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Further mapping of 10q26 supports strong association of *HTRA1* polymorphisms with age-related macular degeneration

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Abstract

Age-related macular degeneration (AMD) is a complex disorder with genetic and environmental influences. The genetic influences affecting AMD are not well understood and few genes have been consistently implicated and replicated for this disease. A polymorphism (rs11200638) in a transcription factor binding site of the *HTRA1* gene has been described, in previous reports, as being most significantly associated with AMD. In this paper, we investigate haplotype association and individual polymorphic association by genotyping additional variants in the AMD risk-associated region of chromosome 10q26. We demonstrate that rs11200638 in the promoter region and rs2293870 in exon 1 of *HTRA1*, are among the most significantly associated variants for advanced forms of AMD.
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Keywords: Age-related macular degeneration; *HTRA1*; Genetics; Single nucleotide polymorphisms

1. Introduction

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the world. Advanced AMD results in central vision loss through two forms of the disease: geographic atrophy and choroidal neovascularization. Soft confluent drusen, which are small, yellowish, extracellular deposits of lipid, protein, and cellular debris are found in the macula and do not result in vision loss. Soft drusen are, however, significant risk factors for the two advanced forms of AMD.

AMD is a complex disease resulting from genetic and environmental influences. While this disease is known to have a genetic basis, few genes have been consistently implicated. The first major susceptibility gene for AMD, *CFH*, has been shown to be associated with geographic atrophy and choroidal neovascularization and has been replicated in multiple studies (Edwards et al., 2005; Hageman et al., 2005; Haines et al., 2005; Klein et al., 2005; Li et al., 2006; Magnusson et al., 2006; Maller et al., 2006). An additional major locus at chromosome 10q26 has been shown in multiple independent studies to confer risk independent of the risk conferred by *CFH* (Fisher et al., 2005; Jakobsdottir et al., 2005; Maller et al., 2006; Rivera et al., 2005; Schmidt et al., 2006).

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Recently a single nucleotide polymorphism (SNP) rs11200638 in a putative transcription factor binding site of the *HTRA1* gene in the 10q26 region was shown to confer significant risk for choroidal neovascularization by our group and others (Dewan et al., 2006; Yang et al., 2006). This association has been consistently replicated in AMD cohorts in Hong Kong Chinese, mainland Chinese, Utah European Americans, and Japanese populations (Lu et al., 2007; Mori et al., 2007; Yoshida et al., 2007). The variant, rs11200638, has also been shown to confer susceptibility to both types of advanced AMD; geographic atrophy and choroidal neovascularization (Cameron et al., 2007). In this paper, we investigate additional variants in chromosome 10q26. These variants span the genes previously reported as being most significantly associated with AMD (*HTRA1*, *LOC387715*, and *PLEKHA1*) in an expanded cohort, in which a subset has been previously shown to have association for the SNP rs11200638 in advanced AMD (Yang et al., 2006). We demonstrate that rs11200638 and the tri-allelic synonymous variant, rs2293870 are among the most significantly associated variants. We also demonstrate the haplotype structure and association patterns of 20 polymorphisms in the chromosome 10q region.

2. Methods

2.1. Patients

This study was approved by the Institutional Review Board of the University of Utah. All subjects provided informed consent prior to participation in the study. AMD patients were recruited in the Moran Eye Center at the University of Utah, as were normal age-matched controls, with eye examinations (individuals age 60 years or older with no drusen or RPE changes). All participants went through a standard examination protocol and visual acuity measurements. Slitlamp biomicroscopy of the fundi using a 90 diopter lens was performed. A stereoscopic color fundus photograph (50°) centered on the fovea was taken of each eye using a Topcon fundus camera (Topcon TRV-50VT, Topcon Optical Company, Tokyo, Japan) by trained ophthalmic photographers. Grading was carried out using a standard grid classification suggested by the International ARM Epidemiological Study Group for the age-related maculopathy (ARM) and age-related macular degeneration group (Bird et al., 1995). All abnormalities in the macula were characterized according to type, size, and number of drusen, hyperpigmentation or hypopigmentation, and AMD stage.

