in mice with a targeted type I collagen mutation. Invest Ophthalmol Vis Sci 2004;45:1841-1845.

(54) Akahori M, Obazawa M, Minami M, Noda T, Noda S, Nakaya N, Tomarev S, Sasaoka M, Shimazaki A, Kawase K, Yamamoto T, Miyake Y, Iwata T. Expression of Mutated Optineurin Leads to Normal Tension Glaucoma and Disruption of Optineurin-Rab8 Interaction in Mice. Submitted.

(55) Grozdanic SD, Betts DM, Sakaguchi DS, Allbaugh RA, Kwon YH, Kardon RH. Laser-induced mouse model of chronic ocular hypertension. Invest Ophthalmol Vis Sci 2003;44:4337-4346.

(56) Gross RL, Ji J, Chang P, Pennesi ME, Yang Z, Zhang J et al. A mouse model of elevated intraocular pressure: retina and optic nerve findings. Trans Am Ophthalmol Soc 2003;101:163-169.

(57) Ruiz-Ederra J, Verkman AS. Mouse model of sustained elevation in intraocular pressure produced by episcleral vein occlusion. Exp Eye Res.2006; 82:879-884.

(58) Anderson MG, Smith RS, Hawes NL, Zabaleta A, Chang B, Wiggs JL et al. Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. Nat Genet 2002;30:81-85.

(59) Jakobs TC, Libby RT, Ben Y, John SW, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. J Cell Biol 2005;171:313-325.

(60) Libby RT, Li Y, Savinova OV, Barter J, Smith RS, Nickells RW et al. Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage. PLoS Genet 2005;1:17-26.

(61) Anderson MG, Libby RT, Gould DB, Smith RS, John SW. High-dose radiation with

bone marrow transfer prevents neurodegeneration in an inherited glaucoma. Proc Natl Acad Sci U S A 2005;102:4566-4571.

(62) Steele MR, Inman DM, Calkins DJ, Horner PJ, Vetter ML. Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. Invest Ophthalmol Vis Sci 2006;47:977-985.

(63) Stasi K, Nagel D, Yang X, Wang RF, Ren L, Podos SM et al. Complement component 1Q (C1Q) upregulation in retina of murine, primate, and human glaucomatous eyes. Invest Ophthalmol Vis Sci 2006;47:1024-1029.

(64) Gould DB, Smith RS, John SW. Anterior segment development relevant to glaucoma. Int J Dev Biol 2004;48:1015-1029.

(65) McMahon C, Semina EV, Link BA. Using zebrafish to study the complex genetics of glaucoma. Comp Biochem Physiol C Toxicol Pharmacol 2004;138:343-350.

COMPLEMENT ACTIVATION OF DRUSEN IN PRIMATE MODEL (*Macaca fascicularis*) FOR AGE-RELATED MACULAR DEGENERATION

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1. INTRODUCTION

Dysfunction of the visual system can alter normal human life style and lower quality of life. The most prevalent causes of visual impairment worldwide are cataracts, glaucoma, and age-related macular degeneration (AMD). These eye diseases are responsible for 69% of blindness globally. Although cataracts are the leading cause of blindness worldwide, recent advances in cataract surgery has significantly reduced the visual impairments caused by cataracts especially in developed countries. The most prevalent eye disease for elderly Europeans and Americans is AMD. This degenerative disease progresses from retinal deposits called drusen to neovascularization and retinal hemorrhages resulting in

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irreversible loss of central vision. In spite of the high incidence of AMD, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues from the AMD donors is often difficult, and even when obtained, they are usually collected many hours or even days after death. Because of limitation for human tissue, the availability of animal models is becomes valuable because they can be used to investigate the molecular mechanisms of the disease and to test new therapeutic intervention.

