235 - A491
Association of CFH, LOC387715, and HTRA1 Polymorphisms With Exudative Age-Related Macular Degeneration in Mainland Chinese


Purpose: To examine the common single-nucleotide polymorphisms in complement factor H (CFH), LOC387715, and HTRA1 genes as potential risk factors for exudative age-related macular degeneration (AMD) in the mainland Chinese.

Methods: A cohort of 121 unrelated patients with exudative AMD and 132 age- and sex-matched control subjects, all unrelated ethnic Chinese from Northern China, enrolled in this study. Genomic DNA was extracted from blood leukocytes. Genotyping for CFH Y402H, LOC387715 A696S, and HTRA1 -625C>A polymorphisms was performed by analyzing amplified genomic fragments using a method of polymerase chain reaction (PCR) followed by allele specific restriction enzyme digestion and direct sequencing.

Results: Frequencies of the CFH variant Y402H in AMD patients and control subjects were 10.3% and 7.6%, respectively, and were not associated with exudative AMD in our study population (p=0.353). Significant associations were detected for exudative AMD in mainland Chinese with LOC387715 variant A696S and the HTRA1 promoter polymorphism -625C>A. Homozygote for the LOC387715 A696S polymorphism was associated with an odds ratio (OR) of 5.45 (95% confidence interval, 2.59-11.49) for exudative AMD. An odds ratio of 7.90 (95% confidence interval, 3.61-17.26) for exudative AMD was found among carriers of the homozygous HTRA1 -625C>A genotype. None of the individuals with both homozygous LOC387715 A696S and homozygous HTRA1 -625C>A polymorphism were associated with an odds ratio of 794 (95% confidence interval, 3.49-19.04).

Conclusions: Our data suggest that the LOC387715 and HTRA1 polymorphisms, but not the CFH Y402H variant, are associated with a higher risk of exudative AMD in the mainland Chinese. The low frequency of CFH Y402H variant was further confirmed among the Chinese population.

CR: N. Liu, None; Y. Xu, None; N. Guan, None; X. Ju, None; X. Yang, None; K. Ma, None; H. Zhou, None; F. Zhang, None.
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237 - A493
Plekha1-loc387715-htra1 Polymorphisms and Exudative Age-Related Macular Degeneration in the French Population

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Purpose: To examine the common single-nucleotide polymorphisms in complement factor H (CFH), LOC387715, and HTRA1 genes as potential risk factors for exudative age-related macular degeneration (AMD) in a French population.

Methods: A cohort of 200 patients affected with exudative AMD were genotyped for the CFH Y402H, LOC387715 A696S, and HTRA1 -625C>A polymorphisms. Based on the genotype, 4 homogygous groups were extracted from the entire cohort: double homozygous for wild alleles of both CFH1 and CFH genes (group 1), homozygous for the at-risk allele of the HTRA1 gene only (group 2), homozygous for at-risk allele of CFH gene only (group 3), and double homozygous for both carriers for at-risk allele of CFH gene (group 4). Based on the genotype, 4 homogygous groups were extracted from the entire cohort: double homozygous for wild alleles of both HTRA1 and CFH genes (group 1), homozygous for the at-risk allele of the HTRA1 gene only (group 2), homozygous for at-risk allele of CFH gene only (group 3), and double homozygous for both carriers for at-risk allele of CFH gene (group 4). Based on fluoroecein angiography, intravascular green angiography and optical coherence tomography, exudative AMD was graded as classic CNV, predominantly classic CNV, minimally classic CNV, retinal angiomasisis proliferation or polypoidal choroidal vasculopathy.

Results: Group 1 (n=9; mean age=72.8) presented 44.4% classic and predominantly classic CNV, 33.3% occult CNV and 11.1% minimally classic CNV. Group 2 (n=12; mean age=71.7) presented 50.0% classic and predominantly classic CNV, 33.3% occult CNV and no minimally classic CNV. Group 3 (n=30; mean age=71.1) presented 10.0% classic and predominantly classic CNV, 63.3% occult CNV and 13.3% of minimally classic CNV. Group 4 (n=20; mean age=69.2) presented 7.0% of classic and predominantly classic CNV, 9.9% of occult CNV and 11.8% of minimally classic CNV. Comparison between group 2 versus group 3 demonstrated that occult CNV and MC CNV were more frequently observed in group 3 than in group 2 (p=0.02). Occult plus minimally classic CNV were more associated with the Y402H1 CFH polymorphism (OR=1.9 [CI=0.5-7.3]) and no significant association between classic and predominantly classic CNV were more prevalent, but not significantly associated with the HTRA1 at-risk alleles (OR 2.5 [CI=0.8-6.4], p=0.14).

