Prime Scholars Library

Advances in Animal Science, Theriogenology, Genetics and Breeding

Full Length Research Paper

Vol. 1 (3), pp. 025-029, September, 2013

Available online at
https://primescholarslibrary.org/

Lack of DLAD mutations in age-related nuclear cataract

Nitza Goldenberg-Cohen, Bat Chen R. Avraham-Lubin, Dorina Calles

The Krieger Eye Research Laboratory, Felsenstein Medical Research Center, Petah Tiqwa, Israel.

Abstract

DNase II like acid DNase (DNase IIβ, DLAD) is expressed in human and murine cells in the lens. Studies in mice have reported that abnormal degeneration of cellular organelles by DLAD reduced lens transparency and that the *DLAD* gene may be involved in cataract formation. The aim of the present study was to search for possible genetic alterations in the *DLAD* gene in human senile cataract. Anterior lens capsule material was collected during surgery from 55 patients with senile cataract, with or without a subcapsular component. Total DNA was extracted, amplified by polymerase chain reaction and sequenced for exon 3 (n = 51) exon 4 (n = 40) and all 6 exons of the *DLAD* gene (n = 27). No mutation was found. There were genomic polymorphisms in all exons except 3 and 4. Nonsynonymous genomic polymorphisms were detected in exon 1 (rs738573) and exon 2 (rs3754274) and synonymous polymorphisms were detected in exon 5 (rs7511984) and exon 6 (rs3768250). In contrast to findings in mice, based on the limited samples analyzed, this study suggests that human age-related nuclear cataract is not associated with *DLAD* mutations.

Keywords: DNAase II like acid DNAase- DNAse IIβ- *DLAD*- senile cataract-nuclear cataract.

Abbreviations: DLAD, DNase II like acid DNase; **LOCS,** lens opacities classification scale; **PCR,** polymerase chain reaction.

INTRODUCTION

The lens is an avascular tissue that focuses light onto the retina. It consists of closely packed fibers that are produced continuously by the differentiation of epithelial cells located near its equator. During their proliferation, differentiation and migration toward the nucleus, the epithelial cells elongate and their light-scattering organelles, including the endoplasmic reticulum, mitochondria, golgi apparatus and nucleus, disappear, together with housekeeping enzymes (RNA polymerase and DNA polymerase, etc.) (Bassnett, 2002).

Concomitantly, the production of lens-specific proteins and crystallins increases. The lens nucleus is composed of fully differentiating fiber-like cells (Nakahara et al., 2007). Because there is no cell turnover, the lens grows throughout life (McAvoy et al., 1999; Piatigorsky, 1981).

Accordingly, the cortex of the adult lens has two populations of fiber cells: a cortical layer of differentiating cells that contain organelles and the nonnucleated fiber cells that do not contain organelles. Several organelle-degradation mechanisms have been proposed, most prominently apoptosis (Dahm, 1999; Ishizaki et al., 1998; Zandy and Bassnett, 2007), cytosolic degradation (van Leyen et al., 1998) and autophagy (Vrensen et al., 1991). Autophagy has been extensively studied in yeast and several molecules involved in this process have been identified (Nagata, 2005). However, the pathways involved in the differentiation of fiber cells are still elusive.

The loss of organelles during fiber cell differentiation apparently occurs to ensure the transparency of the lens (Nagai et al., 2006). Cataract is an alteration in the optical homogeneity of the lens or a decrease in its transparency. It leads to decreased vision and even blindness.

.

Table 1. Primers and polymerase chain reaction (PCR) reaction conditions.

Exon	Primers	Product size bp	Annealing temperature(°C)
1 F	5'-CGCCTTGAAACTCAGACTCC	239	56
1 R	5'-ATGCAGCAGTCCTTCCATTT		
2 F	5'-CTGCCACAGGAAAACAGTCA	307	56
2 R	5'-ACTCTGGTGAAGGCTTTGGA		
3 F	5'-GGGCGTTTGTAAGTGGAAGA	174	56
3 R	5'-TTCCAGTAGGAAAGAGAAACCAA		
4 F	5'-TGTTGCCACTGTGAACCCTA	300	60
4 R	5'-TTTCCAAATCATTTCTTCAACA		
5 F	5'-TGCCTTTGTTTTTGTGTTGTTT	343	56
5 R	5'-CAGCATGTTGTTTGATGATGG		
6 F	5'-CATCCACATATCAGGGGTGA	718	56
6 R	5'-TTGGAGTTGACTAATGTGGAAAAA		

