Postnatal Overexpression of the Human ARMS2 Gene Does Not Induce Abnormalities in Retina and Choroid in Transgenic Mouse Models

We read the article “Overexpression of Htra1 and Exposure to Mainstream Cigarette Smoke Leads to Choroidal Neovascularization and Subretinal Deposits in Aged Mice” by Nakayama et al.1 with great interest. This elegant study explored the function of mouse Htra1 and human ARMS2 gene using transgenic mice. Here we would like to present our study of postnatal overexpression of ARMS2 gene in transgenic mouse models, which supports the conclusion of the article that human ARMS2 gene does not induce abnormalities in retina and choroid in aged mice.

Using whole-genome association mapping strategy, we previously discovered that the LOC387715 variant was in complete linkage disequilibrium with a functional single nucleotide polymorphism (SNP) in the promoter region of Htra1, which is significantly associated with wet-type age-related macular degeneration (ARMD).2 The locus was later designated by the symbol ARMS2, for age-related maculopathy susceptibility 2.3 In addition to ARMD, the ARMS2 gene was suggested to be associated with polypoidal choroidal vasculopathy (PCV) and retinal angiomatous proliferation (RAP) in multiple genetic studies.3,5

To study the function of ARMS2 in vivo, we generated mice with the transgene that placed a loxP-flanked Westphal STOP sequence between a cytomegalovirus (CMV) promoter and human ARMS2 cDNA with an internal ribosomal entry site (IRES)-linked enhanced green fluorescence protein (EGFP) (Figure). To overexpress ARMS2 gene in adults, the ARMS2 transgenic mice were bred with tamoxifen-dependent Rosa26-Cre-ER mice6 and the double transgenic offspring in which the ARMS2 transgene is silent were obtained. After birth, these mice were treated with tamoxifen through the drinking water, and Cre expression was induced. Cre recombinase excises the STOP sequence of the transgene, leading to the expression of ARMS2 and EGFP driven by the CMV promoter (Figure).

All mouse experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. We have successfully obtained nine mice with Cre excision and ARMS2/EGFP expression. At 12 months of age, the entire back wall of the left eyes including retinas and choroid of these mice were dissected and examined by histochemistry. The retinas of the right eyes were isolated for whole-mount staining of isolectin B4. The tissues from single transgenic ARMS2 mice were used as control. Upon examination, none of the nine mice displayed choroidal neovascularization (CNV), PCV, or other abnormalities. In addition, isolectin B4 staining showed a normal vasculature in the retinas of these ARMS2-overexpressing mice. Our results demonstrated that ubiquitous overexpression of ARMS2 does not lead to a typical AMD phenotype.

Though our data are consistent with the results of Nakayama et al.,1 we would like to stress that ARMS2 is a human gene that does not have a mouse analogue.7 A variant in the ARMS2 gene of rhesus macaques significantly associated with macular drusen, suggesting ARMS2 as a common genetic susceptibility factor in primate species.8 In addition, ARMS2 gene is in linkage disequilibrium with Htra1, and its function might be strictly associated with Htra1.9 In the transgenic mice, ARMS2 transgene was randomly inserted into the mouse genome, and its interactions with Htra1 could not be examined. A knock-in model to insert the ARMS2 gene or its mutant into the analogous Htra1 promoter region is an appropriate strategy for studying the function of the ARMS2 gene in mice.

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References


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