Differential Mitochondrial DNA and Gene Expression in Inherited Retinal Dysplasia in Miniature Schnauzer Dogs

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PURPOSE. To investigate the molecular basis of inherited retinal dysplasia in miniature Schnauzers.

METHODS. Retina and retinal pigment epithelial tissues were collected from canine subjects at the age of 3 weeks. Total RNA isolated from these tissues was reverse transcribed to make representative cDNA pools that were compared for differences in gene expression by using a subtractive hybridization technique referred to as representational difference analysis (RDA). Expression differences identified by RDA were confirmed and quantified by real-time reverse-transcription PCR. Mitochondrial morphology from leukocytes and skeletal muscle of normal and affected miniature Schnauzers was examined by transmission electron microscopy.

RESULTS. RDA screening of retinal pigment epithelial cDNA identified differences in mRNA transcript coding for two mitochondrial (mt) proteins—cytochrome oxidase subunit I and NADH dehydrogenase subunit 6—in affected dogs. Contrary to expectations, these identified sequences did not contain mutations. Based on the implication of mt-DNA-encoded proteins by the RDA experiments we used real-time PCR to compare the relative amounts of mt-DNA template in white blood cells from normal and affected dogs. White blood cells of affected dogs contained less than 30% of the normal amount of two specific mtDNA sequences, compared with the content of the nuclear-encoded glyceroldehyde-3-phosphate dehydrogenase (GA-3-PDH) reference gene. Retina and RPE tissue from affected dogs had reduced mRNA transcript levels for the two mitochondrial genes detected in the RDA experiment. Transcript levels for another mtDNA-encoded gene as well as the nuclear-encoded mitochondrial Tfnm transcription factor were reduced in these tissues in affected dogs. Mitochondria from affected dogs were reduced in number and size and were unusually electron dense.

CONCLUSIONS. Reduced levels of nuclear and mitochondrial transcripts in the retina and RPE of miniature Schnauzers affected with retinal dysplasia suggest that the pathogenesis of the disorder may arise from a lowered energy supply to the retina and RPE. (Invest Ophthalmol Vis Sci. 2006;47:1810–1816) DOI:10.1167/iovs.05-0819

Retinal dysplasia is a common clinical syndrome in dogs, and it is manifested clinically as single or multiple retinal folds.1 Morphologic classifications of retinal dysplasia in dogs includes single folds, geographic retinal dysplasia, and retinal dysplasia that is accompanied by retinal detachment.2 There are multiple potential etiologies for retinal dysplasia, including genetic mutations, viruses, and toxins.3–5 Retinal dysplasia is a common congenital disorder and is inherited in many and perhaps all breeds of dogs.6,7 Retinal dysplasia is inherited as an autosomal recessive condition in the miniature Schnauzer,7 Bedlington Terrier,7 American Cocker Spaniel,8 and English Springer Spaniel9 and as an autosomal dominant condition with incomplete penetrance in the Labrador Retriever.10 However, the mode of inheritance is unknown in several breeds, and the mutation(s) that induce retinal dysplasia have not been identified. The identity of the retinal cells that initiate the dysplastic growth is also unknown, although the retinal pigment epithelium (RPE) or the Müller cells have been suggested to be primary cellular instigators of retinal dysplasia.11,12

Recently, we reported retinal dysplasia and persistent hyperplastic primary vitreous in miniature Schnauzers.5 This condition is congenital, with an autosomal recessive mode of inheritance.3 The clinical manifestations vary. Some dogs are minimally affected, with focal areas of retinal dysplasia and expression of persistent hyperplastic primary vitreous. Others are blind from birth, secondary to retinal detachment or nonattachment of the dysplastic retina, or they become blind when the retina detaches later in life.3

Representational difference analysis (RDA) is a subtractive hybridization technique that was designed to identify differences between complex genomes.13 The RDA strategy has been used to try to identify candidate genes in an inherited condition,14 to identify genetic markers informative in purebred dog families,15 and to study genetic polymorphisms in the retinal pigment epithelium (RPE) in young dogs.16

The objective of this study was to use RDA to compare cDNA from the RPE and retina of normal miniature Schnauzers with mRNA from the same tissues in miniature Schnauzers affected with retinal dysplasia. RDA was performed with retinal or RPE cDNA from either affected or nonaffected dogs in excess, to confirm gene expression differences associated with the retinal dysplasia condition. We also completed real-time polymerase chain reaction on these tissues, to confirm and to delineate further the expression differences detected by RDA.