Although cataract may result from normal aging, multiple factors can contribute to this process, including the precipitation and degradation of proteins, exposure to ultraviolet light, exposure to oxidative and other toxic agents, genetic factors, diabetes, smoking, poor nutrition and alterations in endocrinic or enzymatic equilibrium (Cekic et al., 1999; Dilsiz et al., 1999; Garland et al., 1988; Garland, 1999; Tumminia et al., 2001). DNase II-like acid DNase (DLAD; also known as DNase II*β*) is expressed in human and murine cells, specifically in the lens, and in the liver, salivary glands, and lungs (Nakahara et al., 2007; Nagai et al., 2006). Its role in degrading DNA during lens cell differentiation was reported in a study of mice deficient in the *DLAD* gene (Nishimoto et al., 2003). The failure to degrade DNA led to the development of cataract and the accumulation of undegraded DNA in the fiber cells. Nishimoto et al. (2003) identified mutations in the activated enzymatic site of exons 3 and 4 of the *DLAD* gene in cataractous mice.

Recently, Nakahara et al. (2007) found that the DLAD protein is localized to lysosomes of the cortical fiber cells, close to the organelle-free zone. Its absence from epithelial cells indicated that DLAD expression is induced during differentiation of the epithelial cells into fiber cells. Immunohistochemical analysis suggested that this process involves the fusion of the nuclei with the lysosomes and DLAD digestion of the chromosomal DNA.

We sought to determine if findings in murine models could be extended to humans. The aim of the present study was to explore the role of genetic variations in the *DLAD* gene in human age-related cataract formation.

METHODS

Patients

The study group included 55 patients undergoing routine cataract surgery at a major tertiary medical center. All patients signed an informed consent form. All were examined preoperatively by slit-lamp and categorized by cataract type according to the Lens Opacities Classification Scale (LOCS) Background data were derived from the medical files. Anterior lens capsule material excised during surgery (one sample per patient) was analyzed for mutations in exons 1 to 6 of the *DLAD* gene. The study was approved by the institutional and national review boards.

DNA isolation

The capsules containing single-layer lens epithelial cells were suspended in 5 ml of conservation medium until isolation of genomic DNA. DNA was extracted using standard sodium dodecylsulfate (SDS)/proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation.

Detection of DLAD mutations

The 6 exons of the *DLAD* gene were analyzed using polymerase chain reaction (PCR) amplification and direct sequencing. PCR primer sequences were designed with the Primer 3 Program (http://frodo.wi.mit.edu/rgi-bin/primer3/) and are listed in Table 1. PCR amplification was performed in a 50 µL reaction volume containing 100 ng of sample DNA as a template. Details of the conditions for each reaction are summarized in Table 1. The PCR parameters were as follows: denaturation at 95°C for 5 min, 35 cycles of 1 min at 95°C, annealing at 56 - 60°C for 1 min (Table 1) and extension of 1 min at 72°C with Taq polymerase. The PCR product was amplified on 2% agarose gel and visualized with ethidium bromide staining. Direct sequencing of the PCR products was performed with Big Dye Terminator Cycle Sequencing reagents using the ABI PRISM 3700 DNA Analyzer (Applied Biosystems, Foster City, CA).

RESULTS

The patient group consisted of 33 women and 22 men aged 47 to 93 years (mean 75.8 years ± 8.5). All were Caucasian Jews. All cataracts had a nuclear component,

Table 2. Comparison of genotypic variations and frequencies in Caucasian Jews in our study and other ethnic populations.

SNP Data					SNP Frequency (%)				
Exon	SNP ID	Alleles	Amino acid change	Amino acid co-ordinate	Our results	European	Chinese	Afro-American	Japanese
1	RS3738573	C/G	Q/H	3 (3)	CC- 11 CG- 45 GG- 45	CC- 12.5 CG- 50 GG- 37.5	CC- 8.3 CG- 45.8 GG- 45.8	CC- 0 CG-30 GG-70	CC- 18.2 CG- 43.2 GG- 38.6
2	RS3754274	A/G	R/K	51(2)	AA- 13.5 AG- 32 GG- 54.5	AA- 8.3 AG- 41.7 GG- 50	AA- 11.1 AG- 42.2 GG- 46.7	AA- 0 AG- 22.7 GG- 77.3	AA- 13.6 AG- 45.5 GG- 40.9
5	RS7511984	C/T	Y	248(3)	CC- 74 TC- 26 TT- 0	CC- 71.9 TC- 24.6 TT- 3.5	CC- 97.8 TC- 2.2 TT- 0	NO DATA NO DATA NO DATA	CC- 95.5 TC- 4.5 TT- 0
6	RS3768250	C/T	H	305(3)	CC- 15.7 TC- 47.3 TT- 37	CC- 12.5 TC- 58.3 TT- 29.2	CC- 15.6 CT- 51.1 TT- 33.3	CC- 9.1 CT- 40.9 TT- 50	CC- 18.2 CT- 47.7 TT- 34.1

