

Clinical, Histological and Biochemical Observations in Spontaneous Senile Cataract in Mastomys Species

by *Kalidas N. Kohale*

National Centre for Cell Science, Pune University Campus, Pune, India

Summary

Clinical, histological and biochemical examination of spontaneous lens opacity was carried out in *Mastomys coucha* species. The incidence of the spontaneous lens opacity was noticed in adult animals, in the age range of 48-72 weeks, up to a maximum prevalence of 2.6% and 13.71% in male and female animals respectively. The affected animals exhibited mild lens opacity initially, which progressed with age, and later on became dense and looked like a mature cataract. The opacity was irreversible and showed no signs of disappearing in the affected animals. Both male and female were affected but, as noted above, the incidence of the abnormality was higher in the females. The mode of inheritance was not investigated. The ophthalmoscopic examination of the affected animals revealed total lenticular opacity characterized by a shallow anterior chamber and bulging of the lens towards the anterior side due to the swelling of the lens. The serum glucose values in affected animals were within the normal range. The histological examination of opaque lenses revealed total alteration in the micro architecture of the lens cells. The SDS-PAGE analysis of lens crystallins, revealed distribution of major lens crystallins within range of 20-30 kDa molecular weights. The immunoblotting of SDS-PAGE separated proteins with anti-crystallin α , β and γ antibodies indicated expression of major lens crystallins in affected animals. We have concluded that this is a case of spontaneous senile cataract, reported for the first time in *Mastomys* species.

Introduction

Cataracts are frequent diseases in man and often observed in animal models. Cataract can be defined as, the loss of transparency of a crystallin lens which results when the refractive index of the lens varies significantly over distances approximating the wavelength of the transmitted light (*Delaye & Tardieu, 1983; Benedek, 1971*). Refractive index can vary over these distances due to the changes in the lens cell structure, changes in the lens protein constituents, or both. Because of the small size of the families that are usually available for com-

prehensive genetic investigation, it is necessary to look for suitable animal models to identify genes implicated in cataract formation and to explore the mechanisms leading to the opacification of the lens. Animal models with lens abnormality such as cataract are potentially useful models for studying similar disorders in humans.

Many strains of mice and rats with hereditary cataracts have been established. The Blind sterile, *bs* (*Varnum, 1983*), Fidget, *fi* (*Truslove, 1956; Kindiakov & Koniukhov, 1986*), Hereditary Cataract Rat Strain, *scr* (*Shumiya, 1995*), Lens opacity, *lop13* (*Varnum, 1981*), Lens rupture, *lr* (*Fraser & Herer, 1950*), Dysgenetic lens, *dyl* (*Sanyal & Hawkins, 1979; Sanyal et al, 1986*), a recessive cataract, *cac* (*Moser & Gluecksohn-Waelsch, 1967*), Nakano, *nct* (*Piatigorsky et al, 1978*), Vacuolated lens, *vl* (*Dickie, 1967*), and *dcm* (*Kohale, et al, 2004*) are cataract models reported earlier.

In the present study we have noticed spontaneous

*Correspondence: Kalidas N. Kohale

Department of Biological Sciences, Tata Institute of Fundamental Research, Dr. Homi Bhabha Road, Colaba, Mumbai-400 005, India

Tel. +91-022-22782696

Fax +91-022-22804610

E-mail kalidas@tifr.res.in

Web www.tifr.res.in

senile cataract (SSC) in adult *Mastomys* (*Praomys*) species. The clinical examination of the eyes, haematology, serum glucose level, histology of the opaque lens and major lens protein analysis were carried out in the affected animals. The *Mastomys* is a multimammate rodent, widely used as an animal model for parasitic diseases especially for the maintenance of the larval stages of filarial parasite. In *Mastomys* species SSC has not been reported earlier and for the first time we have documented such abnormality, which is characterized by spontaneous total lenticular opacity in aged animals.

