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Role of an extracellular chaperone, Clusterin in the pathogenesis of Pseudoexfoliation Syndrome and Pseudoexfoliation Glaucoma

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ABSTRACT

Pseudoexfoliation (PEX), an age related disorder is a prominent contributor to secondary glaucoma. Earlier studies have suggested involvement of clusterin in the development of PEX. We designed a case-control study to understand the role of clusterin single nucleotide polymorphisms (SNPs) in PEX and analyzed the role of risk alleles in the disease. Genotyping of SNPs in 136 PEX patients and 89 controls of Indian origin revealed a genetic association between rs2279590 and PEX in Indian population with a *p*-value of 0.004. The high risk allele "G" at rs2279590 has an effect on clusterin mRNA expression. There was a twofold higher clusterin mRNA level in "GG" genotyped individuals in comparison to "AA" genotyped individuals ($p = 0.039$). Western blot and immunohistochemistry studies showed an upregulation of Clusterin protein in pseudoexfoliation glaucoma (PXG) affected individuals in both aqueous humor and lens capsules respectively. Together, our results reveal that rs2279590 was found to be associated with PEX in Indian population and the risk allele mediates an allele specific upregulation of the clusterin mRNA. Moreover, upregulation of Clusterin protein in PXG individuals augments further protein deposition.

1. Introduction

Pseudoexfoliation (PEX; OMIM: 177650) is a systemic disorder of extracellular matrix characterized by the presence of fibrillar deposits in the anterior segment of eye. Although the actual source and origin of these deposits is still unknown, it has been reported as a leading cause for secondary glaucoma known as pseudoexfoliation glaucoma (PXG). PXG has been reported to have worse outcomes and faster rate of progression as compared to other types of glaucoma. It has been shown that 50% PEX affected individuals over the age of 70 develop optic nerve degeneration and increased intraocular pressure (Jeng et al., 2007). PEX without glaucoma is known as pseudoexfoliation syndrome (PXF) and has been associated with zonular weakness, cataract formation, elevated serum homocysteine and systemic vascular complications like abdominal aortic aneurysm (Mitchell et al., 1997; Naumann et al., 1998; Schumacher et al., 2001; Schlotzer-Schrehardt and Naumann, 2006; Roedl et al., 2007). Prevalence of PEX varies widely among different population and increases with age; the highest reported in Icelandic population (Arnarsson et al., 2007).

Both familial aggregation studies on Icelandic and Canadian population suggest a prominent genetic contributor underlying the disease (Aasved, 1975; Damji et al., 1999; Allingham et al., 2001; Lemmela et al., 2007). A genome wide association study (GWAS) was performed on Icelandic patients and found three single nucleotide polymorphisms (SNPs), rs1048661, rs3825942 and rs2165241 on chromosome 15q24.1 of lysyl oxidase-like 1 (LoxL1) to be associated with pseudoexfoliation (Thorleifsson et al., 2007). Following this GWAS study, replication studies in Caucasian (Fingert et al., 2007; Challa al., 2008; Hewitt et al., 2008; Mossbock et al., 2008; Pasutto et al., 2008) and Indian populations (Ramprasad et al., 2008) have implicated the "G" allele as major risk allele for both rs3825942 and rs1048661. However, the high risk allele is also present in the normal population with a high prevalence of upto 88% (Challa, 2009). Further, both the SNPs, rs3825942 and rs1048661 have very low specificity in predicting the affected

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status (Challa et al., 2008). Surprisingly, in Black South African population the high risk allele is "A" for rs3825942 (Williams et al., 2010) while "T" is the high risk allele for rs1048661 in Japanese and Chinese population (Hayashi et al., 2008; Ozaki et al., 2008; Chen et al., 2009). These inverse relationships suggest an unclear role of these SNPs in pseudoexfoliation and also hint at the possible role of other candidate gene/s in pseudoexfoliation.

Recently, two groups reported the presence of another protein, Clusterin along with PEX fibrils on the surface of lens capsule (Creasey et al., 2010) as well as in PEX aggregates (Ovodenko et al., 2007). Clusterin or Apolipoprotein-J is a multi-functional protein playing many important roles in a variety of extracellular processes such as lipid transport, apoptosis, stabilization of cell–cell and cell–matrix interactions, inhibition of complement activation and preventing protein misfolding and has been found to play a major role in formation of neurofibrillary tangles in Alzheimer's disease. Furthermore, the expression of Clusterin was found to be decreased in the anterior tissues of PEX individuals which suggests a hampered Clusterin chaperone function responsible for deposition of abnormal extracellular matrix product in anterior segment (Zenkel et al., 2006). Two independent research groups also found two different clusterin SNPs, rs3087554 (Burdon et al., 2008) and rs2279590 (Krumbiegel et al., 2009) having an association with PEX in Australian and German population respectively. Out of these, rs2279590 also has been associated strongly with Alzheimer's disease. Since PEX involves patho-physiological processes similar to those seen in Alzheimer's disease where Clusterin is implicated for protein aggregation, it is logical to explore the role of Clusterin in PEX development.