and 7 also had a cortical component.

No mutations were detected on analysis of the *DLAD* gene. A number of genomic polymorphisms were found in exons 1, 2, 5, and 6; all of them were previously reported in the Genome Database (http://www.ensembl.org/index.html) (Table 2). The PCR amplification products for each exon are presented in Figure 1. DNA alterations and base-pair positions related to the sequences were deposited in the Genebank (Homosapiens DLAD chromosome 1, NM 058248).

The polymorphisms and their frequencies are described in Table 2. Nonsynonymous single-nucleotide polymorphisms (SNPS) were identified in exons 1 and 2 (Table 2, Figure 2). Exon 1 was characterized by a glutamine to histidine change; SNP frequencies were as follows: CC 12, CG 44 and GG 44%. Exon 2 had an arginine-to-lysine change; SNP frequencies were GG 54.5, GA 37.5 and AA 8%. Synonymous polymorphisms were detected in exons 5 and 6 (Table 2, Figure 2): in exon 5 for aspartate, with frequencies of 74% for CC, 26% for CT and 0 for TT, and in exon 6 for histidine, with frequencies of 37% for TT, 47% for TC, and 16% for CC. No polymorphisms were found in exons 3 and 4 in any of the samples tested.

DISCUSSION

We investigated the possible relationship of epithelial cell organelle degradation by lens DLAD and senile nuclear cataract in humans. To our knowledge, this is the first study to explore *DLAD* mutations in this setting.

Our study was prompted by findings in a *DLAD*-deficient mouse model wherein no nuclear DNA degradation occurred during lens cell differentiation (Nishimoto et al., 2003). Specifically, in the *DLAD*-null fiber cells, naked DNA remained in the cytoplasm, but the mitochondria, the endoplasmic reticulum and the nuclear membrane were completely lost. The mice acquired weak cataracts and their response to light was severely reduced (Nishimoto et al., 2003). The data suggested that the nuclei disappear from fiber cells to ensure the transparency of the lens and that cataract develops as a direct consequence of the accumulated DNA. If other organelles are left undigested in the lens, they may also cause cataract. This observation led to the assumption that human cataract, too, might be caused by a genetic defect that impairs degradation of the organelles during lens cell differentiation (Nagata, 2005). The findings were supported by the study of Nakahara et al. (2007), who reported that *DLAD* was a major acid DNase in nuclear cataract in the mouse.

We analyzed DNA extracted from the capsule of nuclear cataracts, with or without cortical components, of 53 patients. No truncating mutations or inactivating mutations were found. By contrast, studies in transgene mice with cataract identified mutation in exons 3 and 4 of the *DLAD* gene (Nishimoto et al., 2003). We speculate that despite the sequence similarity of human and mouse *DLAD* and the apparently similar mechanism that regulates lens transparency in the two species, different genes or different genomic variations in some genes are involved in the process. Interestingly, our results are in line with the conclusion of Nagai et al. (2006) that undigested DNA is not located in the nuclear portion of the lens.

Hawse et al. (2005) analyzed the gene expression profiles of epithelial and fiber cells in the human lens and found global differences between them, although their

.