Materials and Methods

Animals

The breeding colony of *Mastomys* species was maintained at the Experimental Animal Facilities (EAF) of the Institute. The animals were housed in shoebox type autoclavable polypropylene cages covered with stainless steel grills, kept in a well ventilated animal colony with a once-through exhaust system, maintained on positive pressure. The temperature and relative humidity were maintained and monitored regularly at 22°C and 55% respectively through the central air conditioning system. The animals were provided readymade feed in moderate hard pellet form and filtered water (Aqua guard water filters, India) ad-libitum. Humane animal care was provided as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Clinical examination

Animals above one year of age were identified for the study and housed on separate rack in the breeding colony. These animals were kept under observation and monitored regularly for the development of eye and other abnormalities. The affected animals were separated and clinically examined for lens opacity with unaided eye, which was confirmed by examination under the ophthalmoscope. The direct ophthalmoscope examination was not possible due to the total opacity of the lenses. A few affected animals were euthanized and eyes were collected

for observation under the stereomicroscope. The complete details such as sex and the age of the animals were recorded at the time of appearance of opacity. Those animals affected with lens opacity during the period of 1994 to 2004 was taken for the analysis.

Estimation of serum glucose

The animals were fasted overnight and next morning blood samples were collected from the orbital plexus of normal and cataract animals under light anaesthesia. The blood was allowed to clot at 4 °C and serum samples were separated and stored at -20 °C until further use. Serum glucose was estimated as per GOD/POD method using diagnostic kits (Span diagnostic Ltd. India).

Histopathological analysis of eyes

The affected animals were euthanized with CO₂ asphyxiation and eyes were collected and fixed in Bouin's solution for 24 hr. These specimens were subjected to routine histopathological processing for dehydration, embedding in paraffin and sectioning at 5 micron and stained by H & E method. A few eyes were enucleated and lenses removed from both cataract and wild type animals and were reserved for preparation of lens homogenate for the SDS-PAGE analysis.

SDS-PAGE

Lenses were cleaned and homogenized in urea lysis buffer (6M Urea, 2M Thiourea, CHAPS 1 gm and DW 25 ml) using a Teflon tissue homogenizer. The protein content of the homogenate was estimated by the BCA method. The major lens crystallins in lens homogenate was separated by loading samples (~10 ug) on 20% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The gel was stained with Gel code stain (Pierce Chemicals, USA) following standard procedure.

Western blot analysis

The proteins separated by SDS-PAGE were transferred on to nitrocellulose 'Trans-Blot' transfer membrane (Bio-Rad Laboratories, CA) and

used for Western blot analysis. The non-specific sites were blocked with 2% BSA. The membrane was incubated with primary antibodies i.e. Anti-Crystallin α , β and γ (Gifted by Dr. Larry David, Oregon Health University, USA) at the dilution 1:1000, 1:3000 and 1:10000 respectively and ALP conjugated anti-rabbit goat serum, as a secondary antibody (Sigma Chemicals, USA) and stained with BCIP (Sigma Chemicals, USA) for the visualization of the proteins.

Results

Clinical, Histological and Biochemical changes

All the animals affected with lens opacity in the breeding colony of Mastomys, during the period 1994-2005 were taken for the analysis. The total numbers of male and females exhibiting lens opacity were 12 and 54 respectively. The age of the animals at the time of appearance of the opacity varied between 1 to 2 years. The incidence of the lens opacity was increased with age up to a maximum incidence of 2.6% and 13.71% in adult male and female animals respectively. Clinical examination of the affected eyes revealed opacification of the lens. The mild opacity noticed at an earlier stage become more and more dense, and looks like a mature cataract as the age advances. The slit lamp examination of the affected eyes revealed a clear and transparent cornea with no vascularisation. The pupils were widely dilated and not reacting. The iris pattern was hardly seen as the pupils were dilated. The lens was opaque in the cortical region with typical intumescent cataract, characterized by a shallow anterior chamber and bulging of the lens towards the anterior side due to the swelling of the lens (Figure 1A). The direct ophthalmoscope examination of the retina and optic nerve head was not possible, due to the total opacity of the lenses. Both sexes were affected to some degree by this abnormality; however the prevalence was higher in female animals as compared to the male counterpart. In a few animals this abnormality was associated with development of neoplasm on mouth, ear, and inguinal region. The haematological parameters were within normal range in the