Conclusions: This attempt for a genotype-pi-angiographic correlation in a French population of exudative AMD suggests an association between occult CNV and CFH gene and an association between classic and HTRA1 gene.

CR: E. H. Souied, None; N. Leveziel, None; J. Zerbib, None; F. Richard, None; G. Quereux, None; V. Fuseenoue-Bacchi, None; G. Coscas, None; G. Soubran, None; P. Benlian, None.
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238 - A494
Association Between Systemic C-Reactive Protein Levels and Single Nucleotide Polymorphisms in HTRA1 and LOC387715


Purpose: It has been reported that systemic C-reactive protein (CRP) levels are elevated among patients with advanced age-related macular degeneration (AMD) (JAMA 2004, Ophthalmology 2007). On the other hand single nucleotide polymorphisms (SNPs) in the HTRA1 promoter (rs1200638) and adjacent putative gene LOC387715 (rs10490924) are strongly associated with AMD (Science 2006). Our aim was to determine the relationship between the CRP levels and these SNPs.

Methods: One-hundred and seventy-eight patients with neovascular AMD and 56 subjects without AMD or any macular abnormality were studied. The serum CRP level was measured by a high-sensitivity assay using a latex aggregation immunoassay. Genomic DNA was extracted from peripheral blood, and the genotypes were determined for the SNPs in HTRA1 and LOC387715. The CRP levels in the individuals with risk alleles were compared with that without risk alleles using the Kruskal-Wallis nonparametric cross-tabulations between CRP groups and the genotypes were compared using logistic regression analysis by computing the odds ratios (ORs) and 95% confidence intervals (CIs) after the study populations were divided into quartiles.

Results: In the AMD group, the median CRP levels among cases having the risk alleles were: 
- rs1200638 (CA and AA genotypes) and rs10490924 (GT and TT genotypes). The values were significantly higher than that without the risk alleles for rs1200638 (GG genotype: 0.065 mg/l and rs10490924 (GG genotype: 0.065 mg/l, respectively (p<0.05). After adjusting for baseline characteristics, cases in the higher quartiles of CRP had significantly increased risks to have the risk alleles for both rs1200638 (OR, 4.85; 95% CIs, 1.22-19.2) and rs10490924 (OR, 6.30; 95% CIs, 1.61-26.4). In subjects without AMD, the median CRP levels were 0.066mg/l and 0.070mg/l in those having the risk alleles for rs1200638 and rs10490924, respectively, and 0.051mg/l and 0.054mg/l in those without the risk alleles, respectively. The frequency of the risk allele for rs10490924 was significantly increased for the highest quartile of CRP (OR=9.92; 95% CIs, 1.20-81.8).

Conclusions: We found significant associations between elevated serum CRP levels and the associated SNPs in HTRA1 and LOC387715. These results suggest that the SNPs are associated with inflammatory processes that may be underlying pathogenesis of AMD.

CR: T. Yasuma, None; M. Nakamura, None; M. Kikuchi, None; K. Ishikawa, None; M. Kikuchi, None; T. Yamakoshi, None; H. Terasaki, None.
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239 - A495
Variants in the 10q26 Gene Cluster (LOC387715 and HTRA1) Exhibit Enhanced Risk of Age-Related Macular Degeneration Along With CFH in Indian Patients

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Purpose: Single nucleotide polymorphisms (SNPs) in the LOC387715 (rs10490924), HTRA1 (rs11200638) and CFH (rs1061170) have been implicated in age-related macular degeneration (AMD). Earlier we demonstrated the association of rs1061170 in a cohort from India. The present study was undertaken to understand the involvement of LOC387715 and HTRA1 in that cohort.