The retina is composed of nine layers of neural and glial cells that are arranged concentrically at the posterior pole of the eye. Incoming light is focused on the central area of the retina called the fovea which is located in the center of the macula. In humans, the average size of the macula is only 6 mm in diameter. The outer surface of the retina is covered by a monolayer of retinal pigment epithelial (RPE) cells which forms a diffusion barrier between the neural retina and the choroidal blood supply. The RPE regulates the transport of proteins to the retina, and controls the hydration and ionic composition of the subretinal space. The physiological condition of the RPE is closely associated with the pathogenesis of AMD.

2. INTRODUCTION OF AMD

AMD is a blinding disorder characterized by a marked decrease in central vision associated with RPE atrophy with or without choroidal neovascularization (CNV). Many factors including genetic, behavioral, and environmental, are involved in this disease. AMD is characterized by the degeneration of cone photoreceptors in the foveal region of the retina resulting in a decrease of central visual acuity. The progressive impairment of the retinal pigment epithelial (RPE) cells, and damage to Bruch's membrane and choriocapillaris results in retinal atrophy and photoreceptor dysfunction. In some cases, CNV develops, and the new vessels penetrate Bruch's membrane and pass into the

subretinal space.

Two types of AMD are recognized; the non-neovascular type is called the dry-type AMD and includes more than 80% of the cases, and the neovascular type is called the wet-type AMD which is progressive with a higher probability of blindness. The prevalence of AMD differs considerably among the different ethnic groups, but the incidence increases with age in all groups. A lower prevalence of AMD has been reported in individuals of African ancestry than of Anglo-Saxon ancestry. Other risk factors for AMD are cigarette smoking, obesity, hypertension, and atherosclerosis.

3. GENETICS OF AMD

Epidemiological studies have shown that genetic factor play critical role for AMD. Twin studies have previously shown a higher concordance for AMD in monozygotic twins than in dizygotic twins (Heiba, Elston, Klein, and Klein 1994; Seddon, Ajani, and Mitchell 1997; Hammond, Webster, Snieder, Bird, Gilbert, and Spector 2002). In addition, first degree relatives of individuals with AMD have higher incidence of AMD over individuals without a family history of AMD. Genetic segregation studies have also shown a genetic effect that accounts for approximately 60% of AMD with a single major gene accounting for about 55% of the risk of developing AMD. Previous data have suggested that the etiology of AMD has a significant genetic component. Only a small proportion of the families with AMD show Mendelian inheritance, and the majority of the individuals inherit AMD in a complex multi-gene pattern. With the help of the haplotype marker project (HapMap Project), genome wide scanning has identified at least 13 loci linked to AMD on different chromosomes (Iyengar, Song, Klein, Klein, Schick, Humphrey, Millard, Liptak, Russo, Jun, Lee, Fijal, and Elston 2004; Schick, Iyengar, Klein, Klein, Reading, Liptak, Millard, Lee, Tomany, Moore, Fijal, and Elston 2003; Majewski, Schultz, Weleber, Schain, Edwards, Matise, Acott, Ott, and Klein 2003). Recently, a polymorphism of complement factor H (CFH) gene (*Y402H*) was shown to be

associated with an increased risk for AMD (Klein, Zeiss, Chew, Tsai, Sackler, Haynes, Henning, SanGiovanni, Mane, Mayne, Bracken, Ferris, Ott, Barnstable, and Hoh 2005; Edwards, Ritter, Abel, Manning, Panhuysen, and Farrer 2005; Haines, Hauser, Schmidt, Scott, Olson, Gallins, Spencer, Kwan, Noureddine, Gilbert, Schnetz-Boutaud, Agarwal, Postel, and Pericak-Vance 2005; Hageman, Anderson, Johnson, Hancox, Taiber, Hardisty, Hageman, Stockman, Borchardt, Gehrs, Smith, Silvestri, Russell, Klaver, Barbazetto, Chang, Yannuzzi, Barile, Merriam, Smith, Olsh, Bergeron, Zernant, Merriam, Gold, Dean, and Allikmets 2005) .

