Analysis of Eye Lens-Specific Genes in Congenital Hereditary Cataracts and Microphthalmia of the Miniature Schnauzer Dog

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The congenital hereditary cataracts and microphthalmia in the miniature schnauzer dog are inherited by an autosomal recessive mode. To understand the genetic basis of these diseases, the authors purified and analyzed leukocyte deoxyribonucleic acid (DNA) from affected and normal animals using a candidate gene approach. Because the genes that encode the lens-specific proteins, specifically, alpha, beta, and gamma crystallins and the membrane protein (MP26), are known to maintain the structure and function of the lens, the authors used complimentary DNA (cDNA) fragments that corresponded to the above genes to search for the mutations at their loci in the affected animals. They found no evidence of the gene deletion and rearrangement in any of the five loci. In addition, the hybridizable sequences of the dog DNA to the specific probes for the human chromosome 4 and 18 loci, which are reported to be involved in the abnormality of the human eye, seem to be unaffected. These data support the notion that the hereditary cataracts and microphthalmia in the dog may be associated with genes other than those reported for several animal systems. Invest Ophthalmol Vis Sci 32:2662–2665, 1991

In humans, opacification of the lens of the eye due to genetic problems is the leading cause of visual impairment and blindness. Hereditary cataracts in the majority of cases are transmitted as autosomal dominant traits and are heterogeneous even among family members. They are often associated with other inherited ocular diseases, such as retinal degeneration and dislocation of the lens. The molecular mechanisms that govern the cataractogenesis in humans are poorly understood. This result is partly due to the lack of available samples from the operating room. Additionally, genetic studies in humans cannot be undertaken readily because only a small number of progeny becomes available, and in many instances, family histories are difficult to obtain. To better understand the disease processes, several animal models2,3 with inherited cataracts have been used extensively and are of great value. They readily provide tissue samples throughout the course of this disease and are excellent model systems to investigate the underlying causes at the developmental, morphologic, and biochemical level. Moreover, a large number of offspring can be obtained in a relatively short period. They also enable the investigator to manipulate the mating or cross to determine whether an individual animal is pure bred for a specific trait. Among these animal models, rats and mice have received more attention during the last several years. Although the studies on rats and mice have provided important information on the biochemical and morphologic changes that occur during cataract development, investigations on other animal models would be most informative because hereditary cataracts in man are of heterogeneous nature, and hence, no single animal species could be the most suitable model. Hereditary cataracts have been found in several breeds of dog,4 and these occur as inherited autosomal recessive traits. The opacity is primarily in the lens nucleus and posterior cortex. The clinical progression of cataract is variable and is related to the involvement of the equitorial and posterior cortices,4 although at 1–4 weeks, the superficial cortex at the bow appears to be normal. In the miniature schnauzer, the congenital cataracts are seen when the eyelids open at 2 weeks. The affected animals also show other ocular abnormalities, such as microphthalmia, which is also inherited by an autosomal recessive mode. The globe and lens are 10–20% smaller than normal. Although these diseases have been studied at the biochemistry level, no genetic studies have been reported. We analyzed the blood samples of the normal and affected animals to determine the genetic basis of these diseases.

Materials and Methods. The mouse and human crystallin cDNAs were obtained from Drs. J. Piatigorsky of the National Institutes of Health and M. L. Breitman of Canada, respectively. The membrane protein (MP26) and human collagen cDNAs were provided by Drs. Jean-Paul Revel of California Institute of Technology and Ramirez Francesco of Mount Sinai School of Medicine in New York. The human chromosome probes were obtained from the American Tissue Culture Collection (Rockville, MD)/National Institutes of Health (Bethesda, Maryland) repository. High-molecular-weight DNA was isolated from whole blood as described by Bell et al.5 Aliquots (5 μg) were digested with a variety of restriction enzymes according to the procedure recommended by the supplier (Biolabs, Beverly, MA). The fragments were separated by electrophoresis and were Southern
Fig. 1. Hybridization patterns of restricted genomic DNA from normal (N) and affected (A) animals probed with nick-translated gamma crystallin cDNA (A), membrane protein (MP26) cDNA (B), a genomic gamma crystallin gene (C), alpha A crystallin cDNA (D), beta (β23) crystallin cDNA (E), and collagen type II cDNA (F). The markers (M) are shown to the left of the panel. Restriction enzymes used are indicated at the top of each lane.

blotted as described. The blots were hybridized with 32P-labeled cDNAs or genomic DNA inserts in a buffer that contained 3 × SSC (sodium chloride and sodium citrate), 1 × Denhardt's solution, 20 mM phosphate-buffered saline, 0.1% SDS (sodium dodecyl sulfate), 1 mM edetic acid (EDTA), and 200 μg/ml denatured salmon sperm DNA at 68°C overnight. The hybridized membranes were finally washed with a buffer that contained 0.2 × SSC, 0.1% SDS at 60°C for 30 min followed by autoradiography.