The case cohort was comprised of European American individuals with an average age of 81.1 years and was 53.5% female. The control cohort was also European American individuals who had an average age of 75.3 years and was 61.2% male.

2.2. Genotyping

A Utah cohort of 342 advanced AMD patients, which included those with choroidal neovascularization and geographic atrophy, was genotyped and allele frequencies were compared to 215 age and ethnicity matched unaffected controls for all variants. Lab personnel were blinded to case/control status. For SNP rs11200638, we PCR-amplified genomic DNA from AMD patients and age-matched controls using oligonucleotide primers 5′-ATGCCACCCACAACAACTTT-3′ and 5′-CGCGTCCTTCA AACTAATGG-3′ with 5% DMSO. The amplification parameters include denaturing at 95 °C for 3 min, denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s, extending at 72 °C for 45 s for 35 cycles, and 72 °C extension for an additional 10 min. The 385 bp PCR product was digested by restriction endonuclease Eag I, which generated 139 and 246 bp bands representing the normal G allele. An undigested PCR product represented the A allele. For rs10490924, forward primer 5′-TACCCAGGACCGATGG TAAC-3′ and reverse primer 5′-GAGGAAGGCTGAATTGCCTA-3′ were used for PCR amplification, PVUII digestion was used to identify the normal G allele as described previously (Yang et al., 2006). The remaining 18 10q26 polymorphisms were genotyped using the SNaPSHOT method on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Additionally the rs11200638 polymorphism and the rs10490924 variant were re-genotyped for all samples by the SNaPSHOT method in order to verify digestion results.

All SNPs reported in this manuscript had a genotyping success rate >97% and accuracy >98% as judged by random re-genotyping of 10% of the samples.

2.3. Statistical analysis for 10q26.13

The chi-squared test for trend for an additive model over alleles was performed on each polymorphism to assess association with AMD. This statistical analysis utilized the "Trend" program in the PEPI (Programs for EPIdemiologists) package. Linkage disequilibrium and haplotype analysis were examined in Haploview v3.32 using the default parameters.

Polymorphisms of note are deletion/insertion polymorphism (DIP) rs10664316 and SNP rs2293870. rs10664316 is a common variant comprised of the nucleotides AT or a 2 bp deletion. rs2293870 is a tri-allelic polymorphism (C/G/T). For the purposes of Fig. 1 and Haploview (which is limited to only bi-allelic polymorphisms), analysis was performed using G as the non-risk allele and C/T as the risk alleles.

All control cohort bi-allelic genotyping results were screened for deviations from Hardy–Weinberg equilibrium ($p < 0.01$) and SNPs with significant deviation were excluded.

3. Results

3.1. Genotyping and statistical results

We genotyped 19 SNPs and one DIP in the 10q26 region encompassing the genes *PLEKHA1*, *LOC387715*, and *HTRA1* including one SNP, rs11200638, that has previously been reported as being significantly associated with advanced AMD in this cohort. Five SNPs and one DIP reside in the vicinity of *LOC387715*, eight SNPs are in or around *HTRA1*, and six SNPs are in or near *PLELKHA1*. Twelve variants throughout this region were significantly associated with advanced AMD after adjusting for multiple testing ($p < 0.05/19$ (2.6E−03), Table 1). This adjustment is conservative due to the correlation between the SNPs studied. SNP rs11200638 was the most significantly associated polymorphism with a chi-squared test for trend p-value equal to 1.26E−10 (Fig. 1). The SNP with the next lowest p-value was rs2293870 in exon 1 of the *HTRA1* gene, with a p-value of 4.78E−10 for advanced forms of AMD versus age and ethnicity matched controls. This variant is a synonymous SNP coding for the amino acid glycine. Therefore, the highest signals of association appear around or within the *HTRA1* gene.

