Published in final edited form as:

Exp Eye Res. 2012 January ; 94(1): 187–191. doi:10.1016/j.exer.2011.11.005.

A novel *ARMS2* Splice Variant is identified in Human Retina

Gaofeng Wang^{1,*}, William K. Scott¹, Patrice Whitehead¹, Brenda L. Court¹, Jaclyn L. Kovach², Stephen G. Schwartz², Anita Agarwal³, Sander Dubovy², Jonathan L. Haines⁴, and Margaret A. Pericak-Vance¹

¹John P. Hussman Institute for Human Genomics, Dr. John T. Macdonald Foundation Department of Human Genetics, University of Miami Miller School of Medicine, Miami, FL 33136.

²Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, Miami, FL 33136.

³Department of Ophthalmology, Vanderbilt University, Nashville, TN 37232.

⁴Center for Human Genetics Research, Vanderbilt University, Nashville, TN 37232.

Age-related macular degeneration (AMD, MIM 603075) is the major cause of irreversible visual impairment in developed countries. AMD damages photoreceptors located in the central region of the retina (the macula), resulting in an impairment of central visual acuity (Jager et al., 2008). A locus at chromosome 10q26 has been consistently linked to the disease and represents one of the two strongest genetic effects discovered (Weeks et al., 2001; Majewski et al., 2003; Weeks et al., 2004; Kenealy et al., 2004; Fisher et al., 2005). However, it has been difficult to identify with certainty the genetic variant at this locus biologically responsible for modulating risk of AMD. Studies have shown that the AMD-associated haplotype includes the single nucleotide polymorphisms (SNPs) rs10490924 in gene *ARMS2* (*Age-related maculopathy susceptibility 2*, previously known as hypothetical *LOC387715*) and rs11200638 in the promoter region of *HTRA1* (*High-temperature requirement protein A1*) (Jakobsdottir et al., 2005; Rivera et al., 2005; Schmidt et al., 2006; Yang et al., 2006; Dewan et al., 2006; Kanda et al., 2007; Fritsche et al., 2008). Unfortunately, the variations in genes *ARMS2* and *HTRA1* are in such strong linkage disequilibrium (LD) that their effects are indistinguishable using statistical analysis. While studies have suggested a functional effect for each SNP, evidence that the *HTRA1* polymorphism is functional and influences gene expression is inconsistent (Yang et al., 2006; Chan et al., 2007; Kanda et al., 2007; Chowers et al., 2008; Tuo et al., 2008; Kanda et al., 2010; Wang et al., 2010; Yang et al., 2010; Friedrich et al., 2011). This inconsistency, combined with the observation that the *ARMS2* rs10490924 variant changes the corresponding amino acid sequence of the protein, suggests that *ARMS2* is the more likely AMD gene in this region, although this continues to be debated in the literature.

The basic function of *ARMS2* in human retina remains largely unknown. One major reason is the lack of a homologous gene in commonly used model animals such as mouse or *Drosophila* (Ding et al., 2009). Currently *ARMS2* is only annotated in the genomes of

© 2011 Elsevier Ltd. All rights reserved.

* Correspondence should be addressed to: Gaofeng Wang, Ph.D. John P. Hussman Institute for Human Genomics University of Miami Miller School of Medicine Miami, FL 33136, United States of America gwang@med.miami.edu Telephone: (305) 243- 5434 Fax: (305) 243- 2703.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

All other authors have no conflicts of interest to declare.

humans and higher primates (Francis et al., 2008). For an accurate gene annotation, expressed sequence tags (EST) are required in addition to sequence similarity comparisons and computational predictions (Mount 2000; Ashurst et al., 2003). However, in genome databases, all *ARMS2* ESTs are from the placenta and HT1080 cell (a fibrosarcoma cell line) and no EST from the human retina is available, suggesting that the annotation of the *ARMS2* gene in human retina needs experimental verification. Part of *ARMS2* transcripts has been observed using RT-PCR by previous studies (Rivera et al., 2005; Kanda et al., 2007; Fritsche et al., 2008; Wang et al., 2010; Yang et al., 2010). We therefore examined the full-length *ARMS2* transcript in human retinas.

