We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS BOOK CITATION INDEX INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Chapter 7

The Role of Apolipoprotein E Gene Polymorphisms in Primary Glaucoma and Pseudoexfoliation Syndrome

Najwa Mohammed Al- Dabbagh, Sulaiman Al-Saleh, Nourah Al-Dohayan, Misbahul Arfin, Mohammad Tariq and Abdulrahman Al-Asmari

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54614

1. Introduction

Primary glaucoma (PG) is one of the most common eye diseases which may potentially result in bilateral blindness. Glaucoma affects 70 million people and is the second leading cause of blindness worldwide. It is estimated that by the year 2020, this number would rise to around 79.6 million [1]. The prevalence of glaucoma varies widely across the different ethnic groups [2-8] and is significantly higher in blacks (4.7%) as compared to the white (1.3%) population [9]. The prevalence of both primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG) is higher in western region of Saudi Arabia as compared to other Asian countries [10]. To date no national study has been undertaken to determine the exact prevalence of glaucoma in Saudi Arabia, though it is one of the major causes of blindness in this country.

The glaucomas are a group of relatively common optic neuropathies in which pathological loss of retinal ganglion cells cause progressive loss of sight and associated alteration in the retinal nerve fiber layer and optic nerve head. Recent studies clearly suggest that abnormalities in structure and function of retinal nerve fiber layer (RNFL) are proportional to the loss of retinal ganglion cells in glaucoma [11]. Studies on two independent patients' populations also confirmed a close association between RNFL thickness and several visual parameters [12]. The retina is a light capturing tissue consisting of more than fifty different types of cells each performing unique function that ultimately provide the visual centers in the brain the information to achieve image formation and visual perception. Photo production require the

retina to have a high metabolic rate, multiple and complex membrane structures [13,14]. The photo receptor outer segments are enriched in polyunsaturated fatty acids including highly light sensitive docosahexenoic acid [15]. Recent experimental study suggests a clear role of fatty acids and cholesterol in optic nerve head blood flow and retinal nerve fibers structures. Retina has a unique mechanism for lipid uptake of low density lipoproteins which provides blood-borne lipids to all the cellular layers of retina [16,17]. Moreover, to keep its steady state lipid composition retina has the ability to synthesize cholesterol [18]. Defects in lipid metabolism in neural retina result in detrimental consequences on its structure and function. Published data clearly suggest the crucial role of lipids and lipoproteins in the pathophsiology of glaucoma [19]. Evidence from population and family studies supports heredity of glaucoma to be a complex trait. It is a genetically heterogeneous disorder attributed to the effects of individual causative mutations as well as interactions of multiple genes with a variety of environmental factors [20].

Pseudoexfoliation syndrome (PEX) is another common and clinically significant systemic condition and represents a complex, multifactorial, late-onset disease of worldwide significance with an estimated prevalence ranging from 10% to 20% of the general population [21]. It is clinically diagnosed by observation of whitish flake-like deposits of PEX material on anterior segment structures, particularly on the anterior lens surface and the pupillary border of the iris. Despite its worldwide distribution, there is a clear tendency for PEX syndrome to cluster geographically and in certain racial or ethnic subgroups. For example, there is a high prevalence of PEX syndrome in Nordic, Baltic, Mediterranean, and Arabian populations, where it affects up to 30% of individuals over age 60. The reported mean age of PEX patients ranges from 69 to 75years, and most epidemiological surveys demonstrate an increasing prevalence with increasing age. There is a significantly higher frequency and severity of optic nerve damage at the time of diagnosis, worse visual field damage, poor response to medications, more severe clinical course, and more frequent necessity for surgical intervention.

PEX is characterized by the pathological production and accumulation of an abnormal fibrillar extracellular material in the surface lining of the anterior and posterior chambers of the eye. The characteristic fibrillar PEX material is composed of microfibrillar subunits surrounded by an amorphous matrix. The material has a complex glycoprotein/proteoglycan structure composed of a protein core surrounded by glycosaminoglycan [22,23].

The fibrillar portion has been characterized as amyloid laminin, oxytalan, and various elastic tissue and basement membrane components [24-26]. Numerous studies showed positive reactions of PEX material to Congo red, showing its intense fluorescence with thioflavin T and S, and positive immunofluorescence with antiserum to amyloid, affinity for ruthenium red, positive histochemical tests for tyrosine and tryptophan [27-30]. However some other studies failed to demonstrate a positive reaction with Congo red in exfoliative deposits [24,27]. Hypothetically, amyloid might deposit in the vicinity of PEX material fibers because of the affinity they both have for elastic tissues. Moreover amyloid in the skin accumulates close to elastic fibers [31]. It has been suggested that the amyloid component normally present on elastic fibers may serve as a ligand for the amyloid–elastic fiber association [32]. Meratoja and Tarkkanen [30] showed amyloid positive material in sites atypical for PEX disease, such as the

http://dx.doi.org/10.5772/54614

ciliary body stroma, sclera, and cornea, in eyes with PEX. Besides its presence in the eye the PEX material is found in many other parts of the body such as the eyes, skin, heart, lungs, liver, kidney, gall bladder, blood vessels, optic nerves, and meninges [26,33,34].

PEX is a heterogeneous group of disorders with both Mendelian and multifactorial traits. Even within individual families, there can be large variations in the phenotypic presentation of gene mutations. Therefore, multifactorial etiologies must be involved in PEX development. This can include polygenic and environmental factors [35]. Some genes may act as susceptibility factors that allow other genes or environmental influences to produce PEX. Further, familial aggregation and the increased frequency of PEX in relatives of affected subjects compared with relatives of unaffected subjects [36,37] suggest an underlying genetic component [38]. The main problems with studies on the genetic background of PEX have been the asymptomatic nature of PEX and late age of onset which make it difficult to collect multi-generation families with several affected individuals for linkage and association studies. A wide variety of inheritance models have been suggested depending on the study material [39] and, of these, the autosomal dominant mode of inheritance with incomplete penetrance has received the most support [40,41]. However, most of these studies investigating PEX inheritance have been based on small pedigrees making hypotheses about the inheritance model uncertain. Thorleifsson et al. [42] explained the genetic aetiology of PEX in virtually all instances. In Iceland and Sweden, the high-risk haplotype is very common with a frequency that averages about 50% in the general population; approximately 25% are homozygous (two copies) for the haplotype with the highest risk.

Apolipoprotein E (APOE) is the major apolipoprotein in the central nervous system, which plays important role in the uptake and redistribution of cholesterol within neuronal network [43]. Immunologically, APOE is present in many cerebral and systemic amyloidoses; such as late-onset Alzheimer's disease, Down's syndrome, and prion disorders. It is thought that APOE can promote the aggregation of amyloidogenic proteins into the β-pleated sheet conformation that is typical of all amyloid deposits, and is directly involved in the amyloid deposition and fibril formation [44,45]. This widespread association of APOE with biochemically diverse amyloids has led scientists to postulate a more general role for it in the process of amyloid formation.

APOE is synthesized by Muller cells (the predominant glial cells of the retina) and released into the vitreous and then transported into the optic nerve through anterograde rapid transport where it has an important role in axonal nutrition [46]. It has been suggested that APOE plays a role in neuronal survival following ischemia and other chemical insults and particular APOE isoform may be related to neuronal degeneration in glaucoma [47]. APOE, is a 34-kDa glycosylated protein, composed of 299 amino acids encoded by a four exon polymorphic gene on chromosome 19q13.2. The gene encoding APOE has three polymorphic variants in human designated as ε2, ε3, and ε4. These variants differ from one another by the presence of either C or T nucleotide at codons 112 and 158. These three alleles encode different APOE isoforms which vary significantly in structure and function including receptor binding capacity and lipid metabolism [48]. As each individual human being carries two allelic copies in a gene, six possible genotypes (ε2/ε2, ε3/ε3, ε2/ε3, ε3/ε4, ε2/ε4, and ε4/ε4) are formed by different

combinations of these three alleles. The frequency of these genotypes differ significantly among different ethnic groups, however, APOE ε3/ε3 is the most predominant genotype and ε3 the most common allele in majority of populations [49-51]. The ε3 allele is considered to be the ancestral allele; and ε2 and ε4 are considered as variants, on the basis of single point mutations. Global studies on the APOE locus have shown highly significant variations in the allele frequencies of ε2, ε3, and ε4 [52-58].

The complex genetic contributions to glaucoma and PEX have been attributed to the effects of individual causative mutations as well as interactions of multiple genes with a variety of environmental factors. However, most of the identified genes do not appear to have a major role in the complex phenotype. Recent whole genome–association studies have successfully identified a number of single nucleotide polymorphisms as genetic factors conferring susceptibility to complex diseases, such as age-related macular degeneration, and it is expected that this will be a useful approach for glaucoma and PEX as well.

Earlier studies clearly point towards a possible association between APOE alleles and glaucoma. However, the results of these studies are contradictory. Some investigators suggested positive association [47,59,60] while others have shown no link at all [61-63]. Moreover, earlier studies were mainly restricted to white populations from Australia [47], United Kingdom [62,63] and Sweden [61] with only few reports from other ethnic groups restricted to Chinese and Japanese [59,60,64,65]. Similarly APOE polymorphism and the presence of ε 2 alleles have been reported to be significantly associated with the development of PEX in Turkish patients [66]. However, APOE genotypes and PEX seems to differ among study populations and no significant differences in allele and genotype frequencies between PEX and control were observed in European patients from Norway [67] and Germany [68]. Moreover, the information about the association of APOE alleles with glaucoma and PEX in Arabs is very limited. Therefore, this study on underlying genetics in these complex disorders will help analyze the genetic aspect of PEX and glaucoma in Saudi patients. In this study, we evaluated the possible association of alleles/genotypes of APOE with primary glaucoma (POAG and PACG) and PEX in Saudi population.

