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High Incidence of Spontaneous Cataracts in Aging Laboratory Rabbits of an Inbred Strain

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Abstract

Objective—To investigate the occurrence of spontaneous cataracts in a breeding colony of the inbred EIII/JC strain of New Zealand White rabbits (*Oryctolagus cuniculi*) and the congenic strain of EIII/JC-HLA-A2.1 transgenic rabbits.

Procedure—A retrospective study was conducted by collecting and analyzing data from clinical records for individual rabbits filed between January 2011 and October 2013.

Results—Thirteen cases (8 females and 5 males) of cataract were identified in a group of 51 EIII/JC inbred rabbits with a morbidity of 25.5%. The median age of the rabbits identified with unilateral or bilateral cataracts was 43 months in contrast to the median age of 23 months of the entire group of 51 rabbits. Additionally, seven cases (5 females and 2 males) of cataracts were identified in a group of 21 EIII/JC-HLA-A2.1 transgenic rabbits. The EIII/JC-HLA-A2.1 transgenic rabbits showed similar morbidity (33.3%) and median age (41 months) for the development of cataracts as the EIII/JC rabbits. In both groups, none of the rabbits younger than 37 months developed cataracts while 13 (93%) of 14 EIII/JC rabbits aged 37 to 49 months and 7 (63.6%) of 11 EIII/JC-HLA-A2.1 transgenic rabbits aged 37 to 43 months developed cataracts. In contrast, none of 78 outbred rabbits with a median age of 29 months (10 to 67 months) developed cataracts.

Conclusion—Results of this study indicate that the occurrence and high incidence of spontaneous cataracts in this inbred strain (EIII/JC) of rabbits were strictly age related and consistently transmitted through inbreeding.

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INTRODUCTION

Rabbits have been widely used as an animal model in ophthalmology research because of their relatively large eyes and moderate body size.¹ However, a rabbit model for studying spontaneous cataracts has not been available. Although two types of inherited cataracts, *Cat-1* and *Cat-2*, had been reported by Nachtsheim and Gurich in 1939 and Ehling in 1957 respectively (Cited by Brock et al., 2012),² there were few reports presented from further studies of cataracts in rabbits. Munger et al. reported that 5.7% of animals in a group of 670 New Zealand White (NZW) rabbits aged 77 to 288 days exhibited immature cataract in one or both eyes.³ In a further study, the same group found a significant lower incidence (1.1%) of cataracts in 276 F1 rabbits of New Zealand White (NZW) × New Zealand Red (NZR) at the same age range.³ It was concluded, based on the above results, that spontaneous juvenile cataracts occurred in the rabbits and the incidences suggested an autosomal recessive model of inheritance. In a separate study to investigate spontaneous eye diseases in a group of 586 NZW rabbits, it was reported that unilateral or bilateral cataracts were identified in 1.9 percent of the rabbits and most of the affected rabbits were 2-4 months old.⁴ A recent retrospective study including 1022 NZW rabbits and 246 NZW × NZR F1 rabbits aged 6 to 16 weeks from 5 different suppliers demonstrated that the overall incidence of lenticular lesions were 4.6% in NZW rabbits and 3.3% in NZW × NZR F1 rabbits,⁵ which were similar to the incidence as previously reported.³ Additionally, it was demonstrated that NZW rabbits from different suppliers showed significant differences for prevalence rates, ranging from 0 to 5.2%.⁵

Here we report a high incidence of cataracts in an inbred strain of aging rabbits and transmission of the high incidence of cataracts to a newly established congenic strain through continual backcrossing with rabbits of a HLA-A2.1 transgenic line. The purpose of this study was to determine the age and incidence of spontaneous cataracts and inheritance of the cataract transmission in an inbred strain (EIII/JC) of rabbits. We hypothesize that the occurrence of cataracts in this strain of rabbits is age related and also linked to a strong genetic component for inheritance.