The 5' and 3' ends of gene transcripts provide important information about the transcription start site and stop site respectively. To verify the sequence of the *ARMS2* transcript, and to search for alternative *ARMS2* transcripts, we performed 5' RACE (rapid amplification of complementary DNA ends) and 3' RACE assays. The premade adaptor-ligated Marathon-Ready human retina cDNA (Clontech) was used as a template to perform both 5' RACE and 3' RACE. The cDNA library is a pool of 99 Caucasian samples. In combination with AP1 and AP2 primers (provided with the cDNA by Clontech), the two reverse primers that were used in the nested PCR cycles are 5'-GGATGATAGACAGTGTTCAGGTGGT-3' and 5'-TCAGTTACCATCGGTCCTGGGTATAGGCGCAGCAT-3'. The 5' RACE PCR product showed one band at ~400bp and the sequence of the band was perfectly aligned to the *ARMS2* region (Figure 1). PCR bands were cloned into pCR4-topo vectors. Eight independent colonies from the band and eight independent colonies from the smears were sequenced. Analysis of the sequences of the smear below the ~400bp band showed that they are non-specific PCR products. The annotated transcription start site (TSS) for *ARMS2* in the database (UCSC Genome Browser, NCBI GRCh37) is at Chr10:124204169, which is largely based on the sequence of ESTs from placenta. The 5' RACE data showed that the aligned sequence starts at Chr10:124203949. This result indicates that the TSS for *ARMS2* in the retina is 220bp upstream from the TSS annotated in the genome databases.

We further used the Marathon-Ready human retina cDNA (Clontech) for 3' RACE. In combination with AP1 and AP2 primers, the two forward primers that were used in the first and the second PCR cycles are 5'-CCACATTATGTCCCTGTACCCTACAT-3' and 5'-CACTGTCTATCATCCACTGCAGCA-3'. The 3' RACE PCR produced one band at ~500bp and the sequence of the band was perfectly aligned to the *ARMS2* region (Figure 2). The 3' RACE confirmed that the polyadenylation site of *ARMS2* transcript is indeed at Chr10:124206858 as annotated in the database (UCSC Genome Browser, NCBI GRCh37).

To examine the full-length *ARMS2* transcripts, we designed “*ARMS2*-full” primers (Forward: 5'-TTTTTCAAATCCCTGGGTCTCT-3'; Reverse: 5'-AGAGAAAGGAGGGCAAGAAAAC-3') near the 5' and 3' ends of the transcripts based on the RACE data. Using human retinal cDNA, the RT-PCR showed a specific band at ~800 bp which is smaller than the expected size of 966 bp. We then sequenced the ~800 bp band. Aligned to the UCSC genome database by BLAT (BLAST-like alignment tool), the sequence of the band indicated: (1) it matched the extended 5' end of the *ARMS2* transcript obtained from RACE assays, suggesting the *ARMS2* transcript in retina is indeed 220bp longer at the 5' end compared to the RNA sequence annotated in the database; (2) the first 187 nucleotides of exon 2 were spliced out. This result was replicated by RT-PCR using the “*ARMS2*-full” primers and by sequencing 4 additional human retinal cDNA samples. No deletion in the corresponding region of exon 2 was found by sequencing genomic DNA of the retinas (data not shown). This result suggests a novel splice variant of *ARMS2* transcripts.

