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An *Apolipoprotein E* Variant may Protect against Age-Related Macular Degeneration through Cytokine Regulation

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Abstract

Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness among the elderly in Western countries. Genetic factors, age, cigarette smoking, nutrition, and exposure to light have been identified as AMD risk factors. In this study, we investigated the association between *ApoE C112R/R158C* single nucleotide polymorphisms (which determine the E2, E3, and E4 isoforms) and age-related macular degeneration (AMD), and the mechanism underlying the association. Genomic DNA was extracted from 133 clinically screened controls, 94 volunteers with a younger mean age, 120 patients with advanced AMD, and 40 archived ocular AMD slides for single nucleotide polymorphism typing. The effects of recombinant *ApoE* isoforms on CCL2 (a chemokine), CX3CR1 (a chemokine receptor), and VEGF (a cytokine) expression in cultured human retinal pigment epithelium (RPE) cells were tested and serum cholesterol profiles of the clinically screened subjects were analyzed. *ApoE112R* (E4) distribution differed significantly between AMD patients and controls. *ApoE112R* allele frequency was 10.9% in the AMD group when compared with 16.5% in the younger controls and 18.8% in the clinically screened controls. The pathologically diagnosed archived AMD cases had the lowest allele frequency of 5%. No significant differences in *ApoE158C* (E2) distribution were observed among the groups. A meta-analysis of 8 cohorts including 4,289 subjects showed a strong association between AMD and *112R*, but not *158C*. In vitro studies found that recombinant ApoE suppresses CCL2 and VEGF expression in RPE cells. However, the E4 isoform showed more suppression than E3 in both cases. These results further confirm the association between *ApoE112R* and a decreased risk of AMD development. The underlying mechanisms may involve differential regulation of both CCL2 and VEGF by the ApoE isoforms.

Keywords

age-related macular degeneration; apolipoprotein E, single nucleotide polymorphism; genetic susceptibility; cytokines

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INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of visual impairment and blindness among the elderly in Western countries [la Cour et al., 2002]. AMD is a significant health problem in the United States, with a current estimate of roughly 1.75 million persons with advanced AMD in the general population. By the year 2020, it is estimated that ~2.95 million people will have developed AMD [Friedman et al., 2004].

The etiology of AMD remains elusive. To date, age, cigarette smoking, nutrition, and exposure to light have been identified as AMD risk factors [Age-Related Eye Disease Study Research Group, 2000,2001;Seddon et al., 2001;Husain et al., 2002;Hyman and Neborsky, 2002]. Various studies also indicate a significant genetic contribution to AMD risk [Tuo et al., 2004a]. For example, it has been reported that advanced AMD is more prevalent in Caucasian populations when compared with other races that possess higher levels of uveal pigmentation and skin melanin [Friedman et al., 1999;Age-Related Eye Disease Study Research Group, 2000]. Furthermore, ~20% of AMD patients report having a positive family history [Klaver et al., 1998b;de Jong et al., 2001]. The higher occurrence of AMD among monozygotic twins and first-degree relatives of AMD patients when compared with spouses and unrelated individuals also indicates a significant genetic component in AMD risk [Meyers et al., 1995;Seddon et al., 2005]. A few studies have been able to demonstrate an association between AMD and various single nucleotide polymorphisms (SNPs) [Tuo et al., 2004a;Edwards et al., 2005;Haines et al., 2005;Klein et al., 2005;Tuo et al., 2006].

Apolipoprotein E (ApoE) is a multiple function protein highly expressed in the liver, brain, and retina [Anderson et al., 2001;Ribalta et al., 2003]. The *ApoE* gene is known to be polymorphic. SNPs at positions 112 (rs no. 429358) and 158 (rs no. 7412) determine three major isoforms: E2 (*112C, 158C*), the most common E3 (*112C, 158R*), and E4 (*112R, 158R*). These isoforms vary greatly in terms of protein structure and function [Zannis, 1986;Jarvik, 1997;Laws et al., 2003]. The ApoE E4 isoform is correlated with elevated cholesterol concentrations and an increased risk of cardiovascular disease [Mahley and Huang, 1999;Smith, 2000;Ribalta et al., 2003]. The E4 isoform is also associated with various neurodegenerative diseases, most notably Alzheimer Disease (AD) [Chapman et al., 2001;Laws et al., 2003]. Cardiovascular disease, AD, and AMD share a number of similar pathological features [Klaver et al., 1998a,1999;Mullins et al., 2000;Dentchev et al., 2003]. ApoE is also a ubiquitous component of soft drusen, a hallmark of early AMD development [Anderson et al., 2001]. Moreover, various clinical signs of retinal degeneration are mimicked in Apo(*)E3-Leiden knockout mice that carry a dysfunctional form of human ApoE-E3 [Dithmar et al., 2000;Kliffen et al., 2000].