The third highest associated SNP was rs10490924 with a p-value of 6.18E−09. Fig. 1 and Table 1 shows the p-values from the chi-squared test for trend from all polymorphisms genotyped in the region for advanced AMD cases versus age and ethnicity matched controls.

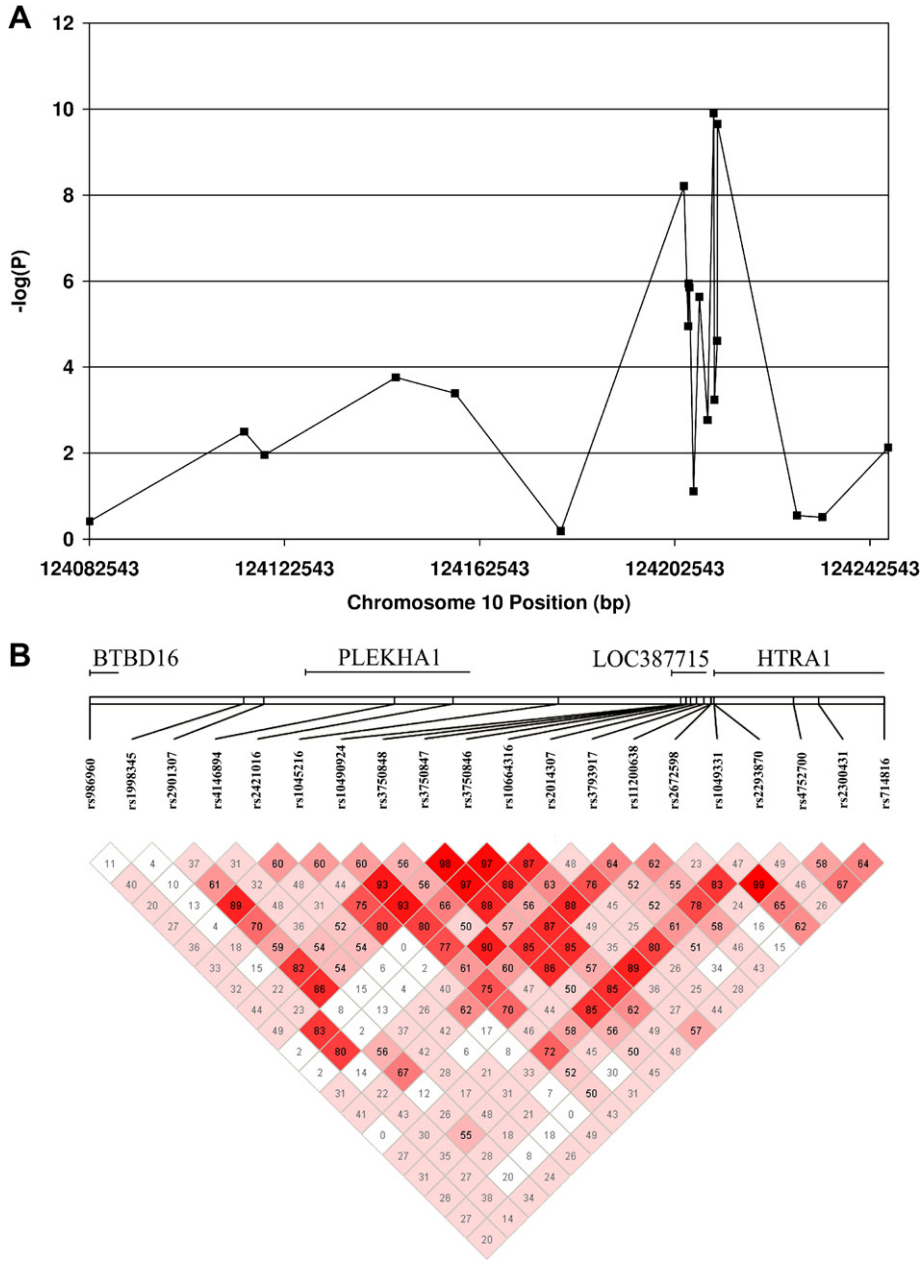

Fig. 1. Association statistics for AMD and SNPs at 10q26. Association statistics for the SNPs at 10q26 in the advanced AMD cohort and control cohort. Nineteen SNPs and one DIP were genotyped in advanced AMD cases and controls in the 10q26 region. Chi-squared tests for trend were performed for each SNP and the $-\log10\,p$-values are shown. The numerical p-values, minor allele frequencies, and locations for the following SNPs can be found in Table 1. From left to right the variants genotyped are: rs986960, rs1998345, rs2901307, rs4146894, rs2421016, rs1045216, rs10490924, rs3750848, rs3750847, rs3750846, rs10664316, rs2014307, rs3793917, rs11200638, rs2672598, rs1049331, rs2293870, rs4752700, rs2300431, rs714816. Aligned below the $-\log(p$-values) graph is the linkage disequilibrium (LD) plot, generated in Haploview v3.32. The numbers listed in each square represent the D'-value for pair wise analysis. Similarly the color corresponds to the degree of linkage disequilibrium. The darker the square is, the higher the LD between the two polymorphisms being analyzed. The polymorphism positions were determined using the "reference" assembly from the National Center for Biotechnology Information website.

Analysis of the LD structure demonstrates relatively lower LD between polymorphic markers in this region in the Utah population (Fig. 1B) than in a previously reported Hong Kong Chinese cohort. In particular, the D'-value was 0.75 between rs10490924 and rs11200638 in AMD cases from the Utah population (Fig. 1B), compared to a $D' > 0.97$ from the Chinese populations (Dewan et al., 2006; Lu et al., 2007).

4. Discussion