These results were confirmed in many of the countries with large Caucasian populations but not in Japan (Okamoto, Umeda, Obazawa, Minami, Noda, Mizota, Honda, Tanaka, Koyama, Takagi, Sakamoto, Saito, Miyake, and Iwata 2006; Gotoh, Yamada, Hiratani, Renault, Kuroiwa, Monet, Toyoda, Chida, Mandai, Otani, Yoshimura, and Matsuda 2006). This gene is located on chromosome 1q25-31 where one of the candidate loci was identified by linkage studies. Another recent study reported that a haplotype association of tandemly located complement 2 and factor B was protective for AMD (Gold, Merriam, Zernant, Hancox, Taiber, Gehrs, Cramer, Neel, Bergeron, Barile, Smith, AMD Genetics Clinical Study Group, Hageman, Dean, Allikmets 2006). HTRA1, a serine protease 11 was recently discovered to be strongly associated with AMD. Unlike the CFH, our study shows strong association with this gene for Japanese AMD patients (Yang, Camp, Sun, Tong, Gibbs, Cameron, Chen, Zhao, Pearson, Li, Chien, Dewan, Harmon, Bernstein, Shridhar, Zabriskie, Hoh, Howes, and Zhang 2006; Dewan, Liu, Hartman, Zhang, Liu, Zhao, Tam, Chan, Lam, Snyder, Barnstable, Pang, and Hoh 2006).

4. BIOCHEMISTRY OF AMD

The early stage of the dry type AMD is characterized by a thickening of Bruch's membrane, aggregation of pigment granules, and increasing numbers of drusen. The thickening of Bruch's membrane obstructs its function as a 'barrier' between the choroid

and the RPE that protects the neural retina from the choriocapillary. Drusen are small yellowish-white deposits that are composed of lipids, proteins, glycoproteins, and glycosaminoglycans. They accumulate in the extracellular space and the inner aspects of Bruch's membrane. Drusen are not directly associated with visual loss but represent a risk factor for both the dry-type and wet-type AMD. The classification of hard and soft drusen is based on their size, shape, and color; hard drusen are yellowish with diameters <50 μm and are found in eyes that are less likely to progress to advanced stages of the disease, while soft drusen are darker yellow and larger in size, and are found in eyes more likely to progress to more advanced stages of AMD. A small percentage of dry-type AMD patients progress to the late stage of the wet-type AMD that is characterized by geographic atrophy or detachment of RPE and the development of CNV in the macular region. The presence of a CNV is the factor that most damages the neural retina because the newly developed vessels grow from the choriocapillaris through Bruch's membrane and extend laterally through the RPE cell layer (classic CNV) or extend between the inner Bruch's membrane and RPE (occult CNV). In advanced stages of AMD, the CNV and fluid leaked into the subretinal or intraretinal regions leads to cell death and retinal detachment.

Recent analyses of the progression of drusen have provided important clues that help understand the molecular pathology of AMD. Using both immunohistochemistry and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes (Russell, Mullins, Schneider, and Hageman 2000; Mullins, Russell, Anderson, and Hageman 2000). These molecules include components of the complement pathway and modulators of complement activation, viz., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1. In addition, molecules triggering inflammation, amyloid P component, a1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. Additional proteins such as crystallins, EEFMP1, and amyloid-beta have been also found

in drusen. The presence of immunoreactive proteins and the oxidative modifications of many proteins in drusen imply that both oxidation and immune functions are involved in the pathogenesis of AMD. These findings suggest that complement activation triggers innate immune responses in the subretinal space. The co-distribution of IgG and terminal complement complexes in drusen indicate that immune responses that directly target antigens in retinal cells might also be occurring. Anti-retinal autoantibodies have been reported in a number of ocular disorders, e.g., macular degeneration in an aged monkey model.