Investigators have attempted to determine the role of *ApoE* in AMD development and pathogenesis [Klaver et al., 1998a;Souied et al., 1998;Baird et al., 2004;Zareparsi et al., 2004]. Interestingly, the E4 allele has been reported by several independent groups as being significantly less prevalent among AMD cases when compared with the corresponding control populations [Klaver et al., 1998a;Souied et al., 1998;Simonelli et al., 2001;Baird et al., 2004;Zareparsi et al., 2004]. The E4 polymorphism is associated with a protective effect against AMD, while the E2 allele is weakly associated with a slightly increased risk for developing late AMD (see our meta-analysis).

Although the majority of previous reports are in agreement, there are studies that have challenged and narrowed the proposed protective effect of the *ApoE* E4 allele [Schmidt et al., 2000;Schultz et al., 2003]. The aim of this present study was first to verify the association between the *ApoE* SNPs and AMD by using an independent sample set, implementing a multiple group design, employing SNP typing with standard DNA controls, and by performing

a meta-analysis of relevant published data. Functional SNP studies determining the effects of recombinant *ApoE* isoforms on the expression of CCL2 (a chemokine), CX3CR1 (a chemokine receptor), and VEGF (a cytokine) in cultured human retinal pigmented epithelium (RPE) cells were performed to explore the potential mechanisms by which the *ApoE* SNPs influence AMD pathogenesis. Lastly, serum cholesterol profiles of the clinically screened subjects were analyzed to elucidate the possible correlation of ApoE isoforms, lipid levels, and AMD development.

MATERIALS AND METHODS

Study Subjects

This multiple case–control study included two AMD groups and two normal control groups. Each participant signed the Informed Consent that was part of the protocol approved by the NEI Institutional Review Board. This research followed the tenets of the Declaration of Helsinki.

Only advanced AMD cases were included in this study. A clinical diagnosis of advanced AMD was defined by geographic atrophy involving the center of the macula and/or choroidal neovascularization with drusen in at least one eye. Stereoscopic fundus photographs of the optic disk and macula were obtained for all AMD patients. These patients are referred to as the clinically diagnosed AMD group (CD AMD). The recruited control subjects were all older than 50 years of age and clinically defined by an absence of drusen or less than 5 small drusen (<63 μm) in the center of the macula and an absence of all other retinal disease affecting the photoreceptors and outer retinal layers. Healthy blood donors (BD) who did not receive a clinical eye examination served as another control group to obtain information on SNP frequencies in a population younger than the average age of AMD onset. This group is referred to as the BD controls.

Archived paraffin-embedded ocular sections were obtained from 40 pathologically diagnosed advanced-stage AMD patients (PD AMD). Twenty-three of the 40 cases were classified as neovascular AMD characterized by loss of photoreceptors and RPE alterations within the macular region and subretinal neovascular fibrous tissue with or without hemorrhage, exudate, and disciform scar. The remaining 17 cases were classified as areolar or dry AMD cases without neovascularization and characterized by a loss of photoreceptors, RPE atrophy or hypotrophy, the presence of diffuse confluent drusen or large drusen, calcification, and fibroglial scar in the macula.

DNA Extraction

Genomic DNA was extracted and isolated from venous whole blood (10 mL) collected from the study subjects using a QIAamp DNA Blood Maxi kit (Qiagen, Valencia, CA). Either laser-capture microscopy (Pix-Cell II; Arcturus, Mountain View, CA) or manual microdissection was used to procure cells from the archived paraffin-embedded ocular sections. Extracted DNA from these cells was then subjected to whole genome amplification using the GenomiPhi™ DNA amplification kit (Amersham, Piscataway, NJ) for subsequent SNP typing.