Results and Discussion. To understand the genetic basis of the above hereditary diseases, we analyzed the leukocyte DNA by using the candidate gene approach. Because the disease primarily affects the eye, we hypothesized that an absence or alteration in one or more structural or functional proteins specific for the eye could contribute to the observed genetic defects. The most abundant lens structural proteins, ie, alpha, beta, and gamma crystallins, have physiologic and structural roles in maintaining the transparency of eye lens. In addition, the biogenesis of the lens fiber cell is accompanied by the appearance of the MP26. Because the congenital cataract is often associated with persistent hyperplastic vitreous and the collagen type II gene is known to be the major constituent of the vitreous body, these could be the most probable candidate genes associated with the primary lesion in the hereditary cataract and microphthalmia.

To test this hypothesis, we used mouse cDNA probes for gamma, beta, and alpha crystallins, MP26 genes, a human genomic probe for the gamma-crystallin gene, and a human cDNA probe for the collagen type II gene. Although these are heterologous probes, their high degree of conservation in the eye among species justifies their use in the dog system.

To detect submicroscopic deletions or rearrangements of the above genes, 32P-labeled probes are hybridized to the purified leukocyte DNA from the normal and affected animals, after digestion with several different restriction endonucleases. The results of one such analysis are shown in Figure 1. The hybridization patterns of the restricted DNA from the normal and affected animal are identical for several different enzymes (panels A–F). We found no evidence for rearrangement, deletion, or substitution at any of the five loci in the affected animals, and also there was no evidence for restriction fragment length polymorphism in the affected animal as shown by several other restriction enzymes. The data described above suggest that the organization of the genes for crystallins, MP26, or the collagen type II genes, although they play an important role in the function of the lens,
is not associated with the occurrence of congenital hereditary cataracts and microphthalmia. This finding is surprising in the light of findings in the other systems, including the human system, where one of the crystallin genes was affected.10-12

Although the synthesis of beta and gamma crystallins is affected2 in the cataractous lenses of the dog, it may be unrelated to the primary cause of the cataractogenesis and microphthalmia. The effect on the crystallin synthesis could be secondary rather than the underlying cause. Similarly, the change in the enzymatic activity reported for certain metabolically important enzymes in the affected lenses could also be due to the secondary effect caused by the alterations in the intracellular concentrations of ions. An altered crystallin synthesis in the chick lens was reported13 14 to be due to changes in the intracellular concentrations of Na+ and K+ ions. However, our findings suggest that the hereditary cataract and microphthalmia in the dog may be associated with different genes than those reported for cataracts in other animals. These data, although compelling, do not exclude these loci as cause for some kind of genetic defect related to cataract and microphthalmia because single base changes that occur outside of the restriction sites and small deletions may have been undetectable in our analyses.

In humans, some forms of cataract and microphthalmia are associated with several other systemic diseases15 that are caused by the deletion of short arm of chromosomes 4 and 18. We analyzed the DNA of the dog using two single-copy probes for each chromosome (specific for the short arm of human chromosomes 4 and 18). The results of one such experiment are summarized in Figure 2. The data suggest that the hybridizable sequences of the DNA of the dog to these specific probes (panels A and B) did not undergo significant changes in their organization at the gross level in the affected animal. Although we do not know the chromosomes in the dog to which the human probes are hybridizing, the hybridization pattern indicates that these sequences are unlikely to be involved in the congenital cataract and microphthalmia. However, it is unclear whether other probes specific for the human chromosomes 4 and 18 are involved in the cataractogenesis and microphthalmia in the dog.

We are extending this study to analyze the DNA by using cloned cDNA probes for other proteins involved in the structural and functional properties of the lens. Studies are also underway to search for mutations of the above candidate genetic loci using polymerase chain reaction approaches.

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References


CORRECTION

In the article “Dose-Related Effects of Prostaglandin F2a Isopropylester on Intraocular Pressure, Refraction, and Pupil Diameter in Monkeys,” which appeared in the March 1991 issue of Investigative Ophthalmology and Visual Science, on page 515, line 13, right column, the sentence should read “Widening of the spaces alone might increase uveoscleral outflow to a lesser degree than actual dissolution of the connective tissue.” The publisher regrets any inconvenience this error might have caused.