5. PRIMATE MODEL FOR AMD

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (Chader 2002). However in mammals, a well-defined fovea is found only in primates (humans and monkeys), and a search for a monkey line affected with macular degeneration has been persistent for a long time. A monkey with macular degeneration was first described by Stafford et al in 1974. They reported that 6.6 % of the elderly monkeys they examined showed pigmentary disorders and drusen-like spots (Stafford, Anness, and Fine 1984). El-Mofty et al reported that the incidence of maculopathy was 50% in a colony of rhesus monkeys at the Caribbean Primate Research Center of the University of Puerto Rico (El-Mofty, Gouras, Eisner, and Balazs 1978). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) (Suzuki Monkeys) in 1986 with a large number of small drusen around the macular region (Nicolas, Fujiki, Murayama, Suzuki, Mineki, Hayakawa, Yoshikawa, Cho, Kanai 1996; Nicolas, Fujiki, Murayama, Suzuki, Shindo, Hotta, Iwata, Fujimura, Yoshikawa, Cho, Kanai 1996; Suzuki, Terao, and Yoshikawa 2003). This single affected monkey has multiplied to a large pedigree of more than 65 affect and 210 unaffected monkeys. Drusen were observed in the

macular region as early as one year after birth, and the numbers increased and spread toward the peripheral retina throughout life. No histological abnormalities have been found in the retina, retinal vessels, or choroidal vasculatures of the eyes with drusen. However, abnormality in electroretinogram (ERG) were observed in sever case showing dysfunction of the macula.

Figure 1. Fundus photograph of both eyes of Suzuki Monkey showing accumulation of drusen (white spot) around the macular region.

Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged macular degeneration sporadically found in older monkeys and also with human drusen (Umeda, Ayyagari, Allikmets, Suzuki, Karoukis, Ambasudhan, Zernant, Okamoto, Ono, Terao, Mizota, Yoshikawa, Tanaka, and Iwata 2005; Umeda, Suzuki, Okamoto, Ono, Mizota, Terao, Yoshikawa, Tanaka, and Iwata 2005; Ambati, Anand, Fernandez, Sakurai, Lynn, Kuziel, Rollins, and Ambati 2003). These observations have shown that the Suzuki Monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate. More than 240 loci are

being investigated to try to identify the disease causing gene and to understand the biological pathways leading to complement activation. Simultaneously, we have been studying a colony of aged monkeys which develop drusen after 15 years of birth.

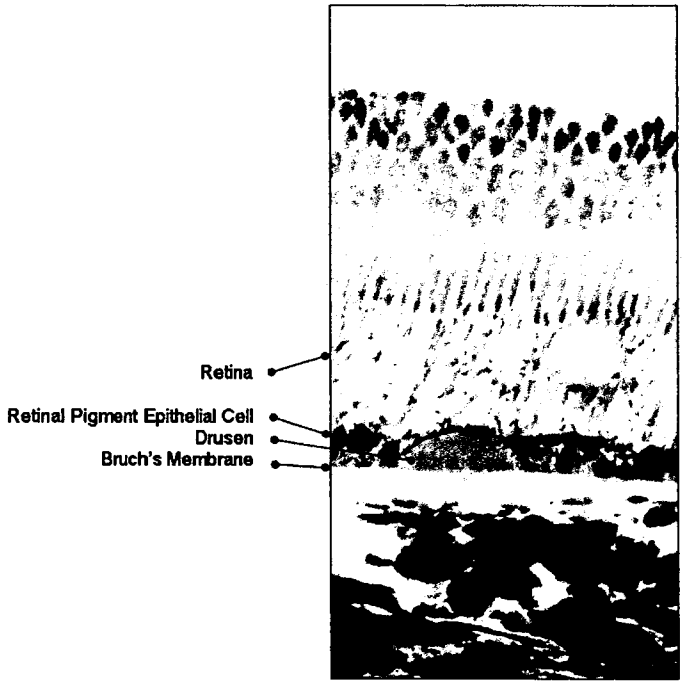

Figure 2. Retinal histological section of affected Suzuki Monkey showing the accumulation of drusen between the retinal pigment epithelium and Bruch's membrane.

