: the Beaver Dam eye study. Ophthalmology. 2002; 109:1767–1779. [PubMed: 12359593]
2. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001; 119:1417–1436. [Erratum, Arch Ophthalmol 2008;126:1251.]. [PubMed: 11594942]
3. Edwards AO, Chen D, Fridley BL, et al. Toll-like receptor polymorphisms and age-related macular degeneration. Invest Ophthalmol Vis Sci. 2008; 49:1652–1659. [PubMed: 18385087]

The Authors Reply

Lewin is correct that polyinosine– polycytidylic acid induces retinal degeneration through *TLR3.* Our earlier study[1] indicated that 21-nucleotide siRNAs activate *TLR3* and induce cytotoxic effects in vivo as effectively as polyinosine–polycytidylic acid. Those findings, coupled with in vitro and structural data[2,3] suggesting that 21-nucleotide siRNAs bind and activate *TLR3,* fuel our concern about potential ocular toxic effects.

Liew and colleagues are correct that there is an age difference between case patients and controls in the two cohorts. However, this difference is unlikely to account for all of the association signals. Klein et al.[4] found that the 10-year incidence of geographic atrophy among 75-year-old patients was only 3.1% and that over a period of 10 years, geographic atrophy did not develop in any of the 2572 persons who had no drusen or had drusen smaller than 63 μm in diameter (these characteristics correspond to those of our controls). Furthermore, the AREDS showed that geographic atrophy developed in less than 0.2% of patients in AREDS category 1 (corresponding to controls in our two replication case–control series) over a period of 5 to 7 years.

The difference between our results and those of Allikmets and colleagues and Edwards and colleagues may be explained by our stringent criteria for controls (i.e., the absence of drusen in the Utah cohort and fewer than 5 drusen smaller than 63 μm in diameter in the control groups).[5] With regard to the difference in allele frequencies and consequent P-value differences in the AREDS case–control series, we note that Edwards and colleagues obtained their genotype data from the dbGaP; this genotyping was performed by the Center for Inherited Disease Research. We obtained AREDS samples from the Coriell Cell Repositories; 50 of 184 of our case patients with geographic atrophy and 91 of 134 of our controls also were genotyped by the Center for Inherited Disease Research. A difference in sample populations and a difference in phenotypic classes may underlie the discrepancy in allele frequencies.

We are disappointed that Edwards and colleagues and Allikmets and colleagues interpret the difference in findings to support a false positive association between *TLR3* and geographic atrophy. Another possibility is that the overall allele effect is modest and not readily detectable in all populations (due to insufficient power). We advise caution in dismissing this second possibility, not least because loci with a modest contribution to disease offer invaluable insights into the mechanisms of disease pathogenesis.

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References

1. Kleinman ME, Yamada K, Takeda A, et al. Sequence- and target-independent angiogenesis suppression by siRNA via TLR3. Nature. 2008; 452:591–597. [PubMed: 18368052]
2. Karikó K, Bhuyan P, Capodici J, Weissman D. Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. J Immunol. 2004; 172:6545–6549. [PubMed: 15153468]
3. Pirher N, Ivicak K, Pohar J, Bencina M, Jerala R. A second binding site for double-stranded RNA in TLR3 and consequences for interferon activation. Nat Struct Mol Biol. 2008; 15:761–763. [PubMed: 18568036]
4. Klein R, Klein BE, Tomany SC, Meuer SM, Huang GH. Ten-year incidence and progression of age-related maculopathy: the Beaver Dam eye study. Ophthalmology. 2002; 109:1767–1779. [PubMed: 12359593]
5. Age-Related Eye Disease Study Research Group. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss: AREDS report no. 8. Arch Ophthalmol. 2001; 119:1417–1436. [Erratum, Arch Ophthalmol 2008;126:1251.]. [PubMed: 11594942]