METHODS

Experimental Design

Tissues were obtained from three 3-week-old affected progeny from an experimental breeding of two known affected miniature Schnauzers.
Three 3-week-old unaffected offspring from a breeding of two known unaffected miniature Schnauzers were used as a source of control tissues. All eyes were examined by an ophthalmologist to verify that the retina was normal in unaffected dogs, whereas the eyes of the affected dogs had the previously described lesions. Total RNA from both retina and RPE of affected and normal subjects was reverse transcribed to cDNA by using an oligo-dT primer to examine potential differences in gene expression associated with the retinal dysplasia condition. Two experiments to measure expression differences were performed with the cDNA. First, an RDA analysis was performed to identify cDNA from genes differentially expressed between diseased and nondiseased states. Second, semiquantitative real-time RT-PCR was performed to confirm increased or decreased expression of several of the identified differentially expressed genes. DNA samples from test and control subjects were also used to investigate the prevalence of mitochondrial DNA within the total (genomic plus mitochondrial) DNA pool, since mitochondrial encoded genes were identified by the cDNA screening procedures. All animals were handled according to the standards set by the Canadian Council on Animal Care and the ARVO Statement for the Use of Animals and Ophthalmic and Vision Research.

**DNA and RNA Extraction**

The dogs were euthanized with an overdose of barbiturates. Retina and RPE were harvested from the eyes in a sterile environment under an operating microscope. Each eye was incised around its circumference at the pars plana, and the anterior segment was removed. The retinas from both eyes of each dog were removed from the posterior segment with a vitrector, cyclodiagnosis spatula, and calibri forceps and placed into 10 mL of extraction reagent (TRIZol; Invitrogen Canada, Burlington, Ontario, Canada). The well created by the posterior segment was then filled with approximately 1 mL of 0.5% trypsin (Invitrogen, Burlington, Ontario, Canada). The white blood cells from each eye were harvested. The RPE cells from both eyes were pooled and placed in 10 mL of the reagent for total RNA isolation. Harvested total RNA was used as a template (5 μg per reaction) in reverse-transcription reactions primed with oligo-dT. The cDNA product of these reactions was frozen at −80 °C for later use in RDA and in real-time PCR reactions.

**Electron Microscopy**

White blood cells were prepared from EDTA-treated whole blood by centrifugation with a dextran-based density separation medium (Lympholyte-Mammal; Cedarlane Laboratories, Hornby, Ontario, Canada). DNA for real-time PCR experiments was extracted from isolated white cell pellets with phenol-chloroform and quantified by complex formation with a double-stranded DNA quantitation reagent (Picogreen; Invitrogen) relative to calibration standards on a fluorometer (Fluroscent Ascent FL; Themo Labsystems, Franklin, MA). For electron microscopy, white blood cell pellets were collected from a 4-year-old female miniature Schnauzer without detectable ocular abnormalities and from a 4-year-old miniature Schnauzer affected with inherited retinal dysplasia. White blood cell pellets were also prepared from three other affected and three other normal dogs. White blood cells were fixed by suspension in 5% glutaraldehyde in 0.2 M s-collidine buffer, embedded in Epon/Araldite, sectioned, and stained with uranyl acetate. A minimum of 20 leukocytes from each dog were examined by electron microscopy and were photographed.

A normal and an affected male were manually ejaculated. Cells and spermatozoa in the ejaculate were fixed by dilution of the ejaculate with glutaraldehyde. Fixed samples were collected by centrifugation and embedded for electron microscopy as just described. Seminiferous tubules were obtained by surgical biopsy from two affected and two normal dogs, fixed, sectioned, stained, and examined for mitochondrial morphology by transmission electron microscopy.