Drusen components of these sporadically found affected monkeys were compared with human and Suzuki Monkeys by classical immunohistochemical techniques and by proteome analysis using mass spectrometer. Significant finding was that drusen contained protein molecules that mediate inflammatory and immune processes. These include immunoglobulins, components of complement pathway, and modulators for complement

activation (e.g., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1), molecules involved in the acute-phase response to inflammation (e.g., amyloid P component, a1-antitrypsin, and apolipoprotein E), major histocompatibility complex class II antigens, and HLA-DR antigens (Umeda et al. 2005). Cellular components have also been identified in drusen, including RPE debris, lipofuscin, and melanin, as well as processes of choroidal dendritic cells, which are felt to contribute to the inflammatory response. In addition to immune components, a number of other proteins were found in drusen. These appear to be vitronectin, clusterin, TIMP-3, serum amyloid P component, apolipoprotein E, IgG, Factor X, crystallins, EEFMP1, and amyloid-beta. The presence of immunoreactive proteins and oxidative modified proteins implicate both oxidation and immune functions in the pathogenesis of AMD.

The eyes of monkey are structurally similar to human eyes which make them extremely valuable for AMD studies. However, there are limitations in using this species over other laboratory animals. Monkeys have a relatively longer life span, have a longer gestation period, have a lower birth numbers resulting in a slower expansion of the pedigree, more difficult to genetically manipulate, and the maintenance cost is high. In the other laboratory animals, the differences in the eye structure, lack of a fovea, and a low cone/rod ratio compared to humans have been considered to be a disadvantage for using them as AMD models. However, they are easier to manipulate genetically and easier and less expensive to maintain. This has made the development of a mouse model of AMD very attractive, and a number of mouse AMD models have been reported recently.

6. MOUSE MODEL FOR AMD

The mouse model described by Ambati et al is deficient either in monocyte chemoattractant protein-1 or its cognate C-C chemokine receptor-2. These mice were

found to develop the cardinal features of AMD including accumulation of lipofuscin in drusen beneath the RPE, photoreceptor atrophy, and CNV (Ambati et al. 2003). An impairment of macrophage recruitment allowed the accumulation of C5a and IgG, which leads to the production of vascular endothelial growth factor by the RPE cells and the development of CNVs. Another mouse model that has three known AMD risk factors: age, high fat cholesterol rich diet, and expression of human apolipoprotein E (apoE2, apoE3, apoE4) has been developed (Malek, Johnson, Mace, Saloupis, Schmechel, Rickman, Toth, Sullivan, and Bowes Rickman 2005). ApoE4-deficient mice are severely affected showing diffuse subretinal pigment epithelial deposits, drusen, thickened Bruch's membrane, and atrophy, hypopigmentation, and hyperpigmentation of the RPE.

Oxidative stress has long been linked to the pathogenesis of AMD. Imamura et al reported a Cu, Zn-superoxide dismutase (SOD1)-deficient mice that had features typical of AMD in human. Senescent Sod1 (-/-) mice had drusen, thickened Bruch's membrane, and choroidal neovascularization (Imamura, Noda, Hashizume, Shinoda, Yamaguchi, Uchiyama, Shimizu, Mizushima, Shirasawa, and Tsubota 2006). The number of drusen increased with age and also after exposure of young Sod1 (-/-) mice to excess light. The retinal pigment epithelial cells of Sod1 (-/-) mice showed oxidative damage, and their beta-catenin-mediated cellular integrity was disrupted. These findings suggested that oxidative stress may affect the junctional proteins necessary for the barrier integrity of the RPE. These observations strongly suggested that oxidative stress may play a major role in AMD.