2. Methods

2.1. Subjects

The present study was undertaken to evaluate the association of APOE allele and genotype in Saudi primary glaucoma and pseudoexfoliation syndrome patients. A total of 200 unrelated Saudi patients with primary glaucoma [primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG)] and 51 pseudoexfoliation syndrome (PEX) were recruited from ophthalmology clinic of the Riyadh Military Hospital, Saudi Arabia. The glaucoma patient group consisted of 100 males and 100 females, with age at diagnosis ranging from 30 to 78 years (mean ± SD: 58±14.4). The control group consisted of 200 unrelated subjects, with 160 males and 40 females, ages ranging from 20 to 58 years (mean ± SD: 45±11.6). The diagnosis of PG was based on clinical observations:

http://dx.doi.org/10.5772/54614

A comprehensive eye examination was done that included best-corrected visual acuity (BCVA) measurements using logarithm of the minimum angle of resolution (logMAR) 4-m charts (Light House Low Vision Products, New York, NY), applanation tonometry, gonioscopy, dilated fundus examination, optic disc photography, and visual field (VF) examination. On gonioscopy, an angle was considered occludable if the pigmented trabecular meshwork was not visible in >180° of angle in dim illumination. Laser iridotomy was performed in subjects with occludable angles after consent was obtained, and they had the rest of the examination on some other day.

2.2. Visual fields

Automated VFs were performed for all the subjects with BCVA of 4/16 (logMAR 0.6) or better, using frequency-doubling perimetry (Carl Zeiss Meditec, Inc., Dublin, CA). All eligible subjects underwent C-20-1 screening (if the results were unreliable or abnormal, the test was repeated) and the N-30 threshold test. The reliability criteria were no fixation or false-positive errors for the C-20-1 screening test and < 20% fixation errors and <33% false-positive and false-negative errors for the threshold N-30 test. Visual fields with no depressed points to any level of sensitivity were considered to be normal. A provisional diagnosis of suspected glaucoma was made when the subject had one or more of the following conditions: intraocular pressure (IOP) ≥ 21 mmHg in either eye; vertical cup-to-disc ratio (VCDR) ≥ 0.7 in either eye or CDR asymmetry ≥ 0.2; and focal thinning, notching, or a splinter hemorrhage. All these subjects were asked to perform a threshold VF test using the Swedish interactive threshold algorithm Standard 30-2 program (model 750, Carl Zeiss Meditec). A glaucomatous field defect was diagnosed using a single reliable threshold VF examination of the central 30° (Swedish interactive threshold algorithm Standard 30-2). The field was considered to be abnormal if the glaucoma Hemi-field test results were outside normal limits and ≥3 abnormal contiguous non-edge points (except the nasal horizontal meridian) were depressed to P< 5% [69]. Reliability criteria were as recommended by the instrument's algorithm (fixation losses <20%; false-positive and false-negative < 33%).

2.3. Diagnostic definitions

The distribution of VCDR and IOP was obtained from those subjects with reliable and normal supra-threshold VF testing using frequency-doubling perimetry. Cases of glaucoma were defined using the International Society of Geographical and Epidemiologic Ophthalmology classification [70]. Glaucoma was classified according to 3 levels of evidence. In category 1, diagnosis was based on structural and functional evidence. It required CDR or CDR asymmetry ≥ 97.5th percentile for the normal population or a neuroretinal rim width reduced to ≥ 0.1 CDR (between 11- and 1-o'clock or 5- and 7-o'clock) with a definite VF defect consistent with glaucoma using the Swedish interactive threshold algorithm 30-2. Category 2 was based on advanced structural damage with unproved field loss. This included those subjects in whom VFs could not be determined or were unreliable, with CDR or CDR asymmetry ≥ 99.5th percentile for the normal population. Lastly, category 3 consisted of persons with an IOP ≥ 99.5th percentile for the normal population, whose optic discs could not be examined because of media opacities.

Blindness was defined as a best-corrected logMAR visual acuity of < 2/40 (log MAR 1.3) and/or constriction of the VF to <10° from fixation in the better eye [71]. Hyperopia was defined as spherical equivalent > 0.50 diopter (D) in a phakic eye [72]. Diabetes mellitus was detected based on current use of antidiabetic medication and/or random blood sugar level > 200 mg/dl [73]. Thus the primary Glaucoma patients were separated in two groups (POAG and PACG) as follows:

POAG: Anterior chamber angles open and appearing normal by gonioscopy, typical features of glaucomatous optic disc as defined earlier, and visual field defects corresponding to the optic disc changes.

PACG: At least two of the criteria mentioned: glaucomatous optic disc damage or glaucomatous visual field defects in combination with anterior chamber angle partly or totally closed, appositional angle closure or synechiae in angle, absence of signs of secondary angle closure (e.g., uveitis, lens related glaucoma; microspherophakia; evidence of neovascularization in the angle and associated retinal ischemia or congenital angle anomalies). Patients with signs of intracranial disease that would cause optic nerve atrophy in x-ray computerized tomography or magnetic resonance imaging were excluded.

Diagnosis of PEX among Saudi patients visiting Primary Care Clinics of Riyadh Military Hospital was undertaken by a team of ophthalmologists. Patients visiting primary care clinic were offered free eye examination to exclude the presence of PEX. Consent was obtained from the patients after describing them the features of PEX syndrome. Patients who suffered ocular trauma or with active eye condition, and/or has undergone ocular surgery were excluded from this study.

All patients were subjected to interviews and initial evaluation was performed by the ophthalmic assistant (OA). Demographic data were collected, complaints of the eye and family history of eye problems were recorded. Visual acuity was recorded. After the preliminary examination and interview all patients were examined by an ophthalmologist for identifying the factors for PEX syndrome by the external eye examination: PEX flakes on pupil margin (undilated examination), Iris transillumination defects, evaluation of anterior chamber depth by Van Herick's technique, measurement of intraocular pressure, poor pupil dilation, and examination of the crystalline lens surface after papillary dilation for the presence of PEX material. After identification of PEX, the patients were short listed and further rechecking and confirmation of PEX syndrome was performed by (1) slit lamp examination of the anterior segment which included flakes on the pupillary margin, iris transillumination defects, flare in the A/C and corneal edema, (2) measurement of intraocular pressure (IOP) with Goldman tanometer (3) gonioscopy to record angle depth PEX flakes and/or hyperpigmentation on the trabecular meshwork which was followed by examination after dilation which included Poor pupillary dilation, flakes on the anterior lens capsule, posterior synechiae, lens opacity, phacodenesis and lens subluxation, bilaterality and symmetry and optic nerve head cupping. Out of 51 confirmed cases of PEX 25 were males and 26 females. The average age of PEX positive males and females patients was 70.43±9.62 years and 65.56±7.45 years respectively.

Venous blood was collected from the confirmed PEX and PG patients as well as healthy controls, stored at -20ºC before extraction of DNA. The study protocol was approved by the

http://dx.doi.org/10.5772/54614

Ethics Committee of the Hospital, and written informed consent was obtained from all study participants.

2.4. Genotyping

The genotypes of the APOE polymorphisms were determined using APOE StripAssay™ kit based on polymerase chain reaction (PCR) and reverse-hybridization technique (ViennaLab Labordiagnostika GmbH, Vienna, Austria). The procedure included three steps: (1) DNA isolation, (2) PCR amplification using biotinylated primers, (3) hybridization of amplification product to a test strip containing allele-specific oligonucleotide probes immobilizd as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin- alkaline phosphatase and color substrates. To cross-check the results the genotypes of the APOE polymorphisms were also determined by PCR and restriction fragment length polymorphism (RFLP) technique. Primers were designed on the basis of the sequence data for APOE available in the GenBank to amplify the coding sequence of APOE. PCR was performed using PuRe Taq Ready-To-Go PCR Beads (Amersham, USA) with following primers:

Forward primer:	5- GAC GCG GGC ACG GCT GTC CAA GGA GCT GCA GGC GAC GCA GGC CCG GCT GGA CGC GGA CAT GGA GGA-3
Backward primer:	5 - AGG CCA CGC TCG ACG CCC TCG CGG GCC CCG GCC TGG TAC ACT-3

Genomic DNA was extracted from whole blood using a commercial kit (Qiamp; Qiagen, Hiden, Germany). The 200–300 ng of genomic DNA was used as a template in 25 μl reaction. Genomic DNA was amplified for 40 cycles. Each cycle consisted of: 94 °C for 30 sec, 68 °C for 10 sec, 72 °C for 1 min; PCR products obtained were separated by electrophoresis on 1.5% agarose gel in TAE buffer, visualized by ethidium bromide fluorescence. Fragments with the expected size were cut from the gel, purified using GFX PCR DNA Gel band purification kit (Amersham, USA). Purified DNA was digested with *Cfo* I (Hha I) enzyme, separated by agarose gel electrophoresis to identify the genotype. On the basis of size and number of various fragments generated, APOE genotypes were determined as ε2/ε2 with 144 bp and 96 bp, ε3/ε3 with 144 bp and 48 bp, ε4/ ε4 with 72 bp and 48 bp, ε2/ε3 with 144 bp, 96 bp and 48 bp, ε3/ ε4 with 144 bp, 72 bp and 48 bp, and ε2/ε4 with 144 bp, 96 bp, 72 bp and 48 bp fragments. The prevalence of various genotypes in patients and controls was determined. Complete matching of results was obtained following both of the above mentioned procedures.