To date, rs11200638, a promoter polymorphism in *HTRA1*, has been shown to be associated with choroidal neovascularization in five independent cohorts (Dewan et al., 2006; Lu et al., 2007; Mori et al., 2007; Yang et al., 2006; Yoshida et al., 2007). Additionally, this polymorphism has been implicated as a risk variant in both

Table 1
Association between variants in chromosome 10q and advanced AMD

SNP	Chromosome 10 position	Case MAF	Control MAF	Allelic difference	Trend *p*-value	Gene
rs986960	124082543	0.459	0.474	0.015	3.89E−01	BTBD16
rs1998345	124114296	0.378	0.333	0.045	3.16E−03	Chr. 10
rs2901307	124118433	0.430	0.498	0.068	1.10E−02	Chr. 10
rs4146894	124145371	0.435	0.500	0.065	1.74E−04	PLEKHA1
rs2421016	124157502	0.549	0.447	0.102	4.07E−04	PLEKHA1
rs1045216	124179187	0.351	0.359	0.008	6.46E−01	PLEKHA1
rs10490924	124204438	0.420	0.253	0.167	6.18E−09	LOC387715
rs3750848	124205305	0.359	0.249	0.110	1.12E−05	LOC387715
rs3750847	124205411	0.397	0.244	0.153	1.14E−06	LOC387715
rs3750846	124205555	0.370	0.243	0.127	1.41E−06	LOC387715
rs10664316	124206375(6)	0.324	0.369	0.045	7.76E−02	LOC387715
rs2014307	124207622	0.298	0.386	0.088	2.34E−06	Chr. 10
rs3793917	124209265	0.338	0.260	0.078	1.70E−03	HTRA1
rs11200638	124210534	0.434	0.222	0.212	1.26E−10	HTRA1
rs2672598	124210672	0.583	0.455	0.128	5.75E−04	HTRA1
rs1049331	124211260	0.428	0.289	0.139	2.46E−05	HTRA1
rs2293870 (G/CT)	124211266	0.506	0.327	0.179	4.78E−10	HTRA1
rs4752700	124227602	0.396	0.443	0.047	2.82E−01	HTRA1
rs2300431	124232807	0.269	0.302	0.033	3.09E−01	HTRA1
rs714816	124246335	0.411	0.333	0.078	7.41E−03	HTRA1

p-values and polymorphism information for the variants genotyped at 10q26.
Association statistics for polymorphisms genotyped for advanced AMD cases and age and ethnicity matched controls. Presented are the individual association statistics, including minor allele frequency and *p*-values for each polymorphism.

forms of advanced AMD (Cameron et al., 2007). To further examine this previously reported *HTRA1* promoter SNP's association compared to other polymorphisms in the region, we genotyped a total of 20 tagging polymorphisms including this promoter SNP to survey their association with advanced AMD in an expanded Utah cohort.