Construction of DNA Standards

Standard DNA SNP templates were made to serve as SNP typing controls. Genomic DNA of heterozygous *ApoE C112R* or *R158C* was PCR-amplified using the primers 5′-GGGCACGGCTGTCCAA-3′ and 5′-ATAAGAATTCCCCGGCCTGGTACACT-3′. The 237-bp PCR fragment was inserted in to the pGEM®-T Easy Vector (Promega, Madison, WI). The ligation product was transformed to JM109 high efficiency competent cells (Promega). Colonies containing *ApoE 112C, 112R, 158R*, and *158C* homozygotes were confirmed by

direct sequencing. Plasmid DNA was extracted from the colonies corresponding to each allele to serve as the specific allele standards. The combination of homozygote plasmids served as the heterozygous standard.

SNP Typing

The typing of *ApoEC112R* was performed using a PCR-RFLP technique. A 213 base-pair DNA fragment containing *ApoE112* was PCR amplified using the following primers: 5′-TGATGGACGAGACCATGAAG-3′ and 5′-CAGCTCCTCGGTGCTCTG-3′. RFLP analysis was conducted using the enzyme Hha1.

ApoER158C was typed using Real-time PCR allelic discrimination assays provided by the Assay-by-Demand℠ service of Applied Biosystems (Foster City, CA). Real-time fluorescence detection was performed. Genotypes were determined based on the fluorescence intensities of FAM and VIC, with FAM signaling *ApoE158C* and VIC *ApoE158R*. Standard DNA controls for both alleles and a heterozygous control were included on each genotyping plate in both the RFLP and Real-time PCR assays.

In Vitro Functional Studies

Human ARPE-19 cells were separately exposed to 40 μg/ml of ApoE E3 and E4 recombinant proteins (Invitrogen, Carlsbad, CA) for 24 hr. Lipopolysaccharide (LPS)-treated (100 ng/ml, *Salmonella typhimurium* LPS endotoxin; Difco Laboratories, Detroit, MI) and untreated control cells were processed in a similar fashion. Cells were subjected to RNA extraction. Real-time PCR for determining gene expression was performed using a Stratagene M×3000™ System and Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA). The primers for CCL2 and CX3CR1 were synthesized by Superarray and supplied as an RT^2 Real-Time™ Gene Expression Assay Kit (Superarray, Fredrick, MD). For the internal control, β-actin was amplified using primers 5′-CCCAGCACAATGAAGATCAA-3′ and 5′-ACATCTGCTGGAAGGTGGAC-3′. Following PCR, a thermal melt profile was performed for amplicon identification. Each sample was analyzed at least twice. The comparative Ct method was used for relative quantification and statistical analysis of the fold changes to present the data graphically. The culture media were collected for ELISA assay of CCL2 and VEGF using commercially available kits (BioSource International, Camarillo, CA).

Serum Profiling

Clinically screened cases and controls fasted for a period of 12 hr before their blood draw. Total serum cholesterol was evaluated, with the normal values ranging from 100 to 200 mg/dl.

Statistical Analysis

Linkage disequilibrium (LD) between *ApoE112* and *ApoE158* was analyzed using the web-based SNPAnalyzer (http://www.istech21.com/phar/phar_a01.html). The normalized disequilibrium coefficient D' was used as the parameter for determining the strength of association between the SNPs. With the range of possible D' values lying between −1 and 1, a greater association (coinheritance) was established when the D' value was closer to 1.

The χ^2 test was performed to compare the carrier and allele frequencies and abnormal rates of the cholesterol level of the cases and controls. Hardy–Weinberg equilibrium was also tested using the χ^2 test with 1 degree of freedom. A P value of < 0.025 was considered significant following Bonferroni correction [McIntyre et al., 2000]. Odds ratios (ORs) were calculated and an estimation of confidence intervals was made based on the unmatched case–control

design [Bland and Altman, 2000]. A t-test was used to compare differences in cholesterol levels between groups.