2.5. Statistical analysis

Frequencies of various alleles and genotypes for each polymorphism were compared between patients and controls and analyzed by Fisher's exact test and the P-values < 0.05 were considered as significant. The strength of the association of disease with respect to a particular allele/ genotype is expressed by odd ratio interpreted as *relative risk* (RR) according to the method of Woolf as outlined by Schallreuter *et al* [74]. The RR was calculated only for those alleles and

genotype which were increased or decreased in patients as compared to normal Saudis. The RR was calculated for all the subjects using the formula given below:

RR = (a) × (d)/(b) × (c)

a = number of patients with expression of allele or genotype

b = number of patients without expression of allele or genotype

c = number of controls with expression of allele or genotype

d = number of controls without expression of allele or genotype.

Etiologic Fraction (EF): The EF indicates the hypothetical genetic component of the disease. Values 0.0-0.99 are of significance. It was calculated for positive association (RR>1) using the following formula [75].

EF = (RR-1)f/RR, where f = a/a+c

Preventive Fraction (PF): The PF indicates the hypothetical protective effect of one specific antigen for the disease. It was calculated for negative association only where RR<1 using following formula [75].

PF = (1-RR)f/RR (1-f) + f, where f = a/a+c

Values <1.0 indicated the protective effect of the genotype/ allele against the manifestation of disease.

3. Results

Out of 200 PG patients 134 were diagnosed as having POAG and 66 as having PACG. Diagnosis of POAG was based on category 1 in 20 subjects (14.93 %) and category 2 in 114 subjects (85.07 %). Between category 1 and category 2 there was no significant different in age, IOP and gender distribution. One subject was blind in both eyes and 1 subject had unilateral blindness due to POAG. There were 66 subjects with PACG. Diagnosis was based on category 1 in 16 subjects (24.24%), category 2 in 46 subjects (69.70%), and category 3 in 4 subjects (6.06%). Three subjects (4.55 %) bilaterally, 4 (6.06 %) were unilaterally blind due to PACG.

The results of frequency of APOE alleles and genotypes in the PG patients and the control subjects are summarized in Tables 1, 2,3,4,5 and 6. The frequency of the ε3 alleles was significantly lower in the glaucoma patients (86.5 %) compared to the control subjects (95.75 %, *P*=0.0001, RR=0.284, PF=0.544). On the other hand the frequencies of the ε4 allele was significantly higher in the glaucoma patients as compared to controls (12.25% vs 4.25%, *P*=0.0001, RR=3.145, EF=0.506). The allele ε2 was present only in 5 patients while totally absent in control groups (Table 1).

http://dx.doi.org/10.5772/54614

Allele	Glaucoma (N=400)		Control (N=400)		*P-value*	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency(%)			
ε4	49	12.25	17	4.25	0.0001‡	3.145	0.506*
ε3	346	86.50	383	95.75	0.0001‡	0.284	0.544
ε2	5	1.25	0	0.0	0.030‡	-	-

N, number of alleles; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡, statistically significant

Table 1. Distribution of APOE allele frequencies in glaucoma patients and matched control subjects.

Our study on various genotypes of APOE also showed variations in patient and control groups (Table 2). The prevalence of ε3/ε3, ε3/ε4, ε4/ε4, ε2/ε3, and ε2/ε4 was 75.5, 20.5, 1.5,1.5 and 1.0% in patients and 91.5, 8.5, 0,0 and 0 % in control group respectively.

Genotype	Glaucoma (N=200)		Control (N=200)		*P-value*	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency (%)			
ε3/ε3	151	75.50	183	91.50	0.0001‡	0.286	0.530
ε3/ε4	41	20.50	17	8.50	0.0006‡	2.775	0.491*
ε4/ε4	3	1.50	0	0.0	0.1240	-	-
ε2/ε3	3	1.50	0	0.0	0.1240	-	-
ε2/ε4	2	1.00	0	0.0	0.2493	-	-
ε2/ε2	0	0.0	0	0.0	-	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡, statistically significant

Table 2. Distribution of APOE genotypes in glaucoma patients and matched controls

Though the frequency of ε3/ε3 genotype was higher in both the test and control Saudi population, the statistical analysis of data showed strongly significant difference in ε3/ ε3 genotype frequencies between patients and controls (P=0.0001, RR=0.286, PF=0.53). The difference in the frequencies of the second common genotype (ε3/ ε4) was also statistically significant between the two groups (*P*=0.0006) being more in glaucoma patients. Genotypes ε4/ε4, ε2/ε3 were found only in 1.5% and ε2/ε4 in 1% of patients while being completely absent in the controls (*P*=0.124). The genotypes ε2/ε2 was absent in both patient and control groups. These results indicated that allele ε4 and genotype ε3/ ε4 are associated with glaucoma and can be a risk factor while allele ε3 and genotype ε3/ ε3 may be protective in Saudis. The frequencies of various genotypes and alleles were not significantly different in male and female patients clearly indicating that gender plays no role in genotype/ allele distributions among populations (Table 3).

Genotype/Allele	Male (N=100)		Female (N=100)		*P*-value
	Number	Frequency (%)	Number	Frequency (%)	
ε3/ε3	71	71.00	80	80.00	0.143
ε3/ε4	26	26.00	15	15.00	0.079
ε4/ε4	0	0.0	3	3.00	0.123
ε2/ε3	2	2.00	1	1.00	0.623
ε2/ε4	1	1.00	1	1.00	0.999
ε3	170	85.00	176	88.00	0.385
ε4	27	13.50	22	11.00	0.451
ε2	3	1.50	2	1.00	0.685

N, number of subjects

Table 3. Distribution of APOE genotypes and alleles in male and female glaucoma patients

Though the distribution of APOE genotypes and alleles was not significantly different in two types of glaucoma (Table 4) however when compared with controls separately, significant difference was found in the frequencies of genotypes ε3/ε4, ε3/ε3 and alleles ε4 and ε3 in POAG and controls.

Genotype/Allele	Open angle glaucoma (134) N (%)	Angle closure glaucoma (66) N (%)	*P-value*
ε3/ε4	30 (22.39)	11 (16.66)	0.456
ε4/ε4	3 (2.24)	00	0.552
ε3/ε3	98 (73.13)	53 (80.30)	0.298
ε2/ε3	2 (1.49)	1 (1.52)	1.000
ε2/ε4	1 (0.75)	1 (1.52)	0.552
ε4	37 (13.81)	12 (9.09)	0.197
ε2	3 (1.12)	2 (1.52)	0.666
ε3	228 (85.07)	118(89.39)	0.277

N, number of subjects

Table 4. Comparison of APOE genotype/ allele frequencies in patients with POAG and PACG

The frequency of genotype ε3/ ε4 and ε 4 allele was significantly more (*P*= 0.0006 and 0.0001 respectively) in POAG patients as compared to controls (Table 5).

http://dx.doi.org/10.5772/54614

Genotype/Allele	Open angle glaucoma (134) N (%)	Controls (200) N (%)	*p-value*	*RR*	*EF*/PF*
ε3/ε4	30 (22.39)	17 (8.50)	0.0006‡	3.105	0.432*
ε4/ε4	3 (2.24)	00	0.063	-	-
ε3/ε3	98 (73.13)	183 (91.50)	0.0001‡	0.252	0.507
ε2/ε3	2 (1.49)	00	0.160	-	-
ε2/ε4	1 (0.75)	00	0.401	-	-
ε4	37 (13.81)	17(4.25)	0.0001‡	3.608	0.495*
ε2	3 (1.12)	00	0.064	-	
ε3	228 (85.07)	383 (95.75)	0.0001‡	0.253	0.524

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡, statistically significant

Table 5. Distribution of APOE genotype/ allele frequencies in patients with POAG and matched controls

The frequency of allele ε3 and ε3/ε3 genotype was significantly higher in controls (*P*=0.0001). Similarly, the frequency of various genotypes of APOE differ between PACG and controls but the differences were not statistically significant except for ε3/ε3(*P*=0.022) (Table 6). However, the frequency of allele ε4 was higher in PACG whereas ε3 in controls indicating that the allele ε4 is also significantly associated with PACG in Saudis while genotype ε3/ε3 and allele ε3 may be protective.

Genotype/Allele	Angle closure glaucoma (66) N (%)	Controls (200) N (%)	*P-value*	*RR*	*EF*/PF*
ε3/ε4	11 (16.66)	17 (8.50)	0.067	2.152	0.210*
ε4/ε4	0	00	-	-	-
ε2/ε3	1 (1.52)	00	0.248	-	-
ε2/ε4	1 (1.52)	00	0.248	-	-
ε3/ε3	53 (80.30)	183 (91.50)	0.022‡	0.378	0.269
ε4	12 (9.09)	17(4.25)	0.045‡	2.252	0.229*
ε2	2 (1.52)	00	0.061	-	-
ε3	118(89.39)	383 (95.75)	0.010‡	0.374	0.282

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction;

Table 6. Distribution of APOE genotype/ allele frequencies in patients with PACG and matched controls

Over all prevalence of PEX in our study was 3.03%. Unilateral PEX was noted in 38% while bilateral PEX in 62% of the PEX patients (Figures.1 & 2). However, there was no significant

difference in the prevalence of PEX in male and female. Prevalence distribution of PEX with the age in Saudi population is summarized in (Table 7). The prevalence of PEX varied from 0.50% to 25% in various age groups. The majority of the patients screened was in the age group of 50-60 years followed by those from <50 years¸ 61-70 years, 71-80 years and 81-100 years groups. The prevalence of PEX increased with progressing of age.