**Representational Difference Analysis**

Representations of the RNA harvested from each animal were performed as previously described, using the procedures described by Hubank and Schatz. Total RNA preparations from RPE and retina were treated with DNase and the concentration determined by UV absorbance before performing reverse-transcription reactions primed with oligo-dT. Briefly, cDNA was digested with the “four-cutter” restriction endonuclease Sau3A I which cuts after its recognition sequence 5′-GATC. The cleaned restriction fragments were ligated to the R Bam adapter primer set of Hubank and Schatz and amplified by PCR using the R Bam 24 primer. Amplified representation of the normal dog RPE or retinal cDNA was used as “driver” and representation from affected dogs used as “tester.” This means that excess amplified driver cDNA from normal dogs, lacking the adapters necessary for second-round PCR amplification was hybridized to complementary tester sequences to prevent their amplification in the second round of PCR. Only unique “tester” sequences with ligated adapters that could not anneal to driver cDNA should be amplified. The experiment was also repeated with the reverse choice of affected driver and normal tester. J Bam adapters and J Bam 24 primer and N Bam adapters and N Bam 24 primer were used respectively in the second and third rounds of amplification of tester fragments and subtractive hybridization with driver sequence. This complete protocol was performed twice to confirm repeatability. Retinal and RPE tester cDNA pools resulting from the third selective amplification were ligated into a commercial cloning vector (PCR 2.1 TA; Invitrogen) and transformed into competent E. coli JM109 cells, and inserts of significant size were selected for sequencing.

**Real-Time PCR**

Glyceraldehyde-3-phosphate dehydrogenase was used as a housekeeping gene to normalize levels of expression of mRNAs coding for cytochrome oxidase subunit 1, ATPase subunit 6, NADH dehydrogenase subunit 4, and transcription factor A mitochondrial (Tfam) in real-time PCR. Primer design criteria included similarity in melting temperature (Tm) levels and avoidance of primer and template secondary structure at the primer Tm. Designed primer pairs were tested in PCR reaction for the ability to produce the proper size of amplification product and for a clean single product, as shown by ethidium bromide visualization after agarose gel electrophoresis. Primer pairs passing the electrophoresis test were tested further for their ability to produce a sharp single melt curve peak at successive PCR cycles in a thermal cycler (I-Cycler; Bio-Rad, Hercules, CA). The primer pairs meeting these criteria for each template follow.

**GA3-3DH: sense (S) 256 5′-GGGATGCGTGGTGGTCTAGTTATM Tm = 59.6 °C and antisense (AS) 439 5′-TGCTGACCATCTTGGGAGAT Tm = 59.3 °C, yielding a 184-bp product with a calculated Tm of 91.8 °C and a measured Tm of 86.5 °C.**

**Tfam: S 519 5′-CATTCAGCCAACTACTTACCTT Tm = 60.2 °C and AS 648 5′-GGGAAAGGCTTTATCACTTTGAGAT Tm = 60.6°C, yielding a 103-bp product, with a calculated Tm of 83.1 °C and a measured Tm of 81.6 °C.**

**Cytochrome oxidase subunit-1: S 893 5′-GATGATCACACACAGGAGGT Tm = 55 °C and AS 970 5′-CCATTGGTGGTCCGACTT Tm = 55 °C, yielding a 77-bp product, with a calculated Tm of 82.4 °C and a measured Tm of 80.3 °C.**

**NADH dehydrogenase subunit-4: S 160 5′-ACATTGCCACCATGATACAACTCG Tm = 60.5 °C and AS 268 5′-CGTAACTGGTCCCG-TAGGTTGTTA Tm = 60.6°C, yielding a 109-bp product, with a calculated Tm of 83.1 °C and a measured Tm of 81.0 °C.**

**ATPase subunit-6: S 274 5′-TTACAGCCCAACACAACTCTC Tm = 60.1 °C and AS 390 5′-GGGTAGAAGGTTCTGTAAGATGC Tm = 60.2°C, yielding a 117-bp product, with a calculated Tm of 84.4 °C and a measured Tm of 82.4 °C.**

PCR conditions for standard cycles in the thermal cycler (I-Cycler; Bio-Rad) were 45 seconds at 60° for a combined annealing and extension cycle, followed by denaturation for 25 seconds at 94°. DNA from...
TABLE 1. Differentially Expressed Transcripts Found in Retina or RPE Cells Harvested from Dogs at 3 Weeks of Age