RESULTS

All enrolled subjects were Caucasians of non-Hispanic descent recruited from the greater Washington, DC area. One hundred twenty sporadic advanced AMD patients (CD AMD), 94 random unscreened BD controls, and 133 screened normal controls were included in this study. Out of the advanced AMD patients, 79 had neovascular (wet) AMD. The remaining 41 had areolar (dry) AMD. Forty archived slides of PD AMD cases also were included in the study. The majority of the sources for these slides were Caucasian. The AMD case and screened control groups were matched as closely as possible for both age and gender. The demographics of these four study groups are summarized in Table I.

Among the groups evaluated for the *ApoE 112* and *158* SNPs, no significant deviation from Hardy–Weinberg equilibrium was noted. LD between *ApoE 112* and *158* was incomplete with a D' of −1 overall, suggesting that no linkage association exists between these two SNPs.

SNP-type distribution analyses showed a decreased prevalence of *112R* carriers and alleles among AMD cases when compared with the controls (Tables II and III). Statistically significant differences in both *112R* carrier and allele frequencies were observed in the total controls when compared with the total AMD cases, with 0.51 and 0.53 ORs, respectively ($P < 0.01$; Tables II and III). No significant differences were detected in *158C* carrier and allele frequencies between the AMD cases and controls (Tables II and III). In the CD AMD group, the OR for the *112R* carriers (*112C/R + 112R/R* vs. *112C/C*) was 0.61 when compared with that for the screened controls (Table II). We observed a lower OR of *112R* carriers for the CD AMD cases vs. the screened controls than vs. the BD controls (0.61 vs. 0.67). The lowest OR (0.21) for the *112R* carriers was observed in the PD AMD group in comparison with the screened controls (Table II). Analysis of allele frequencies yielded a similar pattern (Table III). These results may be due to the PD AMD cases having a more definitive diagnosis when compared with that of the CD AMD patients. The assumption that a certain percentage of BD controls will later develop AMD in advancing age is also consistent with this data.

A meta-analysis of 8 independent published studies based primarily on Caucasian populations examined a total of 1,925 AMD patients and 2,364 normal controls (Table IV). The OR, CI, and *P* values for ApoE E2 and E4 were calculated for this pooled data set (Table IV). Five out of the 8 studies reported an association between E4 and a reduced risk of AMD using $P < 0.05$ as the significance criterion. The ORs ranged from 0.38 to 0.82. Pooling the data did not dramatically change the OR; however, the statistical power of this reported association increased ($P < 0.0001$).

To elucidate the possible mechanisms underlying the association between ApoE isoforms and AMD, the effects of the E3 and E4 isoforms on the expression of selected chemokines, chemokine receptors, and cytokines was evaluated in RPE cells. The in vitro experiments indicated that the CCL2 levels in normal and LPS-stimulated RPE cells were 5,874 and 19,012 pg/ml, respectively (Fig. 1). Recombinant ApoE suppressed the expression of the CCL2 transcript and protein in RPE cells. However, the E4 isoform showed a stronger suppression of CCL2 than E3 (Fig. 1). RPE cell expression of VEGF following ApoE recombinant incubation showed the same pattern as that of CCL2 (Fig. 2). The E4 isoform had a stronger suppression of VEGF than E3. No distinct expression of the CX3CR1 transcript was observed in RPE cells incubated with either E3 or E4 (data not shown).

We also analyzed the correlation between *ApoE112R* and serum cholesterol levels (Tables V and VI). The results showed that the *112R* carriers have significantly elevated cholesterol levels

and have more abnormal cholesterol rates in both the control and AMD groups, regardless of analyzing the data as a continuous variable (Table V) or as a binary variable (≤200 mg/dl vs. > 200 mg/dl) (Table VI).

DISCUSSION

Our results provide further evidence that *ApoE112R* is associated with a reduced risk of AMD development. Although there are a few negative reports, this association has been previously reported. In the present study, a quantitative negative correlation between *ApoE112R* and AMD prevalence was observed with a lower OR for the PD AMD cases than for the CD AMD cases when comparing with the same control group. Furthermore, a higher OR was observed for the CD AMD group when compared with the BD control group than when compared with the screened controls. These results suggest a correlation between allele frequency and disease incidence.