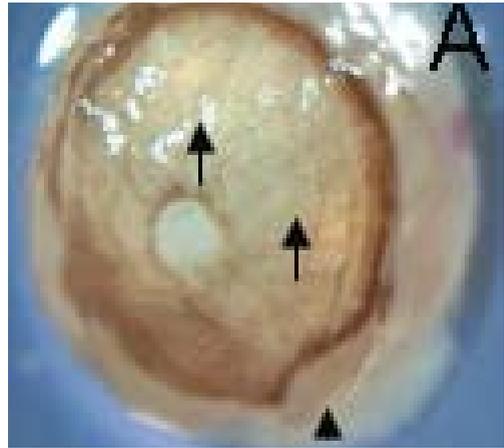


Figure 1A. Photograph of the SSC eye representing opaque lens at the cortical region, characterized by shallow anterior chamber and bulging of lens (Upward arrows) towards anterior side due to swelling of the lens (Magnification, 10X).

affected animals. The fasting serum-glucose levels were 97.64 and 95.85 mg/dl for WT and SSC animals respectively. The values of serum glucose in animals SSC were within the normal range and no significant change was noticed in the serum glucose when compared with wild type animals (Table 1).

Lens Histology: The histological changes in the lenses of SSC animals were characterized by abnormal morphology such as complete destruction of the micro-architecture of the lens core. The normal homogenous appearance of the lens mass as seen in

Table 1. Estimation of glucose level in WT and SSC Mastomys animals.

Sr. No.	Mastomys Animals	Glucose level (mg/dl)
1	WT Animals	97.643± 9.04 (14*)
2	SSC Animals	95.857± 6.790 (14*)

* Values in parenthesis indicate total numbers of animals used for estimation.

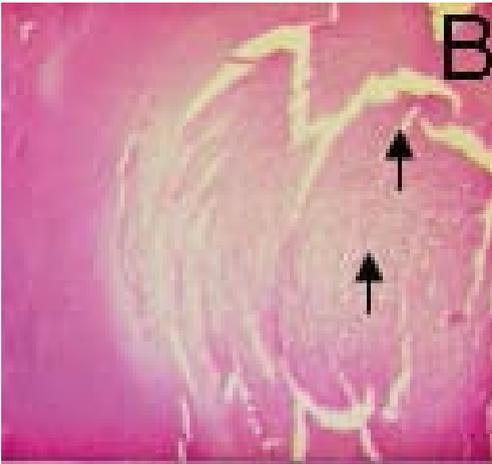


Figure 1B. Photomicrograph of the section of the SSC lens showing abnormal features characterized by complete destruction of microarchitecture (Upward arrows) of lens core (H & E stain; magnification, 20X).

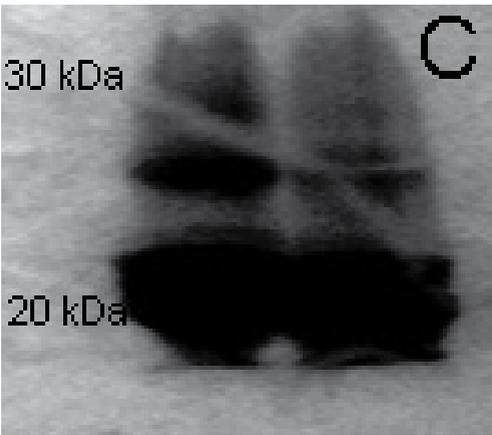


Figure 1C. Photograph representing SDS-PAGE Analysis of the lens crystallin proteins. Notice normal distribution of the lens crystallin proteins between 20-30 kDa molecular weight markers. No significant changes were noticed in the band pattern of SSC (right lane) and WT (left lane) animals.

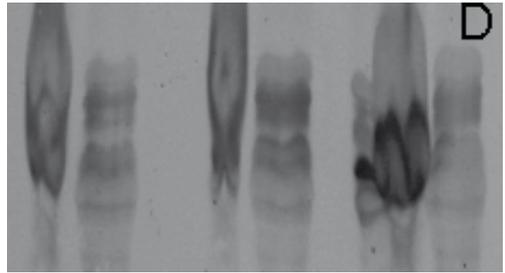


Figure 1D. Photograph of Western blot Analysis of the lens crystallin proteins. SDS-PAGE separated proteins were immunoblotted with anti-crystallin α , β and γ antibodies. The membrane was stained with BCIP (Sigma, USA) stain. Notice normal expression of major lens crystallin proteins in SSC lenses. Figure 1A. Photograph of the SSC eye representing opaque lens at the cortical region, characterized by shallow anterior chamber and budging of lens (Upward arrows) towards anterior side due to swelling of the lens (Magnification, 10X).

the healthy lenses was totally altered (Figure 1B). SDS-PAGE analysis of the lens proteins from SSC animals showed that the major lens crystallin were within the range 20 - 30 kDa molecular weights. No significant change was observed in band pattern in lens proteins of SSC animals when compared with that of WT counterparts (Figure 1C). The immunoblotting studies with anti-crystallin α , β and γ antibodies indicated expression of major lens crystallins in SSC animals and no change was observed in the expression of lens proteins as compared to WT animals (Figure 1D).