From this comparative data, the *HTRA1* promoter SNP, rs11200638, remains the most significantly associated with advanced AMD. In contrast, rs10490924 had the third lowest *p*-value with a *p*-value of 6.18E−09. Interestingly, the second most significant SNP, rs2293870, was located in exon 1 of the *HTRA1* gene, further supporting an association of *HTRA1* with AMD.

Beyond single SNP analysis, haplotype analysis, using Haploview v3.32 and the default parameters, demonstrates inferior haplotypes compared to similar single SNP analysis of rs11200638 and rs2293870 (Table 2). This suggests that the haplotype association can best be described by association from these SNPs.

Previous studies have suggested that the SNP rs10490924 in the gene *LOC387715* is the most significantly associated variant and that *LOC387715* is the second major susceptibility gene for age-related macular degeneration (Kanda et al., 2007; Rivera et al., 2005). Our data in a Utah cohort argues strongly that *HTRA1* is the most likely gene associated with AMD in the 10q region (Fig. 1 and Table 1). In particular, we have identified an additional SNP variant, rs2293870, in *HTRA1* that has a higher association signal than that of *LOC387715* (rs10490924). Kanda et al. propose that *LOC387715* encodes a mitochondrial protein, yet no protein expression data for LOC387715 have been described in the retina or RPE. This

Table 2
Haplotype analysis

rs10490924, rs11200638		~6 KB
TA	2.47E−08	
rs10490924, rs3750848, rs3750847, rs3750846, rs10664316, rs2014307, rs3793917, rs11200638		~6 KB
TAAGACGA	2.38E−06	
rs3750847, rs11200638		~5 KB
AA	1.42E−06	
rs3750847, rs3750846, rs10664316		964 bp
AGA	2.36E−05	
rs10490924, rs11200638, rs2293870		~6 KB
TAT	2.70E−09	
rs11200638, rs2293870		732 bp
AT	8.88E−09	

Haplogroup association data for the most significantly associated haplotypes among the variants genotyped in the chromosome 10q region. No haplotype association *p*-values were lower than the *p*-value of rs11200638 and rs2293870 suggesting that the association in the region can best be explained by the association from these SNPs.

is in contrast to HTRA1 which has been shown to be expressed in drusen, a risk factor for advanced AMD (Cameron et al., 2007; Yang et al., 2006).

The initial findings by Yang et al. and DeWan et al., as well as our current findings, demonstrate that rs11200638 and rs2293870 are the most significant variants within this region, particularly in light of the high amount of genotyping saturation presented. Both SNPs have more significant *p*-values than any of the SNPs genotyped in *LOC387715*.

On the basis of our statistical data, including haplotype analyses and association comparisons presented in this paper, rs11200638 in the promoter region of *HTRA1* and

rs2293870 in exon 1 of *HTRA1* are among the most significant variants in the 10q26 region presented thus far. These data, along with functional data presented to date, make the case for *HTRA1* being the gene most likely associated with advanced AMD in the 10q region. The density of highly significant variants in *HTRA1* supports this gene's involvement in advanced AMD thus highlighting the importance of future study on this gene and its function.

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References

Bird, A. C., Bressler, N. M., Bressler, S. B., Chisholm, I. H., Coscas, G., Davis, M. D., et al. (1995). An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Survey of Ophthalmology, 39*(5), 367–374.

Cameron, D. J., Yang, Z., Gibbs, D., Chen, H., Kaminoh, Y., Jorgensen, A., et al. (2007). HTRA1 variant confers similar risks to geographic atrophy and neovascular age-related macular degeneration. *Cell Cycle, 6*(9), 1122–1125.