In contrast to the protective role of ApoE E4 for AMD, the E4 isoform is associated with an increased risk for AD and various cardiovascular diseases [Mahley and Huang, 1999;Laws et al., 2003]. Current knowledge of the differential functioning among the ApoE isoforms, either in lipid metabolism or neuronal protection, does not readily provide a rationale for the observed protective role of E4 against AMD development [Nathan et al., 1994;Ji et al., 2002;Laws et al., 2003]. ApoE is expressed in photoreceptor outer-segments, the retinal ganglion layer, and both layers of Bruch's membrane. ApoE is also basally secreted by RPE cells [Anderson et al., 2001;Ishida et al., 2004]. It has been postulated that ApoE may facilitate lipid efflux from RPE cells and lipid transport across Bruch's membrane, thus reducing lipid deposit levels [Souied et al., 1998]. However, this hypothesis does not account for the fact that ApoE is a significant component of drusen [Anderson et al., 2001].

Altered ApoE function caused by the *112R* variant may indeed play a role in AMD pathogenesis. Incubation with wild-type ApoE E3 or variant ApoE E4-enriched 3-VLDL results in differential cellular accumulation [Ji et al., 1998;Laws et al., 2003]. A two- to threefold increase in the accumulation of ApoE E3 over ApoE E4 has been observed in various cell types [Ji et al., 2002]. If mechanisms for proper clearance in the retina and Bruch's membrane are not in place, this accumulation may serve as a pathogenic basis for disease development [Ambati et al., 2003;Tuo et al., 2004b]. In fact, the increased accumulation in non-E4 carriers may explain why ApoE is such a ubiquitous component of ocular drusen in AMD-afflicted eyes [Crabb et al., 2002].

ApoE also may be involved in AMD pathogenesis through its association with C-reactive protein (CRP) levels. A chronic inflammation theory has been proposed to account for macular drusen etiology [Anderson et al., 2002]. CRP, a systemic inflammatory marker, is a prominent drusen-associated molecule and is expressed in significantly higher levels among advanced AMD patients [Seddon et al., 2004]. Persons with one or two copies of *ApoE* E4 are associated with reduced CRP levels when compared to individuals lacking these copies [Austin et al., 2004;Judson et al., 2004;Marz et al., 2004]. This finding suggests that an ApoE isoform-mediated inflammatory response may be involved in AMD etiology.

It has been reported that up-regulated macrophage and inflammatory cell function may worsen or exacerbate already existing inflammatory lesions [Espinosa-Heidmann et al., 2003]. This up-regulation can be spurred by both CCL2 and VEGF expression. Our functional study demonstrated a stronger suppression of both CCL2 and VEGF expression in RPE cells by the ApoE E4 isoform (Figs. 1 and 2). These findings implicate a possible mechanism underlying the observed protective effect of this allele. RPE cells constitutively express CCL2, a CC chemokine, and secrete VEGF, a growth factor and cytokine [Holtkamp et al., 1999;Crane et

al., 2000]. Increased levels of both CCL2 and VEGF in RPE cells are related to oxidative stress and hypoxia within the eye [Spilsbury et al., 2000;Uetama et al., 2003]. VEGF is a potent endothelial cell mitogen and angiogenic growth factor strongly expressed in postmortem AMD eyes both with and without choroidal neovascularization [Lip et al., 2001]. Over-expression of VEGF in the RPE, even if only temporarily, is sufficient to induce choroidal neovascularization in the rat eye [Kliffen et al., 1997;Spilsbury et al., 2000]. CCL2 also has been associated with further deterioration and degeneration, especially in advanced disease stages [Ambati et al., 2003]. Considering that our data show nearly a twofold greater suppression of CCL2 mRNA and protein expression by E4 in RPE cells with similar findings for VEGF, we suggest that investigating the varied effects of the ApoE isoforms on chemokine and cytokine regulation may offer insights into the mechanisms responsible for the observed protective role of the E4 isoform.

As mentioned previously, the ApoE E4 isoform has been correlated with elevated total cholesterol levels [Smith, 2000;Ribalta et al., 2003]. This association remains true in this study (Tables V and VI). Although it is difficult to draw any mechanistic conclusions from this correlation, these results do support the validity and accuracy of our SNP typing methodology. These results also suggest a serum cholesterol-independent disease pathway in AMD [van Leeuwen et al., 2004].