Methods: The coding region of LOC387715 and the promoter of HTRA1 were resequenced in cases (n=250) and controls (n=250). Odds ratios were calculated to assess the risk of individual genotypes. Linkage disequilibrium (LD) and haplotype frequencies were estimated with Haploview software. Population attributable risk (PAR) were calculated for the additive effect of these SNPs. Meta analysis was performed to discern the statistical evidence of these associations.

Results: AMD cases exhibited a higher frequency of “risk” alleles of LOC387715 (rs10490924 (p=5.34x10^-10) and HTRA1 (rs11200638 (p=4.32x10^-10) and rs2672998 (p=3.39x10^-10)) and higher disease odds in the corresponding “risk” genotypes. The rs1049024 and rs1200638 SNPs were in light LD (D'=0.90; 95% CI, 0.84-0.93). The “G-C-G-T-A-C” was the risk haplotype (p=0.0045), while the “G-C-G-C-G-T” haplotype was protective (p=2.10x10^-11). The additive effect of CFH and LOC387715 risk genotypes exhibited a PAR of 69.8% (OR=8.34; 95% CI, 5.22-13.31). Genotype, haplotype and meta analysis data suggested that the rs10490924 could be the most AMD-susceptible SNP in the present cohort.

Conclusions: The present data provides an independent validation of the association of LOC387715 and HTRA1 SNPs along with their risk estimates among AMD patients. These associations underscore their significant involvement in AMD susceptibility, which can be useful for predictive testing.

CR: I. Kauri, None; S. Katta, None; A. Hussain, None; N. Hussain, None; A. Mathai, None; R. Nararayanan, None; A.B. Majji, None; R. Reddy, None; S. Chakrabarti, None.

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241 - A497
Elucidation of Light Associated Age Related Macular Degeneration Genetic Risk Loci

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Purpose: Light has been implicated as a risk factor for Age Related Macular Degeneration (AMD). This study tests for genetic association between genes involved in light transduction and AMD.

Methods: Venous blood samples (6ml) were collected from patient samples (n=300) with documented end-stage neovascular AMD, a replicate cohort of end-stage neovascular AMD (n=300) and an age and sex-matched control cohort (n=250). All patients underwent full ocular assessment and participated in a short questionnaire to detail history of smoking, micro and macro angiopathic disease, drug history, refractive status, eye colour, body mass index and family history. Genetic haplotype analysis was undertaken for several chosen candidate genes from pooled DNA samples previously processed on Illumina HD SNP arrays. Haplotype structure was determined using information derived from the HapMap project for each of the candidates. Genotyping was undertaken on a multiplexed SNaPshot technology platform (ABI).

Results: Fourteen SNPs across 2 genes were genotyped in the case and control cohorts. Results indicate a novel association between neovascular AMD and a gene that is involved in light transduction with a common variant of this gene increasing the risk (odds ratio 1.88, p=0.003). Evaluation of the strength of this association is ongoing in a replicate cohort.

Conclusions: Our results show that this simple multiplex analysis can quickly determine the haplotype structure across a number of potential disease-associated loci. Light energy has been considered a risk factor for AMD but the evidence of a causal link is inconsistent. This study points to a potential genetic susceptibility to the effects of increased light exposure in the development of neovascular AMD.

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242 - A498
Association of LOC387715 A6495 With Vitreous Hemorrhage in Polypoidal Choroidal Vasculopathy


Purpose: To investigate whether the LOC387715 polymorphism is associated with polypoidal choroidal vasculopathy (PCV) and with vitreous hemorrhage (VH), one of the most severe clinical phenotypes, in the Japanese population.

Methods: One hundred and nine Japanese patients with PCV, comprised of 9 patients associated with VH (VH group) and 100 patients without VH (non-VH group), and 85 control subjects were analyzed for the LOC387715 polymorphism (rs = 1049024), using denaturing high performance chromatography.

Results: There was a significant difference in the T allele frequency between PCV patients and control subjects (P < 0.001). In comparison with wild-type homozygosity (CC), homozygosity for the at-risk allele genotype (TT) increased the likelihood for PCV 8.4-fold (3.6 to 19.5, 95% confidence interval [CI]) and heterozygosity for the at-risk allele genotype (TG) increased the likelihood for PCV 4.0-fold (1.9 to 9.4, 95% CI). There was a significant difference in the photolytic frequency at the LOC387715 site between the VH and non-VH groups (P = 0.0099, Chi-square test) with the TT genotype occurring in 88.9% in the VH group and 37.0% in the non-VH group. The frequency of the T allele in the VH group was significantly greater than that in the non-VH group (0.944 vs. 0.610, P = 0.0039, Fisher exact test).