<table>
<thead>
<tr>
<th>Identity</th>
<th>Expression Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retina (10 clones analyzed)</td>
<td></td>
</tr>
<tr>
<td>Cytochrome c oxidase subunit 1</td>
<td>2 Transcripts found</td>
</tr>
<tr>
<td>NADH dehydrogenase subunit 4</td>
<td>2 Transcripts found</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>2 Transcripts found, 1 included a portion of the mitochondrial D-loop</td>
</tr>
<tr>
<td>16S rRNA gene</td>
<td>3 Transcripts found</td>
</tr>
<tr>
<td>RPE (9 clones analyzed)</td>
<td></td>
</tr>
<tr>
<td>NADH dehydrogenase subunit 4</td>
<td>2 Transcripts found</td>
</tr>
<tr>
<td>Cytochrome C oxidase subunit 1</td>
<td>4 Transcripts found</td>
</tr>
<tr>
<td>Mitochondrial D-loop</td>
<td>2 Transcripts found</td>
</tr>
<tr>
<td>16S rRNA gene</td>
<td>1 Transcript found</td>
</tr>
</tbody>
</table>

Only transcript fragments over 100 bp were considered.

white blood cells was used as a template in PCR to measure mitochondrial DNA prevalence. Relative mRNA transcript prevalence was measured by real-time PCR in serial dilutions of cDNA produced from oligo-dT primed reverse transcription reactions performed on total RNA isolated from retina and RPE of normal and affected dogs. In addition to template, reactions contained primers and generic RT2 Real-time master mixes (SYBR Green; Applied Biosystems [ABI], Foster City, CA) optimized for the thermal cycler system (I-Cycler; Bio-Rad). The method of Pfaffl18 was used to determine PCR efficiency in calculating mRNA transcript prevalence in RPE and retina from normal and affected dogs.

Results

Representational Difference Analysis

Total RNA yields pooled from two eyes was approximately 60 μg from RPE and 110 μg from retina. cDNA produced from this RNA was processed, to produce the initial representations of each cDNA pool to be compared by RDA. A minimum cloned RDA insert size of ≥100 bp was chosen, to permit unambiguous sequence identification. The results in Table 1 represent findings when the RDA experiment was designed (driver-affected and tester-normal) to detect mutant cDNA or loss of expression in affected dogs.

Ten retinal clones and nine RPE clones from the ligation of the third selective amplification contained inserts that met the minimum size criteria. The clones represent five different genes or transcription areas, all of them coded by the mitochondrial genome. The same basic mitochondrial pattern was observed for both retinal and RPE cDNA pools. Repeated isolation of the same clone, as in six independent instances of identifying cytochrome c oxidase subunit 1 would be consistent with altered expression or with a mutation in the gene coding for this protein. However, the identification of other mitochondrial genes in the same RDA screen would also be expected if there were a decreased mitochondrial genome expression in RPE and in retina of affected miniature Schnauzers.

The mitochondrial genes identified in this RDA test screen contrast significantly with the results of a control RDA study performed to assess background information in dog RPE. The prominent clones identified in the control study as expression differences between same sex siblings were major histocompatibility loci (9/25 clones).16 and no mitochondrial genes were found.

Mitochondrial DNA Prevalence

The RDA test provided evidence of decreased mitochondrial DNA or decreased mitochondrial transcription activity in affected miniature Schnauzer RPE. It would clearly be useful to confirm the RDA observation with other more quantitative procedures. Real-time PCR is designed to make quantitative measurements of DNA or cDNA. The most relevant question to ask in real-time PCR is the relative ratio of genomic to mitochondrial DNA, because neither the number of mitochondria per cell, nor the number of copies of the mitochondrial genome per mitochondrion is a fixed value. An additional related point is that an alteration in mitochondrial transcript prevalence would be more likely to create a general systemic disorder than a tissue-specific abnormality.

The systemic prevalence of the mitochondrial genome in total DNA from normal and affected dogs was investigated in DNA extracted from white blood cells. Total DNA was used as template in real-time PCR, and ΔC_Ts for GA-3-PDH as a genomic marker with ΔC_TS for cytochrome oxidase subunit 1 (Cox-1) as a mitochondrial marker were compared. ΔC_TS values were larger for cytochrome oxidase subunit 1 between normal and affected dogs than for the GA-3-PDH marker. ΔΔC_T calculations from a real-time PCR assay that normalized the Cox-1 template content to GA-3-PDH genomic DNA gave 23% ± 1% as much mitochondrial DNA in affected as in healthy dogs at a PCR DNA template concentration of 2.5 ng/μL, 29% ± 1% as much affected mitochondrial DNA at a DNA concentration of 0.25 ng/μL, and 27% ± 1% as much mitochondrial DNA in affected dogs at a template DNA concentration of 25 pg/μL.