Although difficulty was met in recruiting age-matched controls for an older AMD group, the likelihood of a false-positive was not increased. The incorporation of more closely age-matched controls would certainly increase the statistical power of this study. It is possible that some of the individuals in the BD control group will develop AMD later in life due to the age-related nature of the disease. Therefore, if the *112R* polymorphism is a true protective factor against AMD, an even greater difference in the polymorphic frequencies between the case and control groups is anticipated if a more closely age-matched control population was included. This may explain why a lower OR was obtained for the CD AMD group when compared to the BD controls. With the lowest OR of *112R* carriers in the PD AMD group, our results indicate that a clearly-defined phenotype also will increase the likelihood of successful genetic factor discrimination.

In conclusion, this study confirms that *ApoE112R* is associated with a deceased risk of AMD development. The underlying mechanisms of AMD development may involve altered regulation of CCL2 and VEGF expression in RPE cells by the ApoE isoforms. We are continuing to investigate the potential interaction among *ApoE*, other AMD-associated genes, and environmental factors.

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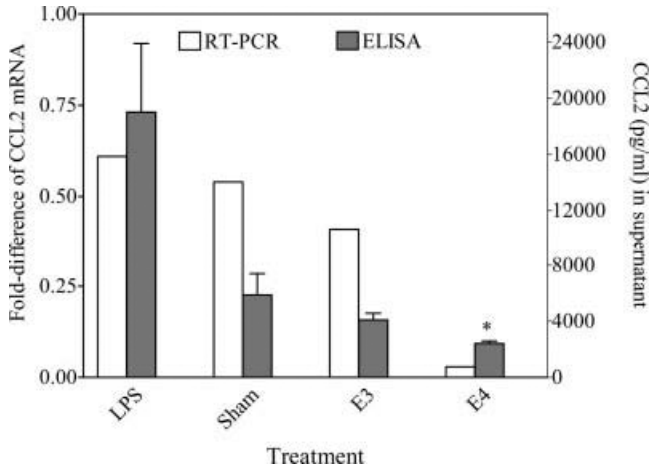

Fig 1.

Fold changes (shown are the means from 2 independent experiments calculated from calibration curves) in mRNA abundance (relative to β-actin) and CCL2 protein (mean ± SD from 4 independent experiments) in the medium of RPE cells incubated with either the E3 or E4 isoform. Significance established at $P < 0.05$. *In comparison with E3 treatment.

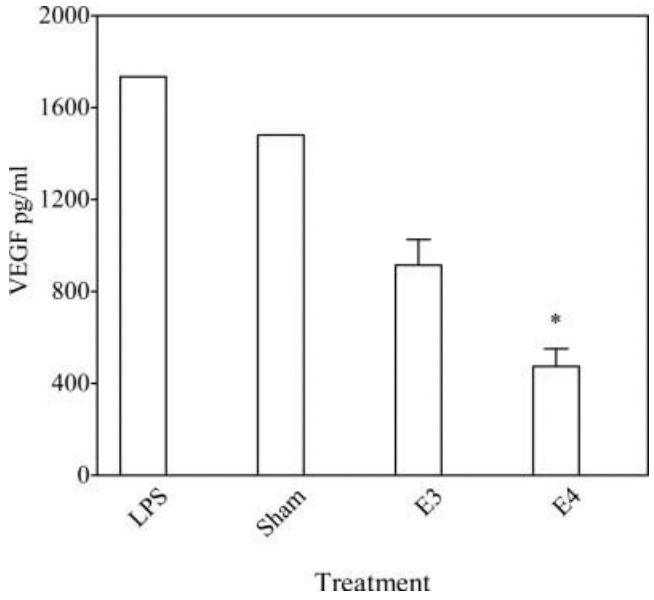

Fig 2.

VEGF protein (mean ± SD from 3 independent experiments) in the RPE cell culture medium of RPE cells incubated with either the E3 or E4 isoform. Significance established at $P < 0.05$. *In comparison with E3 treatment.