Conclusions: The LOC387751 polymorphism is associated with PCV and clinical severity in the subgroups of PCV in the Japanese population.

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243 - A508
Age Related Macular Degeneration: Genetics 1 Organizing Section: BI

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Mitochondrial DNA Polymorphism A4917G Is Independently Associated With Age-Related Macular Degeneration


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Purpose: Mitochondria play central roles in energy production, free radical production, and apoptosis. The objective of this study was to determine if MTND2*LHON4917G (4917G), a specific non-synonymous polymorphism in the mitochondrial genome previously associated with neurodegenerative phenotypes, is associated with increased risk for age-related macular degeneration (AMD).

Methods: AMD patients and unrelated controls were ascertained at both Vanderbilt University Medical Center (VUMC) and Duke University Medical Center (DUMC). All patients and controls had complete ophthalmoscopic exams. Mitochondrial genotyping was performed by the 5'nuclease allelic discrimination Taqman assay, using single nucleotide polymorphisms (SNPs). Effect size for the association is measured as odds ratio (OR) with 95% confidence intervals.

Results: A preliminary study of 303 individuals (293 cases and 100 controls) revealed an increased occurrence of 4917G in cases compared to controls (15.4% vs 9.0%, p = 0.01). Since there was a significant age difference between cases and controls in this initial analysis, we extended the study by selecting 280 tightly age-matched Caucasian pairs from an existing large AMD study population. This well-characterized population was genotyped for 4917G plus specific AMD-associated nuclear genome polymorphisms in CFH, LOC387715 and ApoI. Following adjustment for the listed nuclear genome polymorphisms, 4917G independently predicts the presence of AMD (OR = 2.16, 95% CI 1.20 - 3.71, p = 0.03).

Conclusions: A specific mitochondrial polymorphism previously implicated in other neurodegenerative phenotypes (4917G) appears to convey risk for AMD independent of recently discovered nuclear DNA polymorphisms.

A Strong Association of Vascular Endothelial Growth Factor in Korean Patients With Age-Related Macular Degeneration

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Purpose: Age related macular degeneration (AMD) causes progressive impairment of vision and is the leading cause of irreversible vision loss in older individuals. Although the etiology of AMD has not been clearly elucidated, genetic and environmental factors have been implicated in the AMD. Vascular endothelial growth factor (VEGF), a key regulator of vascular permeability and angiogenesis, has been suggested to play an important role in the pathogenesis of age-related macular degeneration (AMD). In this study, to determine whether VEGF variations are associated with AMD in Korean population, we performed mutation screening of the VEGF gene located on 6p12.

Methods: DNA samples were extracted from buccal swab samples of 90 unrelated patients with Exudative AMD visiting the Department of Ophthalmology at the Catholic University Medical Center. Genotyping of rs11200638 in promoter of the HTRA1 serine peptidease gene was performed using polymerase chain reaction - restriction fragment length polymorphism (RFLP) and direct sequencing. Control individuals were selected from the general population without AMD.

Results: In this study, we investigated three SNPs of VEGF in Korean patients with AMD. Genotypic distribution of the rs113711 in intron 1 was significantly different between AMD patients and controls; TT genotype in AMD patients was significantly increased compared with control subjects (p = 0.004, OR = 3.10, 95% CI 1.47 - 6.70). AMD patients had significantly higher T allele frequency than controls (p = 0.001, OR = 1.40, 95% CI 1.05 - 1.86). But there were no statistically significant differences in the allele and genotype frequencies of rs2010963 and rs833061 in 5′ UTR between the affected individuals and control subjects. The genotype distributions of all polymorphisms of VEGF among the control subjects and the affected individuals were in Hardy-Weinberg equilibrium.

Conclusions: In this study, Korean AMD patients showed significantly difference in rs113711 in intron 1 from the control group, whereas both rs2010963 and rs833061 in 5′ UTR showed no statistically significant association with AMD. Therefore, it is suggested that rs113711 may act as a potential susceptibility variant for Korean AMD patients.