To further characterize *ARMS2* transcription, we designed “*ARMS2*-part” primers (Forward: 5'-CCCTACATGCTGCGCCTATACCCA-3'; Reverse: 5'-TGCACAGAGCAGAAGATGCATCCAATTG-3') to capture both splice variants of *ARMS2*. RT-PCR of human retinal cDNA produced two bands at ~400 bp and ~600 bp. The sequence of the bigger band completely matched the annotated cDNA sequence (designated as isoform-A), while the sequence of the smaller band lacked the same 187 nucleotides of the 5' end of exon 2 compared to the annotated *ARMS2* transcript. This result strongly supports a novel splice variant (designated as isoform-B) of *ARMS2*. By sequence alignment using BLAT, we found that both isoforms use the splice donor site GT at genomic DNA position chr10:124,214,541–124,214,542 (NCBI GRCh37). Isoform-A uses the conventional splice acceptor site AG at the genomic DNA position chr10:124,216,421–124,216,422. Isoform-B uses the alternate splice acceptor site TG at the genomic DNA position chr10:124,216,608–124,216,609 (Figure 3). TG dinucleotides have been reported as alternative 3' splice sites (Szafranski et al., 2007). The sequence of one *ARMS2* EST (BG194076) also suggests the same splice site (Harrington et al., 2001). These results indicate that *ARMS2* expression is more complicated than previously thought, with multiple transcripts.

The presence of the annotated *ARMS2* transcript in retinas has been evaluated by quantitative RT-PCR or other methods (Rivera et al., 2005; 2006; Kanda et al., 2007; Fritsche et al., 2008; Wang et al., 2010; Yang et al., 2010; Friedrich et al., 2011). However, the expression of the novel splice variant of *ARMS2* and more importantly, the ratio of the two *ARMS2* isoforms has not been reported previously. We used quantitative RT-PCR to quantify the *ARMS2* transcripts in human retina. The quantitative RT-PCR assays were custom designed to selectively amplify cDNA fragments of both exons. We first measured *ARMS2* transcript isoforms in punched eye tissues containing choroid/retinal pigment epithelium (RPE)/retina from 5 donor eyes without any clinical history of eye disease by quantitative RT-PCR. The results show that isoform-B is expressed at much higher levels than the isoform-A (Figure 4A, showing the amplification curve of *ARMS2* isoforms). The Δ cycle is about 3-4. Hence, the concentration of isoform A is about 1/8 (2^{-3}) to 1/16 (2^{-4}) of the concentration of isoform B (Kinoshita et al., 1992). This result suggests that the isoform-B is the major transcript form of *ARMS2* in human retinas.

The expression pattern of *ARMS2* transcripts in retinal layers has not yet been presented in the peer-reviewed literature. Previous findings on the distribution of *ARMS2* expression in human retina were performed at the protein level using custom-made antibodies and the results are inconsistent and inconclusive (Fritsche et al., 2008; Kortvely et al., 2010). To examine *ARMS2* expression in retina, we carefully separated retina from choroid/RPE in 5 donor eyes without any clinical history of eye disease. Quantitative RT-PCR (Figure 4B) showed that *ARMS2* is preferentially expressed in choroid/RPE compared to retina (28.3-fold difference, $p < 0.05$). This difference is due to significant differences in isoform-B expression (30.3-fold difference, $p < 0.05$ by Student *t* test), but not isoform-A (4.7-fold difference, not significant). This result suggests that *ARMS2* (particularly isoform-B) is expressed in a higher level in choroid/RPE than in retina.

Accurate gene annotation is critical to functional analysis of associated genetic variants in AMD. Little is known about the basic function of *ARMS2* due to lack of complete knowledge of *ARMS2* transcription. In this study, we found that the *ARMS2* gene transcription starts 220bp upstream from the annotated start site. Importantly, we identified a novel *ARMS2* splice variant which uses TG at the genomic DNA position chr10:124,216,608–124,216,609 as an alternate splice acceptor site. The novel *ARMS2* splice variant is the major transcript isoform in retina, which is expressed in a higher level in choroid/RPE than in retina. Further studies are required to understand the resulting protein

isoforms' function and cellular localization, and the potential pathogenic role of *ARMS2* transcript splice variants in the development of AMD.