In Indians, the prevalence of PEX varies from 0.69% to 3.8% (Arvind et al., 2003; Thomas et al., 2005; Jonas et al., 2013) which increases from 6.28% to 8.45% with age. Further, PEX is also a significant contributor towards developing secondary glaucoma. The following study has been conducted to test the association of two SNPs, rs3087554 and rs2279590 of clusterin as a risk factor towards development of PEX in Indian population as well as to find their role in its pathogenesis.

2. Materials and methods

2.1. Study participants

This study was approved by the ethics review boards of NISER and LV Prasad Eye Institute, Bhubaneswar. All patients underwent detailed ocular examination, including slit lamp examination, ocular biometry, Goldman applanation tonometry, +90D biomicroscopic fundus evaluation and 4 mirror gonioscopy. All procedures were followed according to the tenets of the Declaration of Helsinki and an informed consent was taken from all subjects included in the study. Humphrey visual field 24-2 program was done in all cases.

Inclusion criteria for PXF involved adults >40 years with or without visually significant cataract, best corrected visual acuity >20/100. Clinically evident pseudoexfoliation like material over lens, pupillary ruff with or without poor dilatation; open or closed angles on gonioscopy, normal IOP < 21 mm Hg without any prior anti-glaucoma treatment and no evidence of glaucomatous optic nerve damage or visual field defects.

PXG affected participants comprised of adults >40 years with or without visually significant cataract, having clinically evident pseudoexfoliation like material over lens, pupillary ruff, raised IOP > 21 mm Hg without prior anti-glaucoma treatment and evidence of glaucomatous optic nerve head damage (defined as vertical cup-to-disc ratio of 0.8 or more, cup-to-disc asymmetry of more than 0.2, focal notching, or a combination thereof) with repeatable field defects corresponding to disc damage. Patients with corneal or retinal pathology precluding reliable visual field and disc examination were excluded.

Controls were selected on the basis of adults >40 years with or without visually significant cataract, without clinically evident pseudoexfoliation like material over lens, pupillary ruff, untreated IOP < 21 mm Hg and normal discs and visual field. Demographic as well as clinical features of the study group are shown in Table 1.

2.2. Genotyping

4 ml blood was collected from both case and control subjects and stored at −80°C until further use. Subsequently, genomic DNA was extracted using phenol–chloroform extraction method. For genotyping, the region containing two SNPs (rs3087554 and rs2279590; Fig. 1) were amplified by polymerase chain reaction using two sets of primers (PCR; model Mastercycler® pro; Eppendorf AG, Hamburg) and subsequently sequenced. The primers were designed by Primer-BLAST. Both sequence details and annealing temperature of primers are shown in Table 2. PCR reactions were performed in 25 μl volumes containing Taq Buffer A (GeNei, Bangalore, India), 1.5 mM $MgCl_2$, 0.5 μM of each primer (IDT, USA), 100 mM dNTP mixture (GeNei, Bangalore, India), 100 ng of genomic DNA, 0.5 unit of *Taq* DNA polymerase (GeNei, Bangalore, India) and 2.5 μl of DMSO. The PCR products were subsequently eluted by gel elution kit (QIAquick Gel Extraction Kit, QIAGEN, Hilden) and sequenced unidirectionally using one of the previously mentioned primers (Table 2) with the help of BigDye Terminator v.3.1 cycle sequencing kit (Applied Biosystems, Austin, TX78744, USA). The automated sequencer 3130xl genetic analyzer from Applied Biosystems was used for sequencing and analysis was done using Sequencing analysis software v5.3 (Applied Biosystem) and BioEdit v7.1 (Freely available online at http://www.mbio.ncsu.edu/bioedit/bioedit.html).

2.3. Real-time PCR

Lens capsules were collected from both cases and controls during cataract surgery and immediately kept in RNA later (Invitrogen) and stored in −80°C until further use. Total RNA was isolated from individual lens capsules by using a RNA extraction kit (RNeasy Mini Kit, QIAGEN GmbH, Hilden). cDNA synthesis was performed with 100 ng of total RNA, using a Reverse Transcription

Table 1
Demographic and clinical features of study subjects included for genotyping.

	N =	Age (in years)		Sex		IOP[a] Mean ± SD mm Hg	VCDR[b] Mean ± SD	Manifestation	
		Mean ± SD	Range	Male	Female			Unilateral	Bilateral
PEX Combined Subjects	136	67.1 ± 9.1	40–92	101	35	16 ± 18.3	0.3 ± 0.5	43	93
PXF Subjects	81	68.9 ± 9.1	40–86	55	26	14 ± 4.2	0.20 ± 0.1	20	61
PXG Subjects	55	65.2 ± 8.7	47–92	46	9	23 ± 10.2	0.7 ± 0.2	23	32
Control Subjects	89	57.9 ± 8.9	40–82	52	37	12 ± 3.2	0.1 ± 0.2	NA	NA

[a] IOP = Intraocular pressure.
[b] VCDR = vertical cup disc ratio.