CR: H. Kim, None; J. Mok, None; Y. Kim, None; C. Joo, None.
Support: None.
247 - A503
Replication of Loci Associated With AMD in the 100,000 SNP Genome Wide Association Study of the AREDS Cohort
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**Purpose:** A genome-wide association study on 600 subjects from the age-related eye disease study (AREDS) was deposited into dbGaP. We sought to determine the extent to which SNPs associated with AMD could be replicated in a second cohort.

**Methods:** SNPs associated with AMD using allelic tests (P<0.0001) reported on dbGaP were identified and characterized by level of support based on Hardy-Weinberg equilibrium (HWE), minor allele frequency (MAF), and the presence of nearby SNPs associated with AMD. The raw genotypes for SNPs across these loci were analyzed with genotype-based association methods that are less susceptible to artifacts than the allelic tests reported on dbGaP. SNPs across the genes in the above loci were selected to tag common blocks of DNA in the population and are being genotyped in a replication cohort of 481 cases with and 311 controls without AMD.

**Results:** A total of 46 previously unreported loci had allelic tests of P<0.0001 in the dbGaP. Of these 46, 20 loci were considered most promising based on a stringent HWE criterion (P=0.05), a MAF = 0.01, and one or more adjacent SNPs associated with AMD as reported on dbGaP. Genotypic association tests (Fisher’s exact test and logistic regression with log-odds additive genetic model) suggested that 14 (70%) of these 20 loci had one or more SNPs that were associated with AMD (P<0.0001). Genotyping of the replication cohort on these 14 loci, and other loci with genotypic association, is underway and will be analyzed.

**Conclusions:** In addition to identifying previously established loci (CFH, ARMS2, and BF/C) and a novel variant (C3) replicated recently, at least 14 additional novel loci may be associated with AMD based on robust genotypic association analysis. Allelic tests are dependent upon the assumption of HWE and slight deviation from HWE can result in biased tests, unlike genotypic tests for association. We found that only 70% of the allelic tests of the most promising loci were supported by genotypic analysis. Use of allelic test for genetic association studies (e.g., dbGaP) should be avoided because genotypic tests more accurately reflect the underlying genetic association. The results of a replication study of loci with genotypic association tests will be presented.

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249 - A505
Variations in the Control Region of the Mitochondrial DNA in Age-Related Macular Degeneration Retinas
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**Purpose:** In a replication study of loci with genotypic association tests will be presented. Of these 46, 20 loci were considered most promising based on a stringent HWE criterion (P=0.05), a MAF = 0.01, and one or more adjacent SNPs associated with AMD as reported on dbGaP. Genotypic association tests (Fisher’s exact test and logistic regression with log-odds additive genetic model) suggested that 14 (70%) of these 20 loci had one or more SNPs that were associated with AMD (P<0.0001). Genotyping of the replication cohort on these 14 loci, and other loci with genotypic association, is underway and will be analyzed.

**Methods:** Retinas were isolated from 10 normal eyes (mean age 79±3.8, range 52 to 97) and 11 AMD eyes (mean age 81.5±2.6, range 69-93). Total DNA was extracted and LX-PCR CR: B.L. Fridley, None; A.K. Sharma, None; K.M. James, None; N. Tosakulwong, None; R. Ayyagari, None.

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248 - A504
An Investigation of Selected Candidate Genes for Five-Year Incidence of Age-Related Maculopathy (ARM) in Older Women
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**Purpose:** To investigate the relationship between selected candidate genes and incidence of ARM in a prospective five-year follow-up study.

**Methods:** A retrospective chart review identified 115 AMD cases and 115 age-matched controls. SNPs for 17 genes were evaluated in each group by genotyping. The primary outcome was the incidence of ARM during a follow-up period of five years. Logistic regression analysis was performed to assess the association of genotype of each selected SNP with incident early and late ARM.

**Results:** A total of 46 previously unreported loci had allelic tests of P<0.0001 in the dbGaP. Of these 46, 20 loci were considered most promising based on a stringent HWE criterion (P=0.05), a MAF = 0.01, and one or more adjacent SNPs associated with AMD as reported on dbGaP. Genotypic association tests (Fisher’s exact test and logistic regression with log-odds additive genetic model) suggested that 14 (70%) of these 20 loci had one or more SNPs that were associated with AMD (P<0.0001). Genotyping of the replication cohort on these 14 loci, and other loci with genotypic association, is underway and will be analyzed.