Age group (years)	Patients screened (N)	PEX positive patients (N)	Frequency of PEX (%)
<50	600	3	0.50
51-60	850	27	3.17
61-70	200	16	8.00
71-80	30	4	13.33
81-100	4	1	25
Total	1684	51	3.03

N, number of patients

Table 7. Age specific prevalence of PEX in Saudi patients

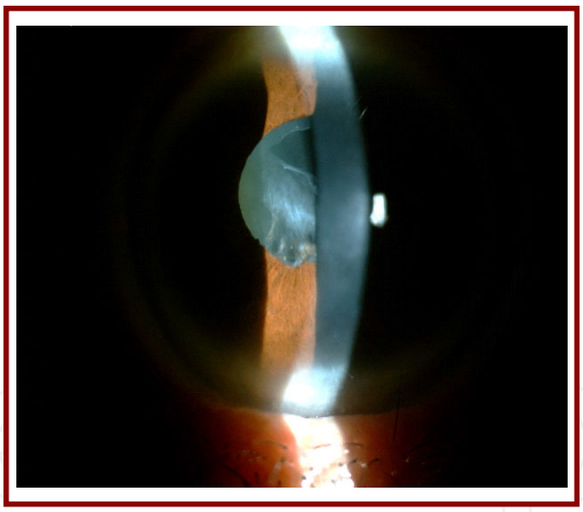

Figure 1. Showing massive PEX material in the papillary area forming a membrane like deposit

The results of frequency of APOE alleles and genotypes in the PEX patients and the control subjects are summarized in Tables 8 and 9. The frequency of the ε3 alleles was significantly lower in the PEX patients (82.35 %) compared to the control subjects (95.75 %, *P*=0.0001, RR=0.207, PF=0.373). On the other hand the frequencies of the ε2 and ε4 allele were significantly higher in the PEX patients as compared to controls (2.94% vs 0.00%, *P*=0.0081 and 14.70% vs 4.25%, *P*=0.0004, RR=3.884, EF=0.347 respectively). The allele ε2 was absent in control group (Table 8).

http://dx.doi.org/10.5772/54614

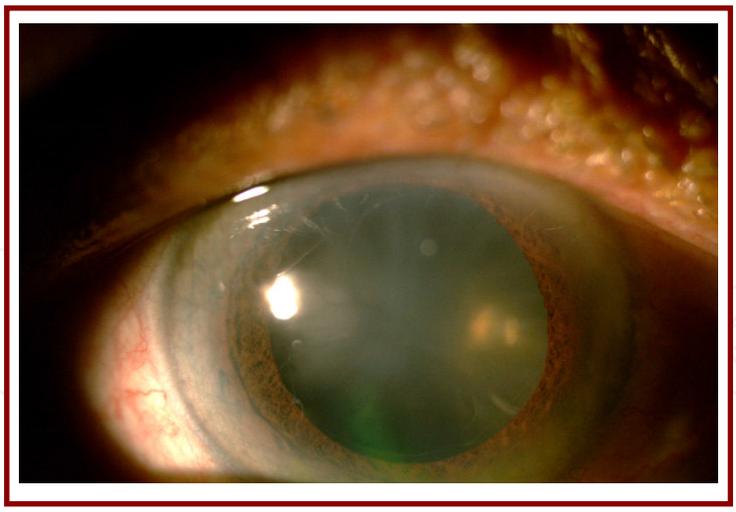

Figure 2. Shows deposition of PEX material more peripherally indicating wide pupillary excursion

Allele	Pseudoexfoliation (N=102)		Control (N=400)		*P-value*	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency(%)			
ε4	15	14.70	17	4.25	0.0004‡	3.884	0.347
ε3	84	82.35	383	95.75	0.0001‡	0.207	0.373
ε2	3	2.94	0	0.0	0.0081‡	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡, statistically significant

Table 8. Distribution of APOE allele frequencies in PEX patients and matched controls

Our study on various genotypes of APOE also showed variations in PEX patient and control groups (Table 9). The prevalence of ε3/ε3, ε3/ε4, ε4/ε4, ε2/ε3 and ε2/ε4 was 70.58, 21.56, 1.96, 1.96 and 3.92% in patients and 91.5, 8.5, 0, 0, and 0 % in control group respectively. Though the frequency of ε3/ε3 genotype was high in both the test and control Saudi population, the statistical analysis of data showed significant difference in ε3/ ε3 genotype frequencies between patients and controls, being more in controls than patients (*P*=0.0002, RR=0.222, PF=0. 363). The difference in the frequencies of the second common genotype ε3/ ε4 was also statistically significant between the two groups and was found to be increased in PEX patient group (*P*=0.012, RR=2.96, EF=0.259). Genotypes ε4/ε4, ε2/ε3 and ε2/ε4 were found only in patients while being completely absent in the controls. The genotype ε2/ε2, was absent in both the groups (Table 9).

These results indicated that alleles ε4 and ε2 and genotype ε3/ ε4 and ε2/ε4 were associated with PEX and can be a risk factor while allele ε3 and genotype ε3/ ε3 may be protective in Saudis. The frequencies of various genotypes and alleles were almost similar in male and female patients clearly indicating that gender plays no role in genotype/ allele distributions among populations.

Genotype	Pseudoexfoliation (N=51)		Control (N=200)		*P-value*	RR	EF*/PF
	Number	Frequency (%)	Number	Frequency (%)			
ε3/ε3	36	70.58	183	91.50	0.0002‡	0.222	0.363
ε3/ε4	11	21.56	17	8.50	0.012‡	2.960	*0.259
ε4/ε4	1	1.96	0	0.0	0.203	-	-
ε2/ε2	0	0	0	0.0	-	-	-
ε2/ε3	1	1.96	0	0.0	0.203	-	-
ε2/ε4	2	3.92	0	0.0	0.040‡	-	-

N, number of subjects; RR, relative risk; EF, etiological fraction; PF, preventive fraction; ‡, statistically significant

Table 9. Distribution of APOE genotype frequencies in PEX patients and matched controls

4. Discussion

The result of this study showed a very high frequency (95.75%) of allele ε3, very low frequency (4.25%) of ε4 and absence of allele ε2 in control population. Global studies on APOE locus have shown highly significant variations in allele frequencies among various populations. Studies from various geographical locations and ethnicities have reported a wide range of frequencies of ε2 (0-12%), ε3 (75-90 %) and ε4 (6-20%) [52-58]. The differences in the APOE genotype/allele frequencies in different populations may be attributed to environmental factors as well as genetic differences. The ε3 allele is the most frequent in all the human groups, especially in populations with a long established agricultural economy, whereas APOE ε4 allele remains higher in populations where the economy of foraging still exists or food supply is/was scarce and sporadically available [76]. Data on APOE allele frequencies collected from literature showed that the APOE allele distributions were different between North and South Europe. Additionally, compared to northern European countries, Mediterranean countries such as Italy, Turkey and Greece had lower frequencies of APOE- ε2 and ε4 alleles [77-79].

Results of present study revealed significant differences in the frequencies of ε3 and ε4 alleles in glaucoma patient as compared to control groups (Table 1). Allele ε3 being more common in controls while ε4 was predominant in glaucoma patients suggesting that the inheritance of the ε4 allele might be a risk factor whereas ε3 might exert a protective effect for glaucoma in Saudi population. Neuroprotective effect of ε3 is also evident from several earlier studies. APOE has an isoform specific effect on neuronal growth with ε3 stimulating neuronal elongation and neurite outgrowth on dorsal root ganglion [80]. In individuals with acute cerebral ischemia, such as an intracerebral hemorrhage, the ε3 allele confers a much higher survival and functional recovery whereas ε4 leads higher rate of disability and mortality [81]. Our results clearly suggest that presence of ε4 is associated with high risk of both POAG and PACG. Vickers *et al* [47] also reported an association between the ε4 allele and NTG in the Tasmanian population. Recently, Yaun *et al* [65] reported that the ε4 may be a latent risk factor in developing primary glaucoma in Chinese population. On the other hand Liew *et al* [82]

http://dx.doi.org/10.5772/54614

found a weak association between APOE ε4 and retinal microvascular degeneration. Contrary to these findings a decrease risk of NTG in Chinese [59,60] and POAG in Japanese with ε4 allele [64] has been reported, whereas some investigators reported no link between APOE polymorphism and glaucoma [61,62].

Besides glaucoma, APOE ε4 allele has been identified as a genetic susceptibility factor for a variety of neurodegenerative disorders in diverse ethnic populations [83-86]. APOE ε4 allele has also been associated with early age-at-onset of AD in a dose dependent manner [87,88]. Interestingly, a high incidence of glaucoma in AD patients clearly suggests a close association between ophthalmic and neurodegenerative disorders [89,90]. It has been hypothesized that the cellular mechanisms involved in the degeneration of optic nerve cells in glaucoma are quite similar to the neurodegenerative changes in AD [47,91,92]. APOE allele ε4 is also strongly linked with increased risk of Parkinson's disease, schizophrenia and coronary artery disease [93-99]. Possession of the ε4 allele is also associated with a retarded recovery after traumatic head injury [100,101]. The exact mechanism by which APOE ε4 exerts its deleterious effect is far from clear. However, APOE alleles has been reported to modulate the biological functions of APOE in part by altering the binding of the different lipoprotein lipid classes [93]. Individuals carrying the ε4 allele have higher plasma and neuronal levels of cholesterol as compared to individuals with ε2 or ε3. APOE immunoreactivity has been localized to basal laminar deposits and soft drusen in age related macular degeneration [102]. APOE has also been localized to the Müller cells (specialized retinal glia) [46,102] and this protein may be increased in Müller cells in glaucomatous eyes [103], indicating that this glial cell may have a role in the retinal response to glaucomatous injury.