TABLE I

Demographic Summary of Study Groups

	N	Female (%)	Mean age	Past or current smokers (%)	Caucasian (%)	Wet AMD (%)
Screened control	133	76 (57.1)	69.7 ± 8.4	62 (46.6)	133 (100.0)	N/A
Random control	94	32 (34.0)	35.1 ± 9.1	NA	94 (100.0)	N/A
Clinical AMD	120	66 (55.0)	75.2 ± 7.2	72 (60.0)	120 (100.0)	79 (65.8)
Archived AMD	40	20 (74.1)[a]	82.8 ± 9.2	NA	26 (96.3)[a]	23 (57.5)

NA, not available; N/A, not applicable.

[a]Values excluding data from 13 specimens with unknown sex and race.

TABLE II

Carrier Frequencies of *ApoE C112R* and *R158C*

		ApoE112					*ApoE158*				
	N	***C/C***	***C/R* (%)**	***R/R* (%)**	***C/R*+*R/R* OR (CI)**	***X*²/*P***	***R/R***	***R/C* (%)**	***C/C* (%)**	***R/C*+*C/C* OR (CI)**	***X*²/*P***
All controls	227	152	69 (30.4)	6 (2.6)			182	44 (19.4)	1 (0.4)		
Controls	133	87	42 (31.6)	4 (3.0)			103	30 (22.6)	0		
Blood donors	94	65	27 (28.7)	2 (2.1)			79	14 (14.9)	1 (1.1)		
All AMD	160	127	31 (19.4)	2 (1.3)	0.51 (0.32–0.79)	7.12/0.009	102	18 (15.0)	0	0.85 (0.51–1.42)	0.58/0.45
CD AMD	120	91	27 (22.5)	2 (1.7)	0.61 (0.35–1.03)[a]	3.20/0.07	102	18 (15.0)	0	0.79 (0.39–1.32)[a]	1.16/0.29[a]
					0.67 (0.37–1.22)[b]	1.7/0.12				0.98 (0.48–1.95)[b]	0.01/0.94[b]
PD AMD	40	36	4 (10)	0	0.21 (0.06–0.63)[a]	8.52/0.004					

[a] In comparison with screened controls.

[b] In comparison with blood donors.

TABLE III

Allele Frequencies of *ApoE C112R* and *R158C*

		ApoE112				*ApoE158*			
	Alleles	**C**	***R* (%)**	**OR (CI)**	**X^2/*P***	***R***	***C* (%)**	***C* carrier OR (CI)**	**X^2/*P***
All controls	454	373	81 (17.8)			408	46 (10.1)		
Controls	266	216	50 (18.8)			236	30 (11.3)		
Blood donors	188	157	31 (16.5)			172	16 (8.5)		
All AMD	320	285	35 (10.9)	0.53 (0.33–0.80)	7.4/0.007	222	18 (7.5)	0.89 (0.49–1.40)	0.44/0.51
CD AMD	240	209	31 (12.9)	0.63 (0.43–1.05)[a]	3.18/0.08[a]	222	18 (7.5)	0.82 (0.40–1.30)[a]	1.03/0.31[a]
				0.68 (0.44–1.19)[b]	1.69/0.19[b]			1.01 (0.51–1.03)[b]	0.01/0.94[b]
PD AMD	80	76	4 (5)	0.23 (0.09–0.69)[a]	8.24/0.004				

[a] In comparison with screened controls.

[b] In comparison with blood donor controls.

TABLE IV

Meta-Analysis of Independent AMD-*ApoE* Association Studies in Caucasian Populations