**Conclusions:** In addition to identifying previously established loci (CFH, ARMS2, and BF/C) and a novel variant (C3) replicated recently, at least 14 additional novel loci may be associated with AMD based on robust genotypic association analysis. Allelic tests are dependent upon the assumption of HWE and slight deviation from HWE can result in biased tests, unlike genotypic tests for association. We found that only 70% of the allelic tests of the most promising loci were supported by genotypic analysis. Use of allelic test for genetic association studies (e.g., dbGaP) should be avoided because genotypic tests more accurately reflect the underlying genetic association. The results of a replication study of loci with genotypic association tests will be presented.

**CR:** B.L. Fridley, None; A.K. Sharma, None; K.M. James, None; N. Tosakulwong, None; R. Ayyagari, None.

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250 - A506
Late-Onset Severe Retinal Degeneration in a Mouse Model Due to a Nonsense Mutation
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"Ophthalmology, University of California San Diego, La Jolla, CA; "Ophthalmology & Visual Sciences, University of Michigan, Ann Arbor, MI; "Jackson Laboratory, Bar Harbor, ME; "Ophthalmology, OU/HSC, Oklahoma City, OK; "Epidemiological & Biostatistical Sciences, Case Western Reserve University, Cleveland, OH.

**Purpose:** To determine the molecular mechanism underlying age-related retinal degeneration (arrd1) segregating in autosomal recessive mode in a naturally occurring mouse model.

**Materials and Methods:** Retinal pathology was evaluated by fundus photography, ERG, morphology, immunohistochemistry and electron microscopy at various ages. Microsatellite marker and candidate gene analyses were carried out to map and clone the arrd1 gene. Molecular pathology of retina was determined by in situ hybridization, qRT-PCR and nonsense mediated decay (NMD) assay. Involvement of arrd1 gene in causing retinal degeneration in patients was evaluated by family-based association analysis. LD structure of ARRD1 gene was examined and custom Goldengate Illumina panel constructed to test for association with patients with the age-related macular degeneration (AMD).

**Results:** The arrd1 mice had a normal ocular fundus appearance, ERG and retinal histology at age 6 months. Attenuation of vessels, RPE atrophy and presence of retinal dots were noted at 8-10 months which progressed to complete loss of photoreceptors and extinguished ERG by 22 months. Levels of photoreceptor specific gene transcripts were significantly low at 12 months and found minimal by 18 months. Genetic analysis revealed a nonsense mutation in the arrd1 gene which results in premature truncation of the putative protein. We identified a novel transcript, which is the predominant transcript of the arrd1 gene in retina. In situ hybridization localized this transcript to the nuclear layers of the retina. The arrd1 mutant transcript was significantly depleted in the retina of affected mice and was observed to undergo NMD. Analysis of two independent cohorts of patients with AMD and several patients with late-onset dominant macular degeneration did not reveal an association between the human ARRD1 gene and AMD.

**Conclusions:** We identified a novel transcript, which is the predominant transcript of the arrd1 gene in retina. Sequencing showed 4.63 versus 9.87 SNPs per individual in the normal retina compared to the AMD retinas. One SNP was significantly more common in the AMD retinas compared to normal retinas OR=11.66, p=0.006. The T44C and A189G SNPs were not found in AMD retinas. There was increased staining of 8-oh-dG in the outer segment of the AMD retinas. The CR functions in the replication and transcription of mtDNA. The CR contains a conserved number of SNPs in the mtDNA CR compared to normal retinas. In addition, the CR mutants had higher levels of 8-oh-dG indicating oxidative stress. The increased levels of SDP's may represent sites for "hot spots" in this critical region of the mtDNA.

**CR:** M.C. Kenney, None; S.R. Atilano, None; M. Chwa, None; D.S. Boyer, None; D. Hwang, None; P. Coskun, None; D.C. Wallace, None; A.B. Neshburn, None; N. Udar, None.