On the other hand, earlier genetic studies support the concept that APOE would directly be involved in the amyloid deposition and fibril formation; and they suggest a close association between one of the main isoforms of APOE encoded by the ε4 allele and both familial and sporadic late-onset Alzheimer's disease (AD) [44,45]. In addition, deposits in various amyloidoses and prion diseases such as Down's syndrome, cystatin C-related Icelandic-type hereditary amyloid angiopathy, Creutzfeldt-Jakob disease, Lewy body dementia, dementia in Parkinson's disease include both biochemically and immunohistochemically detectable amounts of APOE [104-107].

The higher frequency of ε3/ ε3 in controls as compared to the patients indicated a protective effect of ε3/ ε3 on development of glaucoma in Saudis. Though the genotypes ε4/ε4, ε2/ ε3 and ε2/ε4 were only found in glaucoma patients and completely absent in normal Saudi population however, the differences were statistically insignificant. The genotypes ε2/ε2, was absent in both patients and control group. Earlier studies on APOE polymorphism in general healthy population also showed absence of genotypes containing ε2 allele among Saudis [51,108] as well as Native Americans [109].

This study showed that prevalence of PEX in Saudi Population was 3.03%. No significant difference was found in prevalence of PEX between male and female whereas the rate of prevalence varied in different age group. the prevalence of PEX increased with progressing of age. Earlier investigators from Saudi Arabia using a very small hospital based study reported overall prevalence of PEX as 9.3% [110]. PEX occurs worldwide, although reported prevalence

rates vary extensively with geographical location, as well as with ethnicity [21,111]. The prevalence of PEX varies significantly among Asians. The prevalence of PEX has been reported to be 3.01% and 6.28% in two different age groups in Southern Indian population [112], 6.45 % in Pakistani population [113], 3.4% in Japanese [114], 0.4% in Chinese [115] and 0.2 to 0.7% in Chinese Singaporeans [116]. In Scandinavia, the prevalence among persons over age 60 varies from over 20% in Finland to about 25% in Iceland. Aasved [117] found prevalence of 6.3%, 4.0%, and 4.7% in persons over age 60 in Norway, England, and Germany, respectively. Forsius [118] studied prevalence in patients over age 60 years in varied groups and found prevalence ranging from 0% in Greenland Eskimos to 21% in Icelanders. Lantukh and Piatin [119] found a low prevalence in native Siberian Tchutchee, but a much higher rate among immigrants to the area indicating ethnic variations. Similarly in New Mexico, Spanish-American men are nearly six times as likely to develop PEX than are non-Spanish-Americans [120].

The prevalence of PEX may also vary within the same country in similar environments and over short distances as found in present study. Similarly, in France the prevalence in over age 70 years varies from 3.6% in Toulon to 20.6% in Brest [121]. Ringvold et al [122] also found rates of 10.2%, 19.6%, and 21.0% in three closely situated municipalities in central Norway. The reasons underlying true variations, both from one population to another and within more or less homogeneous populations, remain to be explained. Geographic distribution patterns may perhaps be explained either by regional gene pools or by environmental influences. Persons living at lower latitudes (Greece, Saudi Arabia, and Iran) appear to develop PEX at younger ages [123]. Exposure to sunlight (ultraviolet radiation) may or may not be implicated. Forsius and Lukka [124] found no PEX in Eskimos versus 20% among Lapps living at the same latitude.

Similar to our observations, the prevalence of PEX increases with age in most of the studies [112,114,117]. Forsius [125] found PEX incidence to double every decade after age 50. These variations in prevalence rates may consequently be caused, to varying degrees, by genuine differences in genetic, ethnic and environmental factors and by methodological differences in age and sex distribution, diagnostic criteria, experience of the examiners in diagnosing the syndrome and the thoroughness of their examination [126].

This study also indicated that allele ε4 was associated with PEX and can be a risk factor while allele ε3 may be protective for PEX similar to PG in Saudi patients. Allele ε2 was found in only 2.94% of the PEX while totally absent in controls. Contrary to our results, Yilmaz et al [66] reported a close association of ε2 allele with PEX in Turkish population. According to them PEX have significantly higher frequency of ε2 allele (50%). In their study the frequency of genotypes carrying ε2 allele was also significantly higher in PEX. They have suggested that especially when ε2 allele is heterozygous, the possibility of developing PEX increases which could be an indicator for pathogenicity when this allele frequency is over 30% in the PEX group. In our study ε2/ ε3 and ε2/ ε4 genotypes are found only in 1 and 2 cases respectively. As the genotype frequencies are low in these groups, it is difficult to make general conclusion on statistically insignificant data.

On the other hand our results for APOE polymorphism in PEX indicated that genotype ε3/ ε4 was also associated with PEX (P=0.012) and can be a risk factor while genotype ε3/ ε3 may be

http://dx.doi.org/10.5772/54614

protective for PEX (*P*=0.0002) similar to PG in Saudi patients. In addition, the control group had a significantly higher frequency of the ε3 allele (95.75%) than the PEX group (82.35%), showing that this allele had a protective effect for developing the disease (P=0.0001). This is in agreement with Yilmaz et al [66] who reported a protective role of APOE ε3 allele in patients with exfoliation syndrome in Turkish population. However there are reports indicating no association of APOE genotypes and PEX in Germans or Italians [68] and Norwegians [67].

In the literature, ε 4 allele has been shown to be risky for developing amyloidoses in AD [44,45,104,106,107]. Yilmaz et al [66] suggested PEX to belong to the amyloidosis group depending on the deposition of amyloid or amyloid-like material throughout the body. As stated earlier inheritance of the ε4 allele has also been associated with elevated risk to Alzheimer's disease. In this regard, it is interesting that visual deficits have been reported in Alzheimer's disease cases. However, there are conflicting reports as to whether visual field loss observed in a relatively high proportion of Alzheimer's disease cases is associated with retinal or central damage [127-129]. It has also been noted that both Alzheimer's disease and Parkinson's disease cases have increased glaucomatous retinal changes [90]. In the light of the these findings, there may be similar cellular processes involving APOE related to neuronal damage. It has been argued that both Alzheimer's disease and glaucoma/PEX are ultimately axon damaging conditions and it is how nerve cells respond to this injury that leads to overall neuronal degeneration and the clinical picture of progressive loss of function [130]. Müller cells that express particular APOE isoforms may thus have an important role in regulating the response of retinal ganglion cells to injury. However, it cannot be ruled out that APOE may be acting centrally to promote β-amyloid fibril formation in structures such as the lateral geniculate nucleus [131] and that these plaques are causing damage to retinal axons and visual pathways. In this regard, it would be intriguing to determine whether glaucoma and PEX cases may have a higher incidence of Alzheimer-type dementia.

The result of this study suggests that APOE alleles may influence the risk of glaucoma and PEX. The inheritance of the ε4 allele is associated with elevated risk of POAG, PACG and PEX and ε3 may exert protection for both type of glaucoma as well as PEX. Genotypes containing allele ε2 (ε2/ε3, ε2/ε4) were found only in small number of patients (3POAG, 2 PACG and 1PEX) whereas altogether absent in Saudi normal population so it is difficult to derive any conclusion. Further studies involving larger number of patients from different race/tribes of Saudi Arabia are warranted to reach any definite conclusion as the APOE allele frequencies from same population (Turkish) reported by different authors are not uniform [66,132,133]. These differences in the distribution of APOE allele and genotype in single population in different studies have been attributed to geographical/ racial differences and/ or variations in genotyping methodology.

Though the inheritance of the ε4 allele seems to be associated with elevated risk of primary glaucoma and PEX in our Saudi population. However, it will be important to replicate these results in populations from other geographical locations of Saudi Arabia. The significance of inheritance of these APOE allelic isoforms has yet to be established, as is the case for the potential role of this protein in many other neurodegenerative conditions, but it may be linked with associated hypertension, formation of central β-amyloid deposits or a more general role

in the regulation of lipids following axonal injury. However, our results together with similar data elucidated a potential overlap between the degenerative pathways underlying glaucoma/PEX and Alzheimer-type dementia and brain injury.

5. Conclusion

This study clearly showed that the APOE polymorphism represents a major risk factor for ophthalmic/neurodegenerative diseases and this study together with previous studies pointed to a possible association between APOE alleles and PG/PEX in defined populations. However, the association between APOE genotype and PG/PEX seems to differ among studied populations, indicating a modifying rather than a direct genetic effect. Although our results indicated *ε4* allele to be significantly associated with the development of primary glaucoma (POAG and PACG) and PEX in a Saudi population. Further studies are warranted to understand the role of APOE allelic isoforms in various ethnic populations and to predict the predisposition to degenerative eye diseases like PEX and glaucoma.

Acknowledgements

The authors would like to thank S. Sadaf Rizvi and Mohammad Al-Asmari for their help in laboratory work.