The complement components, C3a and C5a, are present in drusen, and were observed in Bruch's membrane of a laser-induced CNV mice model. Neutralization of C3a or C5a by antibody or by blockade of their receptors by a complement inhibitor significantly reduced the CNV (Nozaki, Raisler, Sakurai, Sarma, Barnum, Lambris, Chen, Zhang, Ambati, Baffi, and Ambati 2006). These observations revealed a role for immunological mechanisms for the angiogenesis and provided evidence for future

therapeutic strategies for AMD. Although the pathology of AMD is pronounced in the macula area, it is not confined to this region. Characteristics of human AMD such as thickening of Bruch's membrane, accumulation of drusen, and CNV have been observed in mouse models. Nevertheless, the primate model will still be the choice for AMD studies, especially at the stage when new therapeutic methods are tested and evaluated for the first time. However, it would be wise and more productive to study both primate and mouse models in AMD research. This will be necessary to learn the mechanisms underlying the disease and to identify clinical and molecular markers for the early stages of AMD. The findings from these studies will provide critical information needed to develop therapies for AMD.

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8. REFERENCES

Ambati, J., Anand, A., Fernandez, S., Sakurai, E., Lynn, B. C., Kuziel, W. A., Rollins, B. J., Ambati, B. K. (2003) An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. Nat. Med. 9,1390-1397.

Chader, G. J. (2002) Animal models in research on retinal degenerations: Past progress and future hope. Vision Res 42,393-399.

Dewan A., Liu, M., Hartman, S., Zhang, S. S., Liu, D. T., Zhao, C., Tam, P. O., Chan, W. M., Lam, D. S., Snyder, M., Barnstable, C., Pang, C. P., Hoh, J. (2006) HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 314,989-992.

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 308,421-424.

El-Mofty A., Gouras, P., Eisner, G., Balazs, E. A. (1978) Macular degeneration in rhesus monkey (Macaca mulatta). Exp. Eye Res. 27,499-502.

Gold, B., Merriam, J. E., Zernant, J., Hancox, L. S., Taiber, A. J., Gehrs, K., Cramer, K., Neel, J., Bergeron, J., Barile, G. R., Smith, R. T. (2006) AMD Genetics Clinical Study Group; G. S. Hageman, M. Dean, R. Allikmets. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. Nat. Genet. 38,458-462.

Gotoh, N., Yamada, R., Hiratani, H., Renault, V., Kuroiwa, S., Monet, M., Toyoda, S., Chida, S., Mandai, M., Otani, A., Yoshimura, N., Matsuda, F. (2006) No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese. Hum. Genet. 120,139-143.

Hageman, G. S., Anderson, D. H., Johnson, L. V., Hancox, L. S., Taiber, A. J., Hardisty, L. I., Hageman, J. L., Stockman, H. A., Borchardt, J. D., Gehrs, K. M., Smith, R. J., Silvestri, G., Russell, S. R., Klaver, C. C., Barbazetto, I., Chang, S., Yannuzzi, L. A., Barile, G. R., Merriam, J. C., Smith, R. T., Olsh, A. K., Bergeron, J., Zernant, J., Merriam, J. E., Gold, B., Dean, M., Allikmets, R. (2005) A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. Proc. Natl. Acad. Sci. U S A 102,7227-7232.

Haines, J. L., Hauser, M. A., Schmidt, S., Scott, W. K., Olson, L. M., Gallins, P., Spencer, K. L., Kwan, S. Y., Noureddine, M., Gilbert, J. R., Schnetz-Boutaud, N., Agarwal, A., Postel, E. A., Pericak-Vance, M. A. (2005) Complement factor H variant increases the risk of age-related macular degeneration. Science 308,419-421.

Hammond, C. J., Webster, A. R., Snieder, H., Bird, A. C., Gilbert, C. E., Spector, T. D. (2002) Genetic influence on early age-related maculopathy: A twin study. Ophthalmology 109,730-736.

Heiba, I. M., Elston, R. C., Klein, B. E., Klein, R. (1994) Sibling correlations and segregation analysis of age-related maculopathy: The Beaver Dam Eye Study. Genet. Epidemiol. 11,51-67.

Imamura, Y., Noda, S., Hashizume, K., Shinoda, K., Yamaguchi, M., Uchiyama, S., Shimizu, T., Mizushima, Y., Shirasawa, T., Tsubota, K. (2006) Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. Proc Natl Acad Sci U S A 103,11282-11287.

