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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 metab‐
olism 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 pathophysiology
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 signifi‐
cance 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 75 years, 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 medica‐
tions, 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
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 ε2 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:
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 tonometer (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 -20°C before extraction of DNA. The study protocol was approved by the
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 immobilized 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 GCC ACG GCT GTC CAA GGA GCT GCA GGC GAC GCA GCC 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:

\[ RR = \frac{(a) \times (d)}{(b) \times (c)} \]

\( a \) = 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].

\[ EF = \frac{(RR-1)f}{RR} \]

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].

\[ PF = \frac{(1-RR)f}{RR(1-f) + f} \]

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).
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.

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).
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.

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

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

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.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Patients screened (N)</th>
<th>PEX positive patients (N)</th>
<th>Frequency of PEX (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>600</td>
<td>3</td>
<td>0.50</td>
</tr>
<tr>
<td>51-60</td>
<td>850</td>
<td>27</td>
<td>3.17</td>
</tr>
<tr>
<td>61-70</td>
<td>200</td>
<td>16</td>
<td>8.00</td>
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<tr>
<td>71-80</td>
<td>30</td>
<td>4</td>
<td>13.33</td>
</tr>
<tr>
<td>81-100</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>1684</td>
<td>51</td>
<td>3.03</td>
</tr>
</tbody>
</table>

N, number of patients

Table 7. Age specific prevalence of PEX in Saudi patients

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

<table>
<thead>
<tr>
<th>Allele</th>
<th>Pseudoexfoliation (N=102)</th>
<th>Control (N=400)</th>
<th>P-value</th>
<th>RR</th>
<th>EF*/PF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Frequency (%)</td>
<td>Number</td>
<td>Frequency (%)</td>
<td></td>
</tr>
<tr>
<td>ε4</td>
<td>15</td>
<td>14.70</td>
<td>17</td>
<td>4.25</td>
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<tr>
<td>ε3</td>
<td>84</td>
<td>82.35</td>
<td>383</td>
<td>95.75</td>
<td>0.0001†</td>
</tr>
<tr>
<td>ε2</td>
<td>3</td>
<td>2.94</td>
<td>0</td>
<td>0.00</td>
<td>0.0081†</td>
</tr>
</tbody>
</table>
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]

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Pseudoexfoliation (N=51)</th>
<th>Control (N=200)</th>
<th>P-value</th>
<th>RR</th>
<th>EF*/PF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Frequency (%)</td>
<td>Number</td>
<td>Frequency (%)</td>
<td></td>
</tr>
<tr>
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<td>36</td>
<td>70.58</td>
<td>183</td>
<td>91.50</td>
<td>0.0002*</td>
</tr>
<tr>
<td>ε3/ε4</td>
<td>11</td>
<td>21.56</td>
<td>17</td>
<td>8.50</td>
<td>0.012*</td>
</tr>
<tr>
<td>ε4/ε4</td>
<td>1</td>
<td>1.96</td>
<td>0</td>
<td>0.0</td>
<td>0.203</td>
</tr>
<tr>
<td>ε2/ε2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>ε2/ε3</td>
<td>1</td>
<td>1.96</td>
<td>0</td>
<td>0.0</td>
<td>0.203</td>
</tr>
<tr>
<td>ε2/ε4</td>
<td>2</td>
<td>3.92</td>
<td>0</td>
<td>0.0</td>
<td>0.040*</td>
</tr>
</tbody>
</table>

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

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