Author details

Najwa Mohammed Al- Dabbagh[1], Sulaiman Al-Saleh[1], Nourah Al-Dohayan[1], Misbahul Arfin[2], Mohammad Tariq[2] and Abdulrahman Al-Asmari[2*]

*Address all correspondence to: abdulrahman.alasmari@gmail.com

1 Department of Ophthalmology, Riyadh Military Hospital Riyadh, Saudi Arabia

2 Research Center, Riyadh Military Hospital Riyadh, Saudi Arabia

References

[1] Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. British Journal of Ophthalmology 2006;90: 262-267.

[2] He M, Foster PJ, Huang W, Zheng Y, Freidman DS, et al. Prevalence and Clinical characteristics of Glaucoma in Adult Chinese: A population based study in Liwan

District, Guangzhou. Investigative Ophthalmology & Visual Science 2006;47: 2782-2788.

[3] Wong TY, Loon SC, Saw SM. The epidemiology of age related eye diseases in Asia. British Journal of Ophthalmology 2006;90: 506-511.

[4] Sakata K, Sakata LM, Sakata VM, Santini, Hopker LM. Prevalence of Glaucoma in a South Brazilian Population: Projeto Glaucoma. Investigative ophthalmology & visual Science 2007;48: 4974-4979.

[5] Cedrone C, Mancino R, Cerulli A, Cesareo M, Nucci C. Epidemiology of primary glaucoma : prevalence, incidence, and blinding effects. Progress in Brain Research 2008;173: 3-14.

[6] Vijaya L, George R, Arvind H, Baskaran M, Ramesh V. Prevalence of primary angle-closure disease in an urban South Indian population and comparison with a rural population. The Chennai glaucoma study. Ophthalmology 2008;115(4): 655-660.

[7] Vijaya L, George R, Baskaran M , Arvind H, Raju P. Prevalence of primary open angle glaucoma in an Urban South Indian population and comparison with a rural population. The Chennai glaucoma study. Ophthalmology 2008;115(4): 648-654.

[8] Pekmezci M, Vo B, Lim AK, Hirabayashi D, Tanaka GH. The characteristics of glaucoma in Japanese Americans. Archives of Ophthalmology 2009;127: 167-171.

[9] Kwon YH, Fingert JH, Kuehn MH, Alward WL. Primary open angle glaucoma. New England Journal of Medicine 2009;360: 1113-1124.

[10] Eid TM, El-Harwary I, El-Menawy W. Prevalence of glaucoma types and legal blindness from glaucoma in the Western region of Saudi Arabia: a hospital based study. International Ophthalmology 2009;29: 477-483.

[11] McKinnon SJ, Goldberg LD, Peeples P, Walt JG, Bramley TJ. Current management of glaucoma and the need for complete therapy. The American Journal of Managed Care 2008;14(1 Suppl): S20-7.

[12] Savini G, Zanini M, Carelli V, Sadun AA, Ross-Cisneros FN, et al. Correlation between retinal nerve fibre layer thickness and optic nerve head size: an optical coherence tomography study. British journal of Ophthalmology 2005;89(4): 489-492.

[13] Gordon W C, Bazan NG. Cellular organization and biochemistry of the retina. London: Chapman and Hall;1997.

[14] Yu DY, Cringle SJ. Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. Progress in Retinal and Eye Research 2001;20: 175-208.

[15] Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Progress in Lipid Research 1983;22: 79-131.

[16] Gordiyenko N, Campos M, Lee JW, Fariss R N, Sztein J, et al. RPE cells internalize low-density lipoprotein (LDL) and oxidized LDL (oxLDL) in large quantities in vitro and in vivo. Investigative Ophthalmology & Visual Science 2004;45: 2822-2829.

[17] Tserentsoodol N, Sztein J, Campos M, Gordiyenko NV, Fariss RN, et al. Uptake of cholesterol by the retina occurs primarily via a low density lipoprotein receptor-mediated process. Molecular Vision 2006;12: 1306-1318.

[18] Fliesler SJ, Florman R, Rapp LM, Pittler SJ, Keller RK. In vivo biosynthesis of cholesterol in the rat retina. Febs Letters 1993;335: 234-238.

[19] Fourgeux C, Bron A, Acar N, Creuzot-Garcher C, Bretillon L. 24S-hydroxycholesterol and cholesterol-24S-hydroxylase (CYP46A1) in the retina: from cholesterol homeostasis to pathophysiology of glaucoma. Chemistry and Physics of Lipids 2011;164(6): 496-499.

[20] Tielsch JM, Sommer A, Katz J, Royall RM, Quigley HA, et al. Racial variations in the prevalence of primary open-angle glaucoma. The Baltimore Eye Survey. Journal of the American Medical Association 1991;266: 369-374.

[21] Ritch R, Schlötzer-Schrehardt U. Exfoliation syndrome. Survey of Ophthalmology 2001;45: 265-315.

[22] Li ZY, Streeten BW, Wallace RN. Association of elastin with pseudoexfoliative material: an immunoelectron microscopic study. Current Eye Research 1988;7: 1163-1172.

[23] Netland PA, Ye H, Streeten BW, Hernandez M R. Elastosis of the lamina cribrosa in pseudoexfoliation syndrome with glaucoma. Ophthalmology 1995;102: 878-886.

[24] Dark AJ, Streeten BW, Cornwall CC. Pseudoexfoliative disease of the lens: a study in electron microscopy and histochemistry. British Journal of Ophthalmology 1977;61: 462-472.

[25] Morrison JC, Green WR. Light microscopy of the exfoliation syndrome. Acta Ophthalmologica (Copenh.) 1988;184: 5-27.

[26] Streeten BW, Li ZY, Wallace RN, Eagle RC Jr, Keshgegian AA. Pseudoexfoliative fibrillopathy in visceral organs of a patient with pseudoexfoliation syndrome. Archives of Ophthalmology 1992;110: 1757-1762.

[27] Bertelsen TI, Ehlers N. Morphological and histochemical studes on fibrillopathia epithelocapsularis. Acta Ophthalmologica (Copenh) 1969;47: 476-488.

[28] Ringvold A. Light and electron microscopy of the anterior iris surface in eyes with and without pseudo-exfoliation syndrome. Graefe's Archive For Clinical and Experimental Ophthalmology 1973;188:131-137.

[29] Davanger M, Pedersen OO. Pseudo-exfoliation on the anterior lens surface. Demonstration and examination of an interfibrillar ground substance. Acta Ophthalmologica 1975;53: 3-18.

[30] Meretoja J, Tarkkanen A. Occurrence of amyloid in eyes with pseudoexfoliation. Ophthalmic Research 1977;9: 80-91.

[31] Yanagihara M, Kato F, Shikano Y, Fukushima N, Mori S. Intimate structural association of amyloid and elastic fibers in systemic and cutaneous amyloidoses. Journal of Cutaneous Pathology 1985;12(2): 110-116.

[32] Winkelmann RK, Peters MS, Venencie PY. Amyloid elastosis. A new cutaneous and systemic pattern of amyloidosis. Archives of Dermatology 1985;121(4): 498-502.

[33] Streeten BW, Dark AJ, Wallace RN, Li ZY, Hoepner JA. Pseudoexfoliative fibrillopathy in the skin of patients with ocular pseudoexfoliation. American Journal of Ophthalmology 1990;110: 490-499.

[34] Schlötzer-Schrehardt U, Dörfler S, Naumann GO. Immunohistochemical localization of basement membrane components in pseudoexfoliation material of the lens capsule. Current Eye Research 1992;11: 343-355.

[35] Wani FR, Romana M, Singh T, Wani IR, Wani IR, et al. Prevalence of Exfoliative Glaucoma among Kashmiri Population: A Hospital Based Study. International Journal of Health Sciences (Qassim) 2009;3(1): 51–57.

[36] Pohjanpelta P, Hurskainen L. Studies on relatives of patients with glaucoma simplex and patients with pseudoexfoliation of the lens capsule. Acta Ophthalmologica (Copenh) 1972;50(2): 255-261.

[37] Tarkkanen A. Pseudoexfoliation of the lens capsule. Acta Ophthalmologica. 1962;71: 1-98.

[38] Allingham RR, Loftsdottir M, Gottfredsdottir MS, Thorgeirsson E, Jonasson F, et al. Pseudoexfoliation syndrome in Icelandic families. British Journal of Ophthalmology 2001;85: 702-707.

[39] Damji KF, Bains HS, Stefansson E, Loftsdottir M, Sverrisson T, et al. Is pseudoexfoliation syndrome inherited? A review of genetic and nongenetic factors and a new observation. Ophthalmic Genetics 1998;19: 175-185.

[40] Forsius HR, Fellman JO, Eriksson AW. Genetics of exfoliation syndrome (pseudoexfoliation of the lens). New Trends in Ophthalmology 1993;3: 135-139.

[41] Lemmelä S, Forsman E, Sistonen P, Eriksson A, Forsius H, et al. Genome-wide scan of exfoliation syndrome. Investigative Ophthalmology & Visual Science 2007;48(9): 4136-4142.

[42] Thorleifsson G, Magnusson KP, Sulem P, Walters GB, Gudbjartsson DF, et al. Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science 2007;317: 1397-1400.

[43] Laws SM, Hone E, Gandy S, Martins RN. Expanding the association between the APOE gene and the risk of Alzheimer's disease: possible roles for APOE promoter

polymorphisms and alterations in APOE transcription. Journal of Neurochemistry 2003;84: 1215-1236.