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251 - A507
Mutation Analysis of the Human Serum Amyloid P Component Gene in a United Kingdom Cohort of Age-Related Macular Degeneration (AMD) Patients by Two Different Methods

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Purpose: The retinas of patients with AMD contain deposits known as drusen. These deposits contain lipids and proteins which accumulate in the layer called Bruch's membrane. Drusen components provide a list of candidate genes for the genetic analysis of this degenerative eye disease. One such constituent is an acute phase protein called serum amyloid P component (APCS), whose gene is located at 1q21. We propose to investigate whether any polymorphisms which may confer a predisposition to AMD.

Methods: 200 AMD patients and 200 controls from a UK cohort were analysed by two different mutation scanning techniques. The APCS gene was investigated by amplifying 6 PCR fragments prior to analysis by both single stranded conformational polymorphism (SSCP) and high resolution melt (HRM) analysis. All fragments displaying a mobility shift by either screening method were DNA sequenced in order to discern the mutation involved, and allowed comparison between the two analyses.

Results: Initial results reveal two previously described mutations within the APCS gene in this cohort. A non-synonymous polymorphism within exon 1 (P4S), and a synonymous polymorphism (V144V) in exon 2, both of which are class 1 single nucleotide polymorphisms (SNPs) with a change in melting temperature (Tm) greater than 0.5°C.

Conclusions: The two analytical methods employed detected both of the polymorphisms described. Further studies are underway to establish which mutation scanning method proves to be the most sensitive, reliable and cost effective.

CR: A.J. Cree, None; X. Chen, None; A.J. Lotery, None.


252 - A508
Reduction of MnSOD2 Using Cre-LoxP Recombination System: Evidence of Oxidative Damage and Impaired Visual Response

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Purpose: Generation of oxidative stress in the retina and retinal pigment epithelium (RPE) complex has been suggested as a main factor leading to the development of age-related macular degeneration (AMD). Our goal is to test this theory in the mouse by reducing RPE-specific MnSOD2. Previously, we showed that a MnSOD-specific ribozyme Rz432 diminished the electroretinogram (ERG) response and caused histological damage in the retinas of mice. Here we utilized an alternative approach to determine if knockdown of SO2D causes an increased level of reactive oxygen species in the retina/RPE complex leading to the pathogenesis similar to AMD.

Methods: To confirm that subretinally injected VMD2-CRE (AAV1) virus into the floxed-SOD2 mice mediates RPE-specific recombination, VMD2-CRE (AAV1) virus was injected subretinally into the reporter mouse strain which contains a floxed PGK-neo cassette and an enhanced yellow fluorescent protein (EYFP) gene inserted into the Gt(ROSA)26Sor locus. The expression of EYFP was detected by immunostaining. To reduce MnSOD2 in the RPE, we utilized Cre-loxP recombination system. Cre was packaged into AAV1 under the control of the VMD2 promoter which leads to expression in the RPE. Mice bearing a floxed allele of SOD2 were injected subretinally in the right eye with AAV1-VMD2-cre. Left eyes were injected with AAV1-GFP as controls. Electroretinogram (ERG) analysis was performed at 4 or 8 and 14 weeks post-injection, and eyes were processed at 8 weeks post injection to examine oxidative damage markers. To see the oxidative stress in these mice, immunostaining was done using anti-HNE, a marker of lipid peroxidation. Eyes of mice injected with AAV-Rz432 were examined by histology.

Results: In the reporter mouse line, YFP expression was detected only in the RPE layer after injection with AAV-VMD2-CRE. In mice bearing the floxed SOD2 allele, a 30 percent reduction was observed in both a-wave and b-wave amplitudes of those eyes injected with the VMD2-CRE virus as compared to control. The level of 4-HNE was increased in the right eye as compared with left eye. In continuation of examining the effect of MnSOD2 ribozyme we analyzed retinas of mice 7 and 12 months post injection with AAV-Rz432. By 7 months, retinas exhibited deposits in RPE layer, shortened outer and inner segments of photoreceptors, and the thinning of outer nuclear layer indicating loss of photoreceptor cells. By 12 month of post injection with AAV-Rz432, we detected more pronounced changes to the RPE such as vacuole formation and atrophy.

Conclusions: As previously shown, reduction of MnSOD2 by using ribozyme results in the damage of outer retina of mice. RPE-specific down-regulation of SOD2 by using cre-mediated recombination leads to increased oxidative stress in the RPE and reduced ERG response.

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