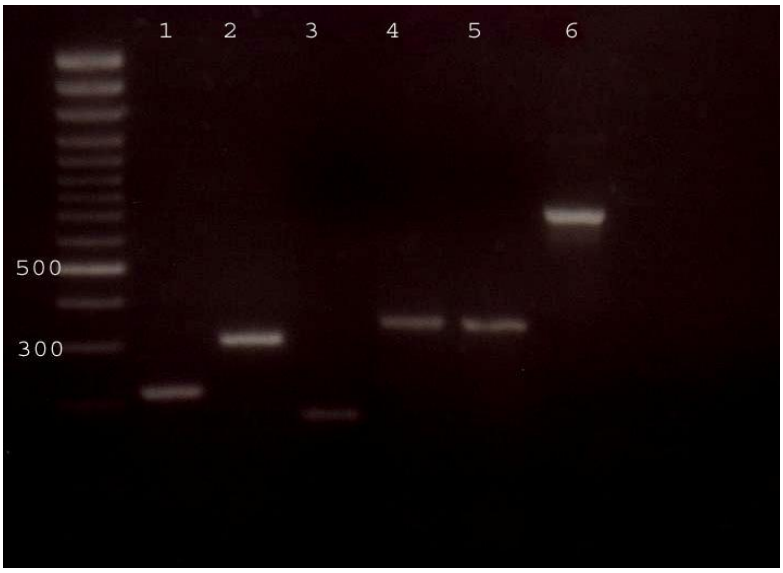

Figure 1. Gel electrophoresis of the PCR products of the 6 *DLAD* exons.

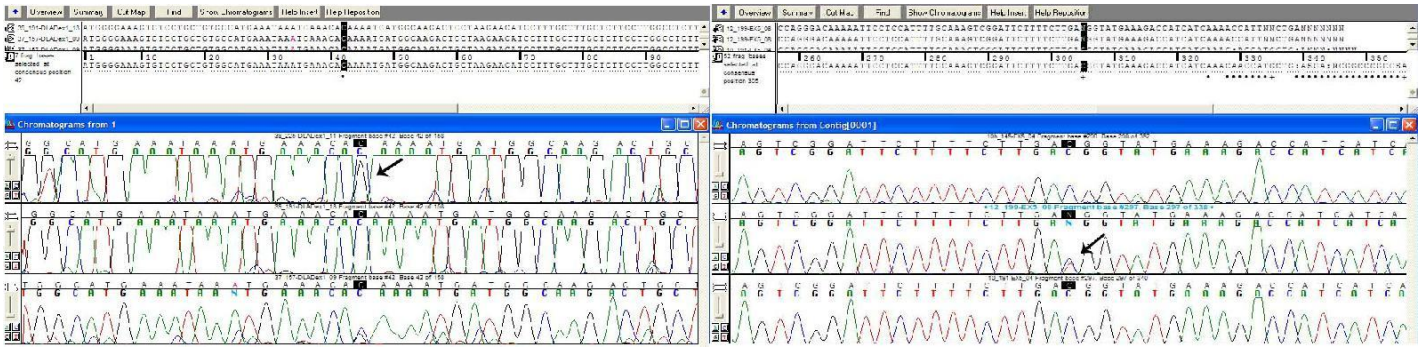

Figure 2. DNA sequence for exon 1 and 5 in a few samples. Note heterozygosity (arrow).

analysis did not clearly point to genes other than *DLAD* that might play a role in organelle degradation. Given that DLAD is active in acid conditions and is probably localized to the lysosomes, the process of DNA degradation may involve a canonical autophagic mechanism (Nakahara et al., 2007). However, Matsui et al. (2006) failed to detect any abnormalities in lens cell differentiation in mice lacking Atg5, a protein essential for autophagosome formation.

The genotype variations and the distribution of the SNP in exons 1, 2, 5 and 6 were similar to those reported previously in studies of the genomic polymorphism of *DLAD* in Chinese and Japanese populations, but different from those in African and African-American populations (Genome Browser). Although we found no association between cataract formation and genotype, the different distributions in the Africans/African-Americans are in line with the different type of cataract (cortical rather than nuclear) that characterizes these populations, which occurs at an earlier age.

The present study was restricted to senile cataract and we cannot extrapolate the findings to other types of cataract, such as cortical or congenital cataract or acquired cataract in younger groups. However, even when the nuclear cataract had a cortical component, no mutation could be detected. Many studies have presented evidence of a genetic component in the risk of congenital cataract, but none has explored the role of *DLAD*.

Our study was also limited by the small size of the sample. Furthermore, although we screened all exons, we did not examine mRNA expression or levels of the protein itself, or enzymatic dysfunction in the lens. Nevertheless, the results may suggest that *DLAD* mutations are not associated with nuclear cataract in elderly people. The question of whether animals are a good model to define genes involved in acquired human disease is still unresolved. The widespread occurrence of cataract in the elderly may point to multiple causative factors. Moreover, given that some eyes are spared, it is possible that a

DLAD mutation is not enough to account for the sequence of events leading to the development of cataract. Recent studies report an increase in the annual incidence of cataract surgery in younger patients (Francis and Moore, 2004), which may coincide with changes in other risk factors.