Acknowledgments

We thank all the individuals and their families who donated eyes for the study. We also thank the Florida Lions Eye Bank which provides the eye tissues for research purposes. We appreciate Dr. Dale Hedges (University of Miami) for his critical suggestions on data interpretation. This research was supported by National Institutes of Health grants (2R01EY012118-11 to M.A.P.-V., J.L.H., W.K.S and A.A.) and was partially supported by NIH center grant P30-EY014801 and by an unrestricted grant to the University of Miami from Research to Prevent Blindness, New York, NY.

S.G.S. is a consultant for Alimera Sciences and Bausch + Lomb, and is co-inventor on a patent related to use of genetic data to detect steroid responders, licensed to IC Labs. W.K.S., M.A.P.-V., A.A., and J.L.H. are co-inventors on a patent related to use of *ARMS2* genotypes for diagnosis of AMD, licensed to ArcticDx.

REFERENCES

- Ashurst JL, Collins JE. Gene annotation: prediction and testing. *Annu. Rev. Genomics Hum. Genet.* 2003; 4:69–88. [PubMed: 14527297]
- Chan CC, Shen D, Zhou M, Ross RJ, Ding X, Zhang K, Green WR, Tuo J. Human HTRA1 in the archived eyes with age-related macular degeneration. *Trans Am. Ophthalmol. Soc.* 2007; 105:92–97. [PubMed: 18427598]
- Chowers I, Meir T, Lederman M, Goldenberg-Cohen N, Cohen Y, Banin E, Averbukh E, Hemo I, Pollack A, Axer-Siegel R, Weinstein O, Hoh J, Zack DJ, Galbinur T. Sequence variants in HTRA1 and LOC387715/*ARMS2* and phenotype and response to photodynamic therapy in neovascular age-related macular degeneration in populations from Israel. *Mol. Vis.* 2008; 14:2263–71. [PubMed: 19065273]
- Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, Tam PO, Chan WM, Lam DS, Snyder M, Barnstable C, Pang CP, Hoh J. HTRA1 promoter polymorphism in wet age-related macular degeneration. *Science.* 2006; 314:989–992. [PubMed: 17053108]
- Ding X, Patel M, Chan CC. Molecular pathology of age-related macular degeneration. *Prog. Retin. Eye Res.* 2009; 28:1–18. [PubMed: 19026761]
- Fisher SA, Abecasis GR, Yashar BM, Zarepari S, Swaroop A, Iyengar SK, Klein BE, Klein R, Lee KE, Majewski J, Schultz DW, Klein ML, Seddon JM, Santangelo SL, Weeks DE, Conley YP, Mah TS, Schmidt S, Haines JL, Pericak-Vance MA, Gorin MB, Schulz HL, Pardi F, Lewis CM, Weber BH. Meta-analysis of genome scans of age-related macular degeneration. *Hum. Mol. Genet.* 2005; 14:2257–2264. [PubMed: 15987700]
- Friedrich U, Myers CA, Fritsche LG, Milenkovich A, Wolf A, Corbo JC, Weber BH. Risk- and non-risk-associated variants at the 10q26 AMD locus influence *ARMS2* mRNA expression but exclude pathogenic effects due to protein deficiency. *Hum. Mol. Genet.* 2011; 20:1387–99. [PubMed: 21252205]
- Fritsche LG, Loenhardt T, Janssen A, Fisher SA, Rivera A, Keilhauer CN, Weber BH. Age-related macular degeneration is associated with an unstable *ARMS2* (LOC387715) mRNA. *Nat. Genet.* 2008; 40:892–896. [PubMed: 18511946]
- Francis PJ, Appukuttan B, Simmons E, Landauer N, Stoddard J, Hamon S, Ott J, Ferguson B, Klein M, Stout JT, Neuringer M. Rhesus monkeys and humans share common susceptibility genes for age-related macular disease. *Hum. Mol. Genet.* 2008; 17:2673–80. [PubMed: 18535016]
- Harrington JJ, Sherf B, Rundlett S, Jackson PD, Perry R, Cain S, Leventhal C, Thornton M, Ramachandran R, Whittington J, Lerner L, Costanzo D, McElligott K, Boozer S, Mays R, Smith E, Veloso N, Klika A, Hess J, Cothren K, Lo K, Offenbacher J, Danzig J, Ducar M. Creation of genome-wide protein expression libraries using random activation of gene expression. *Nat. Biotechnol.* 2001; 19:440–445. [PubMed: 11329013]
- Jager RD, Mieler WF, Miller JW. Age-related macular degeneration. *N. Engl. J. Med.* 2008; 358:2606–2617. [PubMed: 18550876]

- Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. *Am. J. Hum. Genet.* 2005; 77:389–407. [PubMed: 16080115]
- Kanda A, Chen W, Othman M, Branham KE, Brooks M, Khanna R, He S, Lyons R, Abecasis GR, Swaroop A. A variant of mitochondrial protein LOC387715/ARMS2, not HTRA1, is strongly associated with age-related macular degeneration. *Proc. Natl. Acad. Sci. USA.* 2007; 104:16227–16232. [PubMed: 17884985]
- Kanda A, Stambolian D, Chen W, Curcio CA, Abecasis GR, Swaroop A. Age-related macular degeneration-associated variants at chromosome 10q26 do not significantly alter ARMS2 and HTRA1 transcript levels in the human retina. *Mol. Vis.* 2010; 16:1317–23. [PubMed: 20664794]
- Kenealy SJ, Schmidt S, Agarwal A, Postel EA, De La Paz MA, Pericak-Vance MA, Haines JL. Linkage analysis for age-related macular degeneration supports a gene on chromosome 10q26. *Mol Vis.* 2004; 10:57–61. [PubMed: 14758336]
- Kortvely E, Hauck SM, Duetsch G, Gloeckner CJ, Kremmer E, Alge-Priglinger CS, Deeg CA, Ueffing M. ARMS2 is a constituent of the extracellular matrix providing a link between familial and sporadic age-related macular degenerations. *Invest Ophthalmol Vis Sci.* 2010; 51:79–88.
- Majewski J, Schultz DW, Weleber RG, Weleber RG, Schain MB, Edwards AO, Matise TC, Acott TS, Ott J, Klein ML. Age-related macular degeneration—a genome scan in extended families. *Am. J. Hum. Genet.* 2003; 73:540–550. [PubMed: 12900797]
- Mount SM. Genomic Sequence, Splicing, and Gene Annotation. *Am. J. Hum. Genet.* 2000; 67:788–792. [PubMed: 10986039]
- Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, Weber BH. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. *Hum. Mol. Genet.* 2005; 14:3227–3236. [PubMed: 16174643]
- Schmidt S, Hauser MA, Scott WK, Postel EA, Agarwal A, Gallins P, Wong F, Chen YS, Spencer K, Schnetz-Boutaud N, Haines JL, Pericak-Vance MA. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. *Am. J. Hum. Genet.* 2006; 78:852–864. [PubMed: 16642439]
- Kinoshita T, Imamura J, Nagai H, Shimotohno K. Quantification of gene expression over a wide range by the polymerase chain reaction. *Anal Biochem.* 1992; 206:231–5. [PubMed: 1280006]
- Szafranski K, Schindler S, Taudien S, Hiller M, Huse K, Jahn N, Schreiber S, Backofen R, Platzer M. Violating the splicing rules: TG dinucleotides function as alternative 3' splice sites in U2-dependent introns. *Genome Biol.* 2007; 8:R154. [PubMed: 17672918]
- Tuo J, Ross RJ, Reed GF, Yan Q, Wang JJ, Bojanowski CM, Chew EY, Feng X, Olsen TW, Ferris FL 3rd, Mitchell P, Chan CC. The HtrA1 promoter polymorphism, smoking, and age-related macular degeneration in multiple case-control samples. *Ophthalmology.* 2008; 115:1891–8. [PubMed: 18718667]
- Wang G, Spencer KL, Scott WK, Whitehead P, Court BL, Ayala-Haedo J, Mayo P, Schwartz SG, Kovach JL, Gallins P, Polk M, Agarwal A, Postel EA, Haines JL, Pericak-Vance MA. Analysis of the 3' UTR insertion/deletion polymorphism of ARMS2 gene in age-related macular degeneration. *Hum. Genet.* 2010; 127:595–602. [PubMed: 20182747]
- Wang G, Scott WK, Haines JL, Pericak-Vance MA. Genotype at polymorphism rs11200638 is not correlated with HTRA1 expression level. *Arch. Ophthalmol.* 2010; 128:1491–93. [PubMed: 21060055]
- Weeks DE, Conley YP, Tsai HJ, Mah TS, Rosenfeld PJ, Paul TO, Eller AW, Morse LS, Dailey JP, Ferrell RE, Gorin MB. Age-related maculopathy: an expanded genome-wide scan with evidence of susceptibility loci within the 1q31 and 17q25 regions. *Am J Ophthalmol.* 2001; 32:682–92. [PubMed: 11704029]
- Weeks DE, Conley YP, Tsai HJ, Mah TS, Schmidt S, Postel EA, Agarwal A, Haines JL, Pericak-Vance MA, Rosenfeld PJ, Paul TO, Eller AW, Morse LS, Dailey JP, Ferrell RE, Gorin MB. Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. *Am. J. Hum. Genet.* 2004; 75:174–189. [PubMed: 15168325]

- Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howes K, Zhang K. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. *Science*. 2006; 314:992–993. [PubMed: 17053109]
- Yang Z, Tong Z, Chen Y, Zeng J, Lu F, Sun X, Zhao C, Wang K, Davey L, Chen H, London N, Muramatsu D, Salazar F, Carmona R, Kasuga D, Wang X, Bedell M, Dixie M, Zhao P, Yang R, Gibbs D, Liu X, Li Y, Li C, Li Y, Campochiaro B, Constantine R, Zack DJ, Campochiaro P, Fu Y, Li DY, Katsanis N, Zhang K. Genetic and functional dissection of HTRA1 and LOC387715 in age-related macular degeneration. *PLoS. Genet*. 2010; 6:e1000836. [PubMed: 20140183]

Figure 1.

The initial transcription site of *ARMS2* gene in human retina is 220bp upstream than annotated in UCSC genome browser. A, gel image of 5' RACE B, sequence of the 5' RACE band (arrow); the Marathon cDNA adaptor sequence is in italic and primer sequences for nested PCR are underlined; C, Sequence alignment of 5' RACE results and *ARMS2* gene region.

Figure 2.

Validation of polyadenylation site of *ARMS2* transcript in human retina. A, gel image of 3' RACE; B, sequence of the 3' RACE band; the Marathon cDNA adaptor sequence is in italic and primer sequences for nested PCR are underlined; C, Sequence alignment of 3' RACE results and *ARMS2* gene region.

Figure 3.
The RT-PCR gel image and sequence trace showing the novel splicing site for the *ARMS2* transcripts which result in isoform-A and isoform-B mRNAs.

Figure 4. Preferential expression of *ARMS2* in choroid/RPE. A, Amplification curves of quantitative RT-PCR show that *ARMS2* isoform-B is expressed at a much higher level than the isoform-A in 5 donor eyes without any known eye disease history. B, *ARMS2* (total and isoform-B) is preferentially expressed in choroid/RPE than retina (* $P < 0.05$). The data was presented as mean $\pm$ SD (standard deviation).