Dewan, A., Liu, M., Hartman, S., Zhang, S. S., Liu, D. T., Zhao, C., et al. (2006). HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science, 314*(5801), 989–992.

Edwards, A. O., Ritter, R., 3rd, Abel, K. J., Manning, A., Panhuysen, C., & Farrer, L. A. (2005). Complement factor H polymorphism and age-related macular degeneration. *Science, 308*(5720), 421–424.

Fisher, S. A., Abecasis, G. R., Yashar, B. M., Zareparsi, S., Swaroop, A., Iyengar, S. K., et al. (2005). Meta-analysis of genome scans of age-related macular degeneration. *Human Molecular Genetics, 14*(15), 2257–2264.

Hageman, G. S., Anderson, D. H., Johnson, L. V., Hancox, L. S., Taiber, A. J., Hardisty, L. I., et al. (2005). 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, 102*(20), 7227–7232.

Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., et al. (2005). Complement factor H variant increases the risk of age-related macular degeneration. *Science*.

Jakobsdottir, J., Conley, Y. P., Weeks, D. E., Mah, T. S., Ferrell, R. E., & Gorin, M. B. (2005). Susceptibility genes for age-related maculopathy on chromosome 10q26. *American Journal of Human Genetics, 77*(3), 389–407.

Kanda, A., Chen, W., Othman, M., Branham, K. E., Brooks, M., Khanna, R., et al. (2007). A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. *Proceedings of the National Academy of Sciences of the United States of America, 104*(41), 16227–16232.

Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. Y., Sackler, R. S., Haynes, C., et al. (2005). Complement factor H polymorphism in age-related macular degeneration. *Science*.

Li, M., Atmaca-Sonmez, P., Othman, M., Branham, K. E., Khanna, R., Wade, M. S., et al. (2006). CFH haplotypes without the Y402H coding variant show strong association with susceptibility to age-related macular degeneration. *Nature Genetics, 38*(9), 1049–1054.

Lu, F., Hu, J., Zhao, P., Lin, Y., Yang, Y., Liu, X., et al. (2007). HTRA1 variant increases risk to neovascular age-related macular degeneration in Chinese population. *Vision Research, 47*(24), 3120–3123.

Magnusson, K. P., Duan, S., Sigurdsson, H., Petursson, H., Yang, Z., Zhao, Y., et al. (2006). CFH Y402H confers similar risk of soft drusen and both forms of advanced AMD. *PLoS Medicine, 3*(1), e5.

Maller, J., George, S., Purcell, S., Fagerness, J., Altshuler, D., Daly, M. J., et al. (2006). Common variation in three genes, including a noncoding variant in CFH, strongly influences risk of age-related macular degeneration. *Nature Genetics, 38*(9), 1055–1059.

Mori, K., Horie-Inoue, K., Kohda, M., Kawasaki, I., Gehlbach, P. L., Awata, T., et al. (2007). Association of the HTRA1 gene variant with age-related macular degeneration in the Japanese population. *Journal of Human Genetics, 52*(7), 636–641.

Rivera, A., Fisher, S. A., Fritsche, L. G., Keilhauer, C. N., Lichtner, P., Meitinger, T., et al. (2005). 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, 14*(21), 3227–3236.

Schmidt, S., Hauser, M. A., Scott, W. K., Postel, E. A., Agarwal, A., Gallins, P., et al. (2006). Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *American Journal of Human Genetics, 78*(5), 852–864.

Yang, Z., Camp, N. J., Sun, H., Tong, Z., Gibbs, D., Cameron, D. J., et al. (2006). A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science, 314*(5801), 992–993.

Yoshida, T., DeWan, A., Zhang, H., Sakamoto, R., Okamoto, H., Minami, M., et al. (2007). HTRA1 promoter polymorphism predisposes Japanese to age-related macular degeneration. *Molecular Vision, 13*, 545–548.