MATERIALS AND METHODS

Animals

All rabbits included in this study were New Zealand White (NZW), *Oryctolagus cuniculi*. The EIII/JC inbred rabbits were originally obtained from the National Cancer Institute of the NIH in 1988.⁶ The rabbits have been maintained by continual inbreeding in our facility. The EIII/JC-HLA-A2.1 inbred transgenic (EIII/JC-A2) rabbits were produced by continual backcrossing between the HLA-A2.1 transgenic rabbits and EIII/JC inbred rabbits for more than 9 generations.^{7,8} The outbred rabbits were produced by random breeding among outbred NZW rabbits in the facility. All of the rabbits were maintained for breeding and were not involved in any experiment. The animals were housed in an AAALAC accredited animal facility at Penn State Hershey. All protocols using these rabbits were reviewed and approved by the IACUC at Penn State Hershey. Housing and care of the animals were in accordance with the guidelines of the IACUC and the *Guide for the Care and Use of Laboratory Animals*.⁹ The rabbits were singly housed in stainless-steel cages with an

automatic water system and fed a commercial pelleted feed (2031 Teklad High Fiber Rabbit Diet for rabbits without litters, 8630 Teklad 15% Rabbit Diet for rabbits with litters, both diets were made by Harlan Laboratories, Madison, WI). The rabbit rooms were maintained with constant environmental conditions including temperature at $19^{\circ} \pm 3^{\circ}\text{C}$, relative humidity between 30 to 70% and 12:12-h light:dark cycle. Rabbits in this colony are free of *Encephalitozoon cuniculi*, rotavirus, *Treponema* spp., *Pasteurella multocida*, CAR bacillus, coccidia, and other external and intestinal parasites.

Data collection

The data were retrospectively collected from clinical records for individual rabbits filed between January 2011 and October 2013. Only rabbits produced by in-house breeding and aged more than 10 months were included in this study. None of the rabbits were used for any other experiments. The cataracts were identified and recorded by veterinarians specialized in laboratory animal medicine based on the clinical signs and examination by a Welch Allyn Halogen penlight (Skaneateles Falls, NY) without dilation.

Histopathology

The rabbits were euthanized by intravascular injection of Euthasol (Vedco, St Joseph, MO) under general anesthesia by intramuscular injection of ketamine (Ketathesia, Henry Schein Animal Health, Dublin, OH) (35mg/kg) and xylazine (Anased, Akorn, Decatur, IL) (5mg/kg). Eyes were collected and fixed in Bouin's solution, embedded in paraffin, and sectioned at 4-6 μm . The sections were processed and stained with hematoxylin and eosin.

RESULTS

This study included a total of 150 rabbits which were divided into 3 groups based on their genetic background: 1) 51 EIII/JC rabbits, 2) 21 EIII/JC-A2 rabbits, and 3) 78 outbred NZW rabbits. All of the rabbits were at least 10 months with a median age of 23 (11-49) months for EIII/JC rabbits, 35 (19-44) months for EIII/JC-A2 rabbits, and 26 (10-67) months for outbred NZW rabbits (Table 1). In the group of EIII/JC rabbits, 13 rabbits developed cataracts at 37 months or above with an incidence rate of 25.5 percent for the entire group or 93 percent for the 14 rabbits aged 37 to 49 months (median 43) within the same group (Table 1 and Table 2). Similarly, 7 of 21 EIII/JC-A2 rabbits developed cataracts with an incidence of 33.3 percent for the entire group or 63.3 percent for the 11 rabbits aged 37 to 43 months (median 41) (Table 1 and Table 3). Both male and female rabbits were affected for development of cataracts in the two groups (Table 2 and Table 3). In contrast, none of the 78 outbred NZW rabbits, including 25 rabbits aged 37 to 67 months, developed cataracts (Table 1).