Discussion

The present study describes a spontaneous mutation causing abnormal phenotype such as SSC in aged *Mastomys* species. At the time of birth, the eyes of the animals were normal, transparent and no sign of lens opacity was in evidence till they attained the age of approximately one year, however, the loss of lens transparency in affected animals become evident between 1 and 2 years of their age. The affected animals started showing mild lens opacity earlier,

which later became more dense and looked like mature cataract as they aged. These observations suggested that the spontaneous appearance of lens opacity in *Mastomys* species could be due to mutation affecting animals in adult age. The mutation did not affect the eye lens during fetal development or immediately after birth; however, it started causing lens opacity during adult age, leading to the manifestation of clinical cataract. These observations suggested that the effect of mutation was not enough to develop congenital cataract, however, the mutation did make these animals susceptible to some biochemical, physical or environmental changes in adult age leading to the development of cataract. These observations were supported by the studies of Hejtmanick and Kantorow (2004), who found that mutation in crystallins are enough to cause aggregation, they usually result in congenital cataract, while if they merely make them susceptible to environmental factors such as light, hyperglycemic or oxidative damage, they might lead to age-related cataract.

The analysis of data collected between 1994 and 2004, revealed that the incidence of the lens opacity was increased with age up to a maximum incidence of 2.6% and 13.71% in 48 – 72 weeks old male and female animals respectively. Both sexes were affected to some degree by this abnormality; however the prevalence was higher in female animals as compared to the male counterpart. It is possible that the hormonal imbalances, especially low estrogen level in aged female animals, could predispose these animals to the development of old age cataracts; however, further study to explore the role of this factor in the development of cataract would be highly useful. This possibility is supported by the finding (Hales *et al.* 1997), that the ovarian hormone estrogen protects rat lenses against TGF β -induced cataract. In our study, the clinical, haematological and biochemical examination of the affected animals indicated normal physiological parameters except the lens opacity. The level of serum glucose was within normal limit in SSC animals and no significant variation was noticed when compared to normal animals, suggesting that the appearance of

cataracts in aged *Mastomys* species was not the outcome of a diabetes complication in the present case (which is a common sequel to high blood glucose content in aged humans).

Histological changes in the eyes were characterized by aggregation of cellular mass with complete alteration in the micro-architecture of the lens cells. Transparency of the lens results from the highly ordered arrangement of the macro-molecular components of constituent cells and the regular arrangement of lens fibres (Francis *et al.* 1995), which was found to be totally disturbed in SSC. It could be possible that the loss of transparency of the lens in SSC may be due to the progressive deterioration of the lens fibre cells and breakdown of the lens micro-architecture.

Light scattering can occur from the large fluctuation in the optical density due to the vacuole formation in the lens. Both light scattering and loss of transparency can also occur when there are high molecular weight protein aggregates in the lens. A tightly packed bundle of crystallins, which make ~90% of water-soluble lens proteins, are essential to achieve and maintain lens transparency and must exist in a homogeneous phase (Hejtmancik & Kantorow, 2004).

A variety of biochemical or physical changes can cause the phase separation of crystallins into protein-rich and protein-poor areas within the lens fibres. The proteins either remain in solution or form insoluble aggregates or crystals, any of which can lead to the light scattering (Paned *et al.* 2001). Several factors such as daylight, diet, diabetes, dehydration and genetic are postulated to be of importance in the loss of lens transparency in aged humans (Taylor, 1999). However, all these different factors exert their influence predominantly through a common pathway of oxidation of lens proteins (Davis & Truscott, 2001; Harding, 2002) and peroxidation of lipids (Hegde & Verma, 2005). In addition, the deleterious effects of glucose metabolism in the lens and associated changes in lens epithelial cell redox potential also has an exacerbating influence on these oxidative changes (Harding, 1996)

This animal model with age-related cataract would be highly useful in studying the similar pathological processes involved in the mechanism of cataractogenesis in humans. Further studies, including looking for molecular lesions implicated in the mechanism of cataract development in these animals would be highly useful in the understanding cataract development.