[44] Strittmatter WJ, Weisgraber KH, Huang DY, Dong LM, Salvesen GS, et al. Binding of human apolipoprotein E to synthetic amyloid beta peptide: isoform-specific effects and implications for late-onset Alzheimer disease. Proceedings of the National Academy of Sciences of the United States of America 1993;90(17): 8098-8102.

[45] Castano EM, Prelli F, Wisniewski T, Golabek A, Kumar RA, et al. Fibrillogenesis in Alzheimer's disease of amyloid beta peptides and apolipoprotein E. Biochemical Journal 1995;306: 599-604.

[46] Amaratunga A, Abraham CR, Edwards RB, Sandell JH, Schreiber BM, et al. Apolipoprotein E is synthesized in the retina by Muller glial cells, secreted into the vitreous, and rapidly transported into the optic nerve by retinal ganglion cells. Journal of Biological Chemistry 1996;271: 5628-5632.

[47] Vickers JC, Craig JE, Stankovich J, McCormak GH, West AK, et al. The apolipoprotein epsilon4 gene is associated with elevated risk of normal tension glaucoma. Molecular Vision 2002;8: 389-393.

[48] Artiga MJ, Bullido MJ, Sastre I, Recuero M, García MA. Allelic polymorphisms in the transcriptional regulatory region of apolipoprotein E gene. Febs Letters 1998;421: 105-108.

[49] Yin R, Pan S, Wu J, Lin W, Yang D. Apolipoprotein E gene polymorphism and serum lipid levels in the Guangxi Hei Yi Zhuang and Han populations. Experimental Biology and Medicine (Maywood) 2008;233: 409-418.

[50] Raygani VA, Kharrazi H, Rahimi Z, Pourmotabbed T. Frequencies of apolipoprotein E polymorphism in a healthy Kurdish population from Kermanshah, Iran. Human Biology 2007;79: 579-587.

[51] Al-Dabbagh NM, Al-Dohayan N, Arfin M, Tariq M. Apolipoprotein E polymorphisms and primary glaucoma in Saudis. Molecular Vision 2009;15: 912-919.

[52] Gerdes LU, Klausen LC, Sihm I, Faergeman O. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. Genetic Epidemiology 1992;9: 155-167.

[53] Mastana SS, Calderon R, Pena J, Reddy PH, Papiha SS. Antrhopology of the apolipoprotein E (Apo E) gene: low frequency of Apo E4 allele in Basques and in tribal (Baiga) populations of India. Annals of Human Biology 1998;25: 137-143.

[54] Corbo RM, Scacchi R, Mureddu L, Mulas G, Castrechini S, et al. Apolipoprotein B, apolipoprotein E, and angiotensin-converting enzyme polymorphisms in 2 Italian populations at different risk for coronary artery disease and comparison of allele frequencies among European population. Human Biology 1999;71: 933-945.

[55] Singh P, Singh M, Gerdes U, Mastana SS. Apolipoprotein E polymorphism in India: high APOE*E3 allele frequency in Ramgarhia of Punjab. Anthropologischer Anzeiger 2001;59: 27-34.

[56] Singh PP, Singh M, Mastana SS. APOE distribution in world populations with new data from India and the UK. Annals of Human Biology 2006;33: 279-308.

[57] Raygani AV, Zahrai M, Raygani AV, Doosti M, Javadi E, et al. Association between apolipoprotein E polymorphism an Alzheimer disease in Tehran, Iran. Neuroscience Letters 2005;375: 1-6.

[58] Svobodova h, Kucera F, Stule T, Vrablik M, Amartuvshin B, et al. Apolipoprotein E gene polymorphism in the Mongolian population. Folia Biologica (Praha) 2007;53: 138-142.

[59] Fan BJ, Wang DY, Fan DS, Tam PO, Lam DS, et al. SNPs and interaction analyses of myocilin, optineurin, and apolipoprotein E in primary open angle glaucoma patients. Molecular Vision 2005;11: 625-631.

[60] Lam CY, Fan BJ, Wang DY, Tam PO, Yung Tham CC, et al. Association of apolipoprotein E polymorphisms with normal tension glaucoma in a Chinese population. Journal of Glaucoma 2006;15: 218-222.

[61] Zetterberg M, Tasa G, Palmer MS, Juronen E, Teesalu P, et al. Apolipoprotein E polymnorphisms in patients with primary open-angle glaucoma. American Journal of Ophthalmology 2007;143: 1059-1060.

[62] Lake S, Liverani D, Desai M, Casson R, James B, et al. Normal tension glaucoma is not associated with the common apolipoprotein E gene polymorphisms. British Journal of Ophthalmology 2004;88: 491-493.

[63] Ressiniotis T, Griffiths PG, Birch M, Keers S, Chimnery PF. The role of apolipoprotein E gene polymorphisms in primary open-angle glaucoma. Archives of Ophthalmology 2004;122: 258-261.

[64] Mabuchi F, Tang S, Ando D, Yamakita M, Wang J, et al. The apolipoprotein E gene polymorphism is associated with open angle glaucoma in the Japanese population. Molecular Vision 2005;11: 609-612.

[65] Yuan HP, Xiao Z, Yang BB. A study on the association of apolipoprotein E genotypes with primary open-angle glaucoma and primary angle-closure glaucoma in northeast of China. Zhonghua Yan Ke Za Zhi 2007;43: 416-420.

[66] Yilmaz A, Tamer L, Aras Ates N, Camdeviren H, Degirmenci U. Effects of apolipoprotein E genotypes on the development of exfoliation syndrome. Experimental Eye Research 2005;80: 871-875.

[67] Ritland JS, Utheim TP, Utheim OA, Espeseth T, Lydersen S, et al. Effects of APOE and CHRNA4 genotypes on retinal nerve fibre layer thickness at the optic disc and

on risk for developing exfoliation syndrome. Acta Ophthalmologica Scandinavica 2007;85(3): 257-261.

[68] Krumbiegel M, Pasutto F, Mardin CY, Weisschuh N, Paoli D, et al. Apolipoprotein E genotypes in pseudoexfoliation syndrome and pseudoexfoliation glaucoma. Journal of Glaucoma 2010;19: 561-565.

[69] Anderson DR, Patella VM. Automated static perimetry. St. louis, MO: Mosby; 1999. p10-35.

[70] Foster PJ, Buhrmann R, Quigley HA, Johnson GJ. The definition and classification of glaucoma in prevalence surveys. British Journal of Ophthalmology 2002;86(2): 238-242.

[71] Vijay L, George R, Arvind H, Baskaran M, Raju P, et al. Prevalence and causes of blindness in the rural population of the Chennai Glaucoma study. British Journal of Ophthalmology 2006;90: 407-410.

[72] Attebo K, Ivers RQ, Mitchell P. Refractive errors in an older population: the Blue Mountains Eye Study. Ophthalmology 1999;106(6): 1066-1072.

[73] Lamb EJ, Day AP. New diagnostic criteria for diabetes mellitus: are we any further forward? Annals of Clinical Biochemistry 2000;37(5): 588-592.

[74] Schallreuter KU, Levenig C, Kuhnl P, Loliger C, Hohl-Tehari M, et al. Histocompatability antigens in vitiligo: Hamburg study on 102 patients from Northern Germany. Dermatology 1993;187: 186-192.

[75] Savejgaard A, Platz P, Ryder LP. HLA and disease A survey. Immunological Reviews 1982;70: 193-218.

[76] Corbo RM, Schachi R. Apolipoprotein distribution around the world. Is APOE 4 a thrifty allele? Annals of Human Genetics 1999;63: 301-310.

[77] Lehtinen S, Luoma P, Lehtimaki T, Nayha S, Hassi J, et al. Differences in genetic variations of apolipoprotein E in Lapps and Finns. Atherosclerosis 1994;109: 263-268.

[78] Corbo RM, Scacchi R, Mureddu L, Mulas G, Alfano G. Apolipoprotein E polymorphism in Italy investigated in native plasma by a simple polyacrylamide gel isoelectric focusing technique. Comparison with frequency data of other European populations. Annals of Human Genetics 1995;59: 197-209.

[79] Sklavounou E, Economou-Peterse, Karadima G, Panas M, Avramopoulos D, et al. Apolipoprotein E polymorphism in the Greek population. Clinical Genetics 1997;52: 216-218.

[80] Nathan BP, Bellosta S, Sanan DA, Weisgraber KH, Mahley RW, et al. Differential effects of apolipoprotein E3 and E4 on neuronal growth in vitro. Science 1994;264: 850-852.

[81] Roses AD, Saunders AM. ApoE, Alzheimer's disease, and recovery from brain stress. Annals of the New York Academy of Sciences 1997;826: 200-212.

[82] Liew G, Shankar A, Wang JJ, Klein R, Bray MS, et al. Apolipoprotein E gene polymorphisms and retinal vascular signs: the atherosclerosis risk in communities (ARIC) study. Archives of Ophthalmology 2007;125: 813-818.

[83] Hong CJ, Liu TY, Liu HC, Wang SJ, Fuh JL, et al. Epsilon 4 allele of apolipoprotein E increases risk of Alzheimer's disease in a Chinese population. Neurology 1996;46: 1749-1751.

[84] Katzman R, Zhang MY, Chen PJ, Gu N, Jiang S, et al. Effects of apolipoprotein E on dementia and aging in the Shanghai Survey of Dementia. Neurology 1997;49: 779-785.

[85] Lehmann DJ, Smith AD, Combrinck M, Barnetson L, Joachim C. Apoliporpotein E epsilon 2 may be a risk factor for sporadic frontotemporal dementia. Journal of Neurology, Neurosurgery and Psychiatry 2000;69: 404-405.