The cataracts were usually noted in the lens unilaterally at first (Figure 1A) and developed in the contralateral eye after approximately 1-2 months (Figure 1B). In this study, 4 of 13 EIII/JC rabbits and 2 of 7 EIII/JC-A2 transgenic rabbits were initially found with bilateral cataracts (Table 2 and Table 3). The rabbits with unilateral cataracts were frequently euthanized soon after diagnosis, which could bias the incidence of bilateral cataracts. All of the rabbits were initially observed with immature cataracts consisting of local lenticular

opacities at an early stage (Figure 2A). The cataracts gradually became mature within about 2 months (Figures 2B and 2C), and occasionally hypermature with a longer time (Figure 2D). Randomly selected cataracts were confirmed on histology by the presence of lenticular Morgagnian globules in all five evaluated rabbits (Figure 3).

The rabbits were closely monitored for development of blepharospasm and intraocular pressures (IOPs) were intermittently measured by tonometry (Tono-Pen XL Applanation Tonometer, Medtronic Solan, Jacksonville, FL) when cataracts started to develop. None of the rabbits developed blepharospasm and the IOPs were always within the normal tonometry reference range of 15-23 mmHg.¹⁹ Therefore, cataracts secondary to glaucoma could be excluded in these rabbits.

DISCUSSION

Cataracts are characterized by opacification of all or part of the lens of the eye and remain the leading cause of blindness in humans.¹⁰ Although many causes have been identified for the development of secondary cataracts, most cataracts are related to aging (senile cataracts). It has been reported that approximately 75% of people over the age of 75 years have sight-impairing lens opacification.¹¹ Prevalence of age-related cataracts has also been reported in dogs and cats.^{12,13} Similarly, the high incidence of hereditary cataract was reported in aged mice of the SAM (Senescence Accelerated Mouse)-P3 line with 27.5% at 12 months and 70.6% at 16 months.¹⁴ A spontaneous mutation in the mouse *Bfsp2* gene has been characterized in the 129X1/SvJ strain, which is linked to a slow but progressive loss of optical clarity of lenses with age.^{15,16} To our knowledge, the EIII/JC rabbit cataracts presented in this report is the only inheritable cataract affecting aging rabbits.

The EIII/JC inbred rabbits were originally imported from the National Cancer Institute (NCI) of the NIH in late 1980s.⁶ Soon after the NCI stopped breeding this type of rabbit, several breeding pairs were transferred to Penn State Hershey. The Hershey colony had been the only one in the world until several rabbits were shipped from the Penn State Hershey facility to Belgium in 2012 and 2013 in an attempt to establish a new colony. Rabbits in the colony at Penn State Hershey have been maintained by continual inbreeding and retained the inherited characteristics consistently demonstrated by this strain, such as small body size (about 3 kg in adults), small litter size (2-5 kits per litter) and sensitivity to sharp sound (audiogenic). In this study, we report a high incidence of cataracts in aging rabbits of the EIII/JC strain and a congenic strain (EIII/JC-A2) established by backcrossing.

We initially retrospectively analyzed clinical records of 51 EIII/JC rabbits with a median age of 23 (11-49) months and 78 outbred NZW rabbits with a median age of 26 (10-67) months. The results showed that 13 of the 51 EIII/JC rabbits (25.5%) had unilateral or bilateral cataracts at a minimum age of 37 months and a median age of 43 months in contrast to the median age of 23 months in the entire group. When the rabbits were between 37 to 49 months of age, 93 percent of them developed cataracts. The outbred rabbits, representing our control group, did not develop any cataracts; this group included rabbits older than the oldest of the EIII/JC rabbits. These results were similar to those reported in the SAM-P3 mice as described above.¹⁴

In a separate study, we worked to transfer a transgene, HLA.A2.1, from the outbred NZW rabbits to the EIII/JC background by continual backcrossing. Similarly, as in the EIII/JC rabbits, 63.3 percent of the EIII/JC-A2 rabbits aged 37 to 43 months in the F9 or above generation developed cataracts. Cataracts were not found in rabbits younger than 38 months or prior to the F9 generation. It is very likely that the incidence will continue to increase with further inbreeding and as more rabbits are allowed to live for a longer time. The reason that no rabbits prior to the F9 generation developed cataracts might be due to fact that all of the rabbits were sacrificed at the age of about one year during the process of backcrossing. Cataract inheritance by an autosomal recessive gene in an inbred strain of ICR rats was demonstrated by backcross mating.¹⁷ However, the mode of inheritance of cataracts in the EIII/JC rabbits could not be determined based on the current data because none of the heterozygous rabbits produced during the course of backcrossing were allowed to survive to old enough ages for development of cataracts.