Iyengar, S. K., Song, D., Klein, B. E., Klein, R., Schick, J. H., Humphrey, J., Millard, C., Liptak, R., Russo, K., Jun, G., Lee, K. E., Fijal, B., Elston, R. C. (2004) Dissection of genomewide-scan data in extended families reveals a major locus and oligogenic susceptibility for age-related macular degeneration. Am. J. Hum. Genet. 74,20-39.

Klein, R. J., Zeiss, C., Chew, E. Y., Tsai, J. Y., Sackler, R. S., Haynes, C., Henning, A. K., SanGiovanni, J. P., Mane, S. M., Mayne, S. T., Bracken, M. B., Ferris, F. L., Ott, J., Barnstable, C., Hoh, J. (2005) Complement factor H polymorphism in age-related macular degeneration. Science 308,385-389.

Schick, J. H., Iyengar, S. K., Klein, B. E., Klein, R., Reading, K., Liptak, R., Millard, C., Lee, K. E., Tomany, S. C., Moore, E. L., Fijal, B. A., Elston, R. C. (2003) A whole-genome screen of a quantitative trait of age-related maculopathy in sibships from the Beaver Dam Eye Study. Am. J. Hum. Genet. 72,1412-1424.

Majewski, J., Schultz, D. W., Weleber, R. G, Schain, M. B., Edwards, A. O., Matise, T. C., Acott, T. S., Ott, J., Klein, M. L. (2003) Age-related macular degeneration--a genome scan in extended families. Am. J. Hum. Genet. 73,540-550.

Malek, G., Johnson, L. V., Mace, B. E., Saloupis, P., Schmechel, D. E., Rickman, D. W., Toth, C. A., Sullivan, P. M., Bowes Rickman, C. (2005) Apolipoprotein E allele-dependent pathogenesis: a model for age-related retinal degeneration. Proc. Natl. Acad. Sci. U S A. 102,11900-11905.

Mullins, R. F., Russell, S. R., Anderson, D. H., Hageman, G. S. (2000) Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis, amyloidosis, and dense deposit disease. FASEB J 14,835-846.

Nicolas, M. G, Fujiki, K., Murayama, K., Suzuki, M. T., Mineki, R., Hayakawa, M., Yoshikawa, Y., Cho, F., Kanai, A. (1996) Studies on the mechanism of early onset macular degeneration in cynomolgus (Macaca fascicularis) monkeys. I. Abnormal concentrations of two proteins in the retina. Exp. Eye Res. 62,211-219.

Nicolas, M. G, Fujiki, K., Murayama, K., Suzuki, M. T., Shindo, N., Hotta, Y., Iwata, F., Fujimura, T., Yoshikawa, Y., Cho, F., Kanai, (1996) A. Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. Exp. Eye Res.

62,399-408.

Nozaki, M., Raisler, B. J., Sakurai, E., Sarma, J. V., Barnum, S. R., Lambris, J. D., Chen, Y., Zhang, K., Ambati, B. K., Baffi, J. Z., Ambati J. (2006) Drusen complement components C3a and C5a promote choroidal neovascularization. Proc. Natl. Acad. Sci. U S A 103,2328-2333.

Okamoto, H., Umeda, S., Obazawa, M., Minami, M., Noda, T., Mizota, A., Honda, M., Tanaka, M., Koyama, R., Takagi, I., Sakamoto, Y., Saito, Y., Miyake, Y., Iwata, T. (2006) Complement factor H polymorphisms in Japanese population with age-related macular degeneration. Mol. Vis. 12,156-158.

Russell, S. R., Mullins, R. F., Schneider, B. L., Hageman, G. S. (2000) Location, substructure, and composition of basal laminar drusen compared with drusen associated with aging and age-related macular degeneration. Am J Ophthalmol 129,205-214.

Seddon, J. M., Ajani, U. A., Mitchell, B. D. (1997) Familial aggregation of age-related maculopathy. Am. J. Ophthalmology 123,199-206.