Acknowledgements

The author acknowledged the help rendered by Dr. Asha Kelkar, Ophthalmic Surgeon, Kelkar Nursing Home, Pune, India for the ophthalmoscopic examination of the affected eyes and Dr. A. D. Ingle, ACTREC, Mumbai, for histopathological examinations of the eye sections.

References

- Benedek GB*: Theory of transparency of the eye. *Applied Optics* 1971, *10*, 459-473.
- Davis MJ & RJ Truscott*: Photo-oxidation of proteins and its role in cataractogenesis. *Journal of Phytochemistry and Photobiology B* 2001, *63*, 114-125.
- Delaye M & A Tardieu*: Short range order of crystallin proteins accounts for eye lens transparency. *Nature* 1983, *302*, 415-417.
- Dickie MM*: Vacuolated Lens. *Mouse News Letter* 1967, *36*:39.
- Francis JF, V Berry, AT Moore & S Bhattacharya*: Lens biology. Development and human cataractogenesis. *Trends in Genetics* 1999, *15*, 191-196.
- Fraser FC & ML Herer*: The inheritance and expression of the "lens rupture" gene in the house mouse. *Journal of Heredity* 1950, *41*, 3-7.
- Harding JJ*: Testing time for the sunlight hypothesis of cataract. *Current Opinions in Ophthalmology* 1996, *7*, 59-62.
- Harding JJ*: Viewing molecular mechanism of ageing through a lens. *Aeging Research Review* 2002, *1*, 465-479.
- Hegde KR & SD Verma*: Combination of glycemic and oxidative stress in lens: implications in augmentation of cataract formation in diabetes. *Free Radical Research* 2005, *39*, 513-517.
- Hejtmann JF & M Kantorow*: Molecular genetics of age-related cataract. *Experimental Eye Research* 2004, *79*, 3-9.
- Kindiakov BN & BV Koniukhov*: Mutant gene expression in murine aggregation chimeras.5.The ocular retardation and fidget genes. *Ontogenez* 1986, *17*, 47-55.
- Kohale KN, AD Ingle, A Kelkar & PB Parab*: Dense cataract and microphthalmia, new spontaneous mutation in BALB/c mice. *Comparative Medicine* 2004, *54* (3), 275-279.
- Moser GC & S Gluecksohn-Waelsch*: Electrophoretic pattern of lens protein from genetically caused cataracts in the mouse. *Experimental Eye Research* 1967, *6*, 297-298.
- Pande A, J Pande, N Asherie, A Lomakin, O Ogun, J King & GB Benedek*: Crystal cataracts: human genetic cataract caused by protein crystallization. *Proceedings of National Academy of Sciences USA* 2001, *98*, 6116-6120.
- Piatigorsky J, HN Fukui & JH Kinoshita*: Differential metabolism and leakage of Protein in an inherited cataract and a normal lens cultured with ouabain. *Nature* 1978, *224*, 558-562.
- Sanyal S & RK Hawkins*: Dysgenetic lens (dyl) - a new gene in the mouse. *Investigative Ophthalmology & Visual Science* 1979, *18*, 642-645.
- Sanyal S, RV Nie, JD Moes & RK Hawkins*: Map position of dysgenetic lens (dyl) locus on chromosome 4 in the mouse. *Genetic Research* 1986, *48*, 199-200.
- Shumiya S*: Establishment of the Hereditary Cataract Rat Strain (SCR) and genetic analysis. *Laboratory Animal Science* 1995, *45*, 671-673
- Taylor HR*: Epidemiology of age related cataract. *Eye* 1999, *13*, 445-448.
- Truslove GM*: The anatomy of the development of the fidget mouse. *Journal of Genetics* 1956, *54*, 64-68.
- Varnum D*: Nuclear cataract (nuc). *Mouse News Letter* 1981, *64*, 59.
- Varnum D*: Blind-sterile. a new mutation on chromosome 2 of the house mouse. *Journal of Heredity* 1983, *74*, 206-207.