Congenital glaucoma (buphthalmia) has been considered as a cause of secondary cataracts in New Zealand White rabbits.¹⁸ This condition is typically evident by 3 to 5 months of age. However, due to incomplete penetrance, there is great variation in the age of onset and some affected animals do not develop buphthalmia. In the rabbits with buphthalmia, the aqueous humor is unable to drain normally and therefore begins to build-up within the globe, resulting in increased intraocular pressures (IOPs). However, IOPs measured in our rabbits were consistently within the normal range of 15-23 mmHg;¹⁹ therefore excluding glaucoma as a secondary cause of cataracts in the EIII/JC rabbits.

It has been reported that *Encephalitozoon cuniculi* (*E. cuniculi*) infection of the lens and anterior segment in rabbits is a possible cause of cataracts.²⁰ None of the rabbits in our colonies were positive for *E. cuniculi* by serology (IDEXX RADIL, Columbia, MO), nor were the organisms present on histologic evaluation of selected globes from the rabbits in our study. Therefore, it is clear that *E. cuniculi* infection is not associated with development of cataracts in the EIII/JC strain of rabbits.

This study documented the high incidence of spontaneous cataracts in an inbred strain (EIII/JC) of aging NZW rabbits. It also demonstrated that the high incidence of cataracts in aging rabbits can be transmitted to a new congenic strain of rabbits (EII/JC-A2) by continual backcrossing between the HLA-A2.1 transgenic rabbits and the EIII/JC rabbits. The mode of inheritance of cataracts in this strain of rabbits still needs to be determined.

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Figure 1. Cataracts developed in the right eye (A) and left eye (B) of the same EIII/JC inbred rabbit. These antemortem photographs were taken immediately prior to euthanasia 70 days and 17 days after initial diagnosis of cataracts in the right eye and left eye, respectively.

Figure 2. Different stages of cataracts in the EIII/JC inbred rabbits. A) Immature cataract; B) and C) Mature cataract; D) Hypermature cataract.

Figure 3. Hematoxylin and eosin stain of lens from an EIII/JC inbred rabbit with mature cataract. The section shows numerous subcapsular Morgagnian globules within the lens.

Table 1

Total Rabbits in the Colony

<u>Type of Rabbits</u>	<u>Total Number</u>	<u>Male</u>	<u>Female</u>	<u>Age Range</u>	<u>Median Age</u>	<u>Cataract Cases</u>
EIII/JC Inbred	51	24	27	11-49	23	13
EIII/JC-HLA-A2.1	21	6	15	19-44	35	7
Outbred	78	39	40	10-67	26	0

Age = months

Table 2

Cases of Cataract in EIII/JC Inbred Rabbits

Case	Sex	Age [#]	Side(s)
1	F	45	B [*]
2	F	41	U
3	F	40	B
4	M	49	B
5	F	46	B
6	F	48	U
7	M	43	B [*]
8	M	43	B [*]
9	F	38	B [*]
10	M	44	B
11	M	43	B
12	F	37	U
13	F	39	U

B = Bilateral

U = Unilateral

[#] Months of age at which cataract(s) were first documented^{*} Bilateral forming around the same time

Table 3

Cases of Cataract in EIII/JC I-A2 Transgenic Rabbits

Case	Sex	Age [#]	Side(s)
1	M	43	U
2	F	42	B [*]
3	F	40	B [*]
4	F	38	U
5	F	43	B
6	F	41	U
7	M	38	U

B = Bilateral

U = Unilateral

[#] Months of age at which cataract(s) were first documented^{*} Bilateral forming around the same time