		Control Allele no (%)	AMD Allele no (%)	OR (CI)	X^2/P	Comments
Klaver et al. [1998a]	E2	162 (9.0)	22 (12.5)	1.44 (0.74–2.83)	2.33/0.13	Cases and controls derived from the population-based Rotterdam Study in the Netherlands.
	E3	1,357 (75.3)	142 (80.6)			
	E4	281 (15.6)	12 (6.8)	0.39 (0.17–0.92)	9.8/0.002	
	Total	1,800	176			
Souied et al. [1998]	E2	21 (6.2)	23 (9.9)	1.65 (0.89–3.06)	2.58/0.11	Unrelated patients with exudative AMD compared to gender and age-matched controls.
	E3	265 (78.9)	192 (82.8)			
	E4	50 (14.9)	17 (7.3)	0.45 (0.25–0.81)	7.53/0.006	
	Total	336	232			
Schmidt et al. [2000]	E2	60 (8.1)	40 (8.7)	1.09 (0.71–1.65)	0.15/0.70	Familial and sporadic cases of AMD were pooled as well as analyzed separately. Significant differences in age and gender at time of exam. However, estimated age/sex-adjusted ORs were made via logistic regression analysis.
	E3	575 (77.3)	366 (79.6)			
	E4	109 (14.6)	54 (11.7)	0.77 (0.55–1.10)	2.06/0.15	
	Total	744	460			
Simonelli et al. [2001]	E2	2 (2)	17 (9.8)	5.20 (1.17–22.99)	5.76/0.02	Italian population, age-matched controls.
	E3	89 (90.9)	152 (87.3)			
	E4	7 (7.1)	5 (2.9)	0.38 (0.12–1.25)	2.71/0.10	
	Total	98	174			
Schultz et al. [2003]	E2	56 (8.8)	68 (9.4)	1.08 (0.74–1.56)	0.16/0.69	Familial and unrelated AMD patients were included. Here, the groups are pooled. Trends of E4 in protection were found.
	E3	497 (77.7)	575 (79.2)			
	E4	87 (13.6)	83 (11.4)	0.82 (0.59–1.13)	1.46/0.23	
	Total	640	726			
Baird et al. [2004]	E2	17 (6.9)	50 (9.9)	1.48 (0.84–2.63)	1.84/0.17	All participants were Anglo-Celtic in origin with all four grandparents born in either the UK or Ireland.
	E3	185 (75.2)	396 (78.6)			
	E4	44 (17.9)	58 (11.5)	0.68 (0.44–1.03)	3.84/0.05	
	Total	246	504			
Zareparsi et al. [2004]	E2	33 (8.0)	116 (9.2)	1.16 (0.77–1.74)	0.34/0.56	White patient cohort recruited from a single center. Subsequent OR calculations were adjusted for both gender and age.
	E3	320 (78.0)	1,022 (81.2)			
	E4	57 (14)	120 (9.5)	0.47 (0.33–0.66)	4.78/0.03	
	Total	410	1,258			
Current study	E2	46 (10.1)	18 (5.6)	0.89 (0.49–1.40)	0.44/0.51	Multiple case–control groups including advanced AMD cases only.
	E3	327 (72.0)	267 (83.5)			
	E4	81 (17.9)	35 (10.9)	0.53 (0.33–0.80)	7.4/0.007	
	Total	450	320			
Pooled data	E2	397 (8.3)	351 (9.1)	1.11 (0.96–1.29)	1.9/0.17	
	E3	3,615 (76.5)	3,109 (80.8)			
	E4	716 (15.2)	390 (10.1)	0.63 (0.55–0.71)	48.4/0.000	

	Control Allele no (%)	AMD Allele no (%)	OR (CI)	X^2/P	Comments
Total	4,874	3,850			

TABLE V

Relationship Between Cholesterol Level (mg/dL) and *ApoE112* Genotype

	AMD		Control		Total	
	N	**Mean ± SD**	***N***	**Mean ± SD**	***N***	**Mean ± SD**
112 *CR* + *R/R*	29	200.8 ± 31.9	43	206.2 ± 32.9	72	203.5 ± 32.4
112 *C/C*	91	192.7 ± 38.2	90	193.3 ± 35.0	181	193 ± 36.6[a]

[a] $P < 0.01$, t-test, in comparison of Cholesterol means between 112 *CR+R/R* and 112 *C/C*.

TABLE VI

Abnormal Rates of Cholesterol (>200 mg/dl) in Individuals with Different *ApoE112* SNP-types

	AMD			Control			Total		
	Abnormal	**Normal**	**All**	**Abnormal**	**Normal**	**All**	**Abnormal**	**Normal**	**All**
CR+R/R	15 (51.7)	14	29	25 (58.1)	18	43	40 (55.6)	32	72
C/C	33 (36.3)	58	91	36 (40.0)	54	90	69 (38.1)	112	181
X^2/P^a		2.19/0.14			4.05/0.04			6.75/0.01	

[a]Comparison of abnormal rates between *CR+R/R* and *CC* Carriers.