Further studies in larger populations are needed to evaluate the role of DLAD in different types of the cataract and the possible involvement of protein dysfunction.

REFERENCES

Bassnett S (2002). Lens organelle degradation. Exp. Eye Res. 74: 1-6.

Cekic O, Bardak Y, Totan Y, Akyol O, Zilelioglu G (1999). Superoxide dismutase, catalase, glutathione peroxidase and xanthine oxidase in diabetic rat lenses. Ophthalmic. Res. 31: 346-350.

Dahm R (1999). Lens fibre cell differentiation - A link with apoptosis? Ophthalmic. Res. 31: 163-183.

Dilsiz N, Olcucu A, Cay M, Naziroglu M, Cobanoglu D (1999). Protective effects of selenium, vitamin C and vitamin E against oxidative stress of cigarette smoke in rats. Cell Biochem. Funct. 17: 1-7.

Francis PJ, Moore AT (2004). Genetics of childhood cataract. Curr. Opin. Ophthalmol. 15: 10-15.

Garland D (1999). Role of site-specific, metal-catalyzed oxidation in lens aging and cataract: a hypothesis. Exp. Eye Res. 50: 677-682.

Garland D, Russell P, Zigler JS Jr (1988). The oxidative modification of lens proteins. Basic Life Sci. 49: 347-352.

Hawse JR, DeAmicis-Tress C, Cowell TL, Kantorow M (2005). Identification of global gene expression differences between human lens epithelial and cortical fiber cells reveals specific genes and their associated pathways important for specialized lens cell functions. Mol. Vis. 11: 274-283.

Ishizaki Y, Jacobson MD, Raff MC (1998). A role for caspases in lens fiber differentiation. J. Cell Biol. 140: 153-158.

Matsui M, Yamamoto A, Kuma A, Ohsumi Y, Mizushima N (2006). Organelle degradation during the lens and erythroid differentiation is independent of autophagy. Biochem. Biophys. Res. Commun. 339: 485-489.

McAvoy JW, Chamberlain CG, de Iongh RU, Hales AM, Lovicu FJ (1999). Lens development. Eye (Lond) 13(Pt 3b): 425-437.

Nagai N, Takeuchi N, Kamei A, Ito Y (2006). Involvement of DNase II-like acid DNase in the cataract formation of the UPL rat and the Shumiya cataract rat. Biol. Pharm. Bull. 29: 2367-2371.

Nagata S (2005). DNA degradation in development and programmed cell death. Annu. Rev. Immunol. 23: 853-875.

Nakahara M, Nagasaka A, Koike M, Uchida K, Kawane K, Uchiyama Y, Nagata S (2007). Degradation of nuclear DNA by DNase II-like acid DNase in cortical fiber cells of mouse eye lens. FEBS J. 274: 3055-3064.

Nishimoto S, Kawane K, Watanabe-Fukunaga R, Fukuyama H, Ohsawa Y, Uchiyama Y, Hashida N, Ohguro N, Tano Y, Morimoto T, Fukuda Y, Nagata S (2003). Nuclear cataract caused by a lack of DNA degradation in the mouse eye lens. Nature 424: 1071-1074.

Piatigorsky J (1981). Lens differentiation in vertebrates. A review of cellular and molecular features. Differentiation 19: 134-153.

Tumminia SJ, Clark JI, Richiert DM, Mitton KP, Duglas-Tabor Y, Kowalak JA, Garland DL, Russell P (2001). Three distinct stages of lens opacification in transgenic mice expressing the HIV-1 protease. Exp. Eye Res. 72: 115-121.

van Leyen K, Duvoisin RM, Engelhardt H, Wiedmann M (1998). A function for lipoxygenase in programmed organelle degradation. Nature 395: 392-395.

Vrensen GF, Graw J, De Wolf A (1991). Nuclear breakdown during terminal differentiation of primary lens fibres in mice: a transmission electron microscopic study. Exp. Eye Res. 52: 647-659.

Zandy AJ, Bassnett S (2007). Proteolytic mechanisms underlying mitochondrial degradation in the ocular lens. Invest. Ophthalmol. Vis. Sci. 48: 293-302.