[86] Mak YT, Chiu H, Woo J. Apolipoprotein E genotype and Alzheimer's disease in Hong Kong elderly Chinese. Neurology 1996;46: 146-149.

[87] Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A Meta-Analysis. APOE and Alzheimer Disease Meta Analysis Consortium. Journal of the American Medical Association 1997;278: 1349-1356.

[88] Tilley L, Morgan K, Grainger J, Marsters P, Morgan L, et al. Evaluation of polymorphisms in the presenilin-1 gene and the butyrylcholinesterase gene as risk factors in sporadic Alzheimer's disease. European Journal of Human Genetics 1999;7: 659-663.

[89] Bayer AU, Ferrari F. Severe progression of glaucomatous optic neuropathy in patients with Alzheimer's disease. Eye 2002;16: 201-212.

[90] [90]Bayer AU, Keller ON, Ferrari F, Maag KP. Association of glaucoma with neurodegenerative diseases with apoptotic cell death: Alzheimer's disease and Parkinson's disease. American Journal of Ophthalmology 2002;133: 135-137.

[91] McKinnon SJ. Glaucoma: ocular Alzheimer's disease? Frontiers in Bioscience 2003;8: 1140-1156.

[92] Tatton W, Chen D, Chalmers-Redman R, Wheeler L, Nixon R, et al. Hypothesis for a common basis for neuroprotection in glaucoma and Alzheimer's disease: anti-apoptosis by alpha-2-adrenergic receptor activation. Survey of Ophthalmology 2003;48: 25-37.

[93] Strittmatter WJ, Roses AD. Apolipoprotein E and Alzheimer's disease. Annual Review of Neuroscience 1996;19: 53-77

[94] Saunders AM, Schmader K, Breitner JC, Benson MD, Brown WT. Apolipoprotein E e4 allele distributions in late-onset Alzheimer's disease and in other amyloid-forming diseases. Lancet 1993;342: 710-711.

[95] [95]Saunders AM, Strittmatter WJ, Schmechel D, George-Hyslop PH, Pericak-Vance MA, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43: 1467-1472.

[96] Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, et al. Gene dose of apolipoprotein E type-4 allele and the risk of Alzheimer's disease in late onset families. Science 1993;261: 921-923.

[97] Harrington CR, Roth M, Xuereb JH, McKema PJ, Wischik CM. Apolipoprotein E type epsilon 4 allele frequency is increased in patients with schizophrenia. Neuroscience Letters 1995;202(1-2): 101-104.

[98] Liu W, Breen G, Zhang J, Li S, Gu N, et al. Association of APOE gene with schizophrenia in Chinese: a possible risk factor in times of malnutrition. Schizophrenia Research 2003;62: 225-230.

[99] Papapetropoulos S, Farrer MJ, Stone JT, Milkovic NM, Ross OA, et al. Phenotypic associations of tau and ApoE in Parkinson's disease. Neuroscience Letters 2007; 414(2): 141-144.

[100] Teasdale GM, Nicoll JA, Murray G, Fiddes M. Association of apolipoprotein E polymorphism with outcome after head injury. Lancet 1997;350: 1069-1071.

[101] Friedman JS, Walter MA. Glaucoma genetics, present and future. Clinical Genetics 1999;55: 71-79.

[102] Klaver CC, Kliffen M, van Duijn CM, Hofman A, Cruts M, et al. Genetic association of apolipoprotein E with age-related macular degeneration. American Journal of Human Genetics 1998; 63: 200-206

[103] Kuhrt H, Härtig W, Grimm D, Faude F, Kasper M, Reichenbach A. Changes in CD44 and ApoE immunoreactivities due to retinal pathology of man and rat. Journal für Hirnforschung 1997;38(2): 223-229.

[104] Wisniewski T, Frangione B. Apolipoprotein E: a pathological chaperone protein in patients with cerebral and systemic amyloid. Neuroscience Letters 1992;135: 235-238.

[105] Arai H, Muramatsu T, Higuchi S, Sasaki H, Trojanowski JQ. Apolipoprotein E gene in Parkinson's disease with or without dementia. Lancet 1994;344(8926): 889

[106] Benjamin R, Leake A, Ince PG, Perry RH, McKeith IG, et al. Effects of apolipoprotein E genotype on cortical neuropathology in senile dementia of the Lewy body and Alzheimer's disease. Neurodegeneration 1995;4: 443-448.

[107] Schupf N, Kapell D, Lee JH, Zigman W, Canto B. Onset of dementia is associated with apolipoprotein E epsilon4 in Down's syndrome. Annals of Neurology 1996; 40: 799-801.

[108] Al-Khedhairy AA. Apolipoprotein E polymorphism in Saudis. Molecular Biology Reports 2004;31: 257-260.

[109] Gamboa R, Hernandez-Pacheco G, Hesiquio R, Zuniga J, Masso F, et al. Apolipoprotein E polymorphism in the Indian and Mestizo population of Mexico. Human Biology 2000;72: 975-981.

[110] Summanen P, Tonjum AM. Exfoliation syndrome among Saudis. Acta Ophthalmologica 1988;184(Suppl): 107-111.

[111] Desai MA, Lee RK. The medical and surgical management of pseudoexfoliation glaucoma. International Ophthalmology Clinics 2008;48(4): 95-113.

[112] Thomas R, Kumar RS, Chandrasekhar G, Parikh R. Applying the recent clinical trials on primary open angle glaucoma: the developing world perspective. Journal of Glaucoma 2005;14(4): 324-7.

[113] Rao RQ, Arain TM, Ahad MA. The prevalence of pseudoexfoliation syndrome in Pakistan. Hospital based study. BMC Ophthalmology 2006;6: 27.

[114] Miyazaki M, Kubota T, Kubo M, Kiyohara Y, Iida M, Nose Y, Ishibashi T.The prevalence of pseudoexfoliation syndrome in a Japanese population: the Hisayama study. J Glaucoma. 2005;14(6): 482-484.

[115] Young AL, Tang WW, Lam DS.The prevalence of pseudoexfoliation syndrome in Chinese people. British Journal of Ophthalmology 2004;88(2): 193-195.

[116] Foster PJ, Sheah SKL. The prevalence of pseudoexfoliation syndrome in Chinese people: the Tanjong Pagar Survey. Briitish Journal of Ophthalmology 2005;89: 239-240.

[117] Aasved H. Prevalence of bibrilopathia epitheliocapsularies (pseudoexfoliation) and capsular glaucoma. Transaction of Ophthalmological Society UK 1975; 99: 293-295.

[118] Forsius H. Prevalence of pseudoexfoliation of the lens in Finns, Lapps, Icelanders, Eskimos and Russinas. Transaction of Ophthalmological Society UK1979;99: 296-298.

[119] Lantukh VV, Piatin MM. Features of ocular pathology among the indigenous inhabitants of Chukotka. Vestnik Oftalmologii 1982; 18-20.

[120] Ringvold A, Blika S, Elsas T. The middle-Norway eye-screening study. III. The prevalence of capsular glaucoma is influenced by blood-group antigens. Acta Ophthalmologica (Copenh)1993; 71: 207-213.

[121] Colin J, Le Gall G, Le Jeune B, Cambria MD. The prevalence of exfoliation syndrome in different areas of France. Acta Ophthalmologica1988;184(Suppl): 86-89.

[122] Ringvold A, Blika S, Elsas TL The prevalence of pseudoexfolation in three separate municipalities of Middle-Norway. A preliminary report. Acta Ophthalmologica 1987;182(Suppl): 17-20.

[123] Ringvold A: Epidemiology of the pseudo-exfoliation syndrome. Acta Ophthalmologica Scandinavica 1999;77:371-375.

[124] Forsius H, Lukka H. Pseudoexfoliation of the anterior capsule of the lens in Lapps and Eskimos. Canadian Journal of Ophthalmology 1973; 8:274-277.

[125] Forsius H. Exfoliation syndrome in various ethnic populations. Acta Ophthalmologica 1988;184(Suppl): 71-85.

[126] Ritch R. Exfoliation syndrome and occludable angles. Transactions of American Ophthalmology Society1994;92: 845-944

[127] Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-nerve degeneration in Alzheimer's disease. New England Journal of Medicine 1986;315: 485-487.

[128] Sadun AA, Bassi CJ. Optic nerve damage in Alzheimer's disease. Ophthalmology 1990; 97: 9-17.

[129] Davies DC, McCoubrie P, McDonald B, Jobst KA. Myelinated axon number in the optic nerve is unaffected by Alzheimer's disease. British Journal of Ophthalmology 1995;79: 596-600.

[130] Vickers JC, Lazzarini RA, Riederer BM, Morrison JH. Intraperikaryal neurofilamentous accumulations in a subset of retinal ganglion cells in aged mice that express a human neurofilament gene. Experimental Neurology 1995;136(2): 266-269.

[131] Leuba G, Saini K. Pathology of subcortical visual centres in relation to cortical degeneration in Alzheimer's disease. Neuropathology and Applied Neurobiology 1995;21: 410-422.

[132] Brega A, Scacchi R, Cuccia M, Kirdar B, Peloso G. Study of 15 protein polymorphisms in a sample of the Turkish population. Human Biology 1998;70: 715-728

[133] Attila G, Acartürk E, Eskandari G, Akpinar O, Tuli A, et al. Effects of apolipoprotein E genotypes and other risk factors on the development of coronary artery disease in Southern Turkey. Clinica Chimica Acta 2001;312: 191-196.