Stafford, T. J., Anness, S. H., Fine, B. S. (1984) Spontaneous degenerative maculopathy in the monkey. Ophthalmology 91,513-521.

Yang, Z., Camp, N. J., Sun, H., Tong, Z., Gibbs, D., Cameron, D. J., Chen, H., Zhao, Y., Pearson, E., Li, X., Chien, J., Dewan, A., Harmon, J., Bernstein, P. S., Shridhar, V., Zabriskie, N. A., Hoh, J., Howes, K., Zhang, K.. (2006) A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science. 314,992-993.

Umeda, S., Ayyagari, R., Allikmets, R., Suzuki, M. T., Karoukis, A. J., Ambasudhan, R., Zernant, J., Okamoto, H., Ono, F., Terao, K., Mizota, A., Yoshikawa, Y., Tanaka, Y., Iwata, T. (2005) Early-onset macular degeneration

with drusen in a cynomolgus monkey (Macaca fascicularis) pedigree: exclusion of 13 candidate genes and loci. Invest. Ophthalmol. Vis. Sci. 46,683-691.

Umeda, S., Suzuki, M. T., Okamoto, H., Ono, F., Mizota, A., Terao, K., Yoshikawa, Y., Tanaka, Y., Iwata, T. (2005) Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (Macaca fascicularis). FASEB J. 19,1683-1685.

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網膜・硝子体のプロテオーム解析

1）はじめに

ヒトゲノムプロジェクトが終了し、約 2 万 3 千個の遺伝子が発見された。この遺伝子から転写される RNA（トランスクリプトーム）から 10 万種類以上のタンパク質が生成されると予測されている（図１）。近年、タンパク質のイオン化技術や質量分析計の精度が向上し、さらにそれを制御・解析するソフトウエアの改良によって、質量分析計の専門技術者でなくても細胞、組織、体液などのタンパク質（プロテオーム）を網羅的に測定し、データを解析することがある程度可能になってきた。健常と病気のプロテオームを比較し、その違いを明らかにすることは、疾患の発症機序を解明するための手がかりとなる情報をもたらすだけでなく、疾患バイオマーカーとして早期診断法の確立にも応用が期待される。硝子体プロテオームは疾患網膜の状態を反映してダイナミックに変化していると考えられる。また、網膜疾患には脈絡膜毛細血管と深い関わりのあるものがあり、網膜成分の血管への漏出による血漿成分の変化として捉えられることが期待されている。本編ではここ数年間の質量分析計を用いた網膜・硝子体の網羅的タンパク質解析に焦点を絞り、その利用方法と臨床応用への可能性について紹介したい。

2）硝子体のプロテオーム

硝子体は眼球内で最も体積を占める透明なゼリー状の組織であり、網膜と接しているために網膜疾患によってその組成は大きく変化していると考えられる。硝子体、房水、血漿の蛋白量をそろえて 1 次元電気泳動を行うと見分けがつかないほど泳動パターンは類似している（図２）。これは房水も硝子体も血漿由来の体液であり、血漿を構成する蛋白が硝子体や房水にも多く含まれていることを意味している。これまで我々が扱った房水や硝子体検体の蛋白濃度は出血の有無などによって 0.1-1.0mg/ml であるのに対し、血漿は 50-70mg/ml と 50 倍以上の蛋白濃度が測定されている。高蛋白濃度の血漿はプロテオミクス（プロテオーム研究）の分野で最も解析が先行しており、1 万種類のタンパク質がすでに同定されている。血漿は２２種類の蛋白が９９％を占めており、微量蛋白は残り 1％に含まれている（図３）。質量分析計の性質上、高い濃度で存在する蛋白から検出されるので、血漿を無分画のまま測定するとアルブミン、免疫グロブリン、トランスフェリンなどが検出され、微量タンパク質は検出されにくい。この数年間に２２種類の蛋白を除去するための前処理技術として、各タ