The finding of reduced amounts of mitochondrial DNA in white blood cells of miniature Schnauzers affected with retinyl dysplasia, combined with the identification of differential expression of three mitochondrial genes in RDA experiments suggests a problem with achieving normal mitochondrial transcript production in affected dogs. We have investigated the extent of this problem by the use of semiquantitative real-time RT-PCR.

**FIGURE 1.** Effect of template concentration and disease status on real-time PCR amplification of a 77-bp fragment (bases 893–970) of the mitochondrial-encoded cytochrome oxidase subunit 1 gene. Template for the PCR reaction was cDNA produced by reverse transcribing either 1000, 200, or 40 ng of total RNA isolated from retina of normal (squares) or affected (diamonds) miniature Schnauzers. Data in the left-hand curve producing the lowest C_T comes from the highest template concentration in each case.
Marker Gene Expression Levels

The number of transcripts from the reduced relative amounts of mitochondrial DNA were investigated for three mitochondrial marker genes. Levels of mRNA for cytochrome oxidase subunit I and NADH dehydrogenase subunit 4 were measured because these genes had been identified in the RDA experiment. Expression of the mRNA for a third mitochondrial gene, subunit 6 of ATPase, was added to determine whether expression of genes not detected by RDA was also affected. Expression of a fourth gene coding for the Tfam transcription factor was also determined. Although coded on nuclear DNA, the Tfam transcription factor is reported to be important for both the number of copies of DNA within each mitochondrion and the transcript production from the mitochondrial DNA template.19

Real-time PCR measures relative cDNA prevalence. Larger amounts of template in a real-time PCR reaction produce more product per cycle, and cross-detection threshold levels at a lower cycle number (C_t). Fluorescence output of a double-stranded (ds)DNA intercalating dye (SYBR Green; ABI) versus cycle number is shown during real-time PCR for a cytochrome oxidase subunit 1 product from normal and affected dog retina (Fig 1).

Quantitation of the PCR product by fluorescent dye binding depends on the production of a homogeneous product from primer-specific template. Homogeneity of the cytochrome oxidase subunit 1 PCR product is demonstrated by the DNA melting curve shown in Figure 2. Use of primer sets chosen for quality of PCR product generated the real-time PCR results from the RPE and from retinal cDNA shown in Table 2. Differences in C_t between normal and affected dogs were corrected for relative amounts of GA-3-PDH housekeeping gene cDNA expression in total RNA samples, generating ΔC_t values for each of the four genes compared with GA-3-PDH. The negative ΔΔC_t for each of the four genes compared with GA-3-PDH indicate that affected dogs expressed relatively lower levels of each of these four test genes compared with the housekeeping gene in both retina and RPE from affected dogs.

PCR efficiencies for each template and primer set were derived from a separate set of experiments based on a template dilution series. PCR efficiency for each template was determined from relative real-time C_t for each primer pair, using serial doubling cDNA template dilutions from 250 ng down to 8 ng of cDNA. The resulting efficiency values were used to assign relative cDNA amounts to ΔC_t for each template. The calculated mRNA expression levels relative to levels measured in normal dogs are shown in Figure 3. On normalizing the expression of three mitochondrial encoded templates to GA-3-PDH, the calculated mRNA expression levels relative to levels measured in normal dogs are shown in Figure 3. On normalizing the expression of three mitochondrial encoded templates to GA-3-PDH, the calculated mRNA expression levels relative to levels measured in normal dogs are shown in Figure 3.
PDH expression, it was observed that retinal tissue from affected dogs expressed similar relative amounts of the three mRNA transcripts equivalent to approximately 10% of the level found in normal dogs. There were also severe reductions in relative mitochondrial transcript expression in RPE tissue from affected dogs, although there were wider variations observed between the expression of the three transcripts in the RPE.

The Tfam transcription factor controls mitochondrial DNA copy number as well as transcription levels from the two mitochondrial DNA promoters, in concert with TfB1 and TfB2 mitochondrial transcription factors. The relative expression of the nuclear encoded Tfam transcription factor was also depressed significantly in both retinal and RPE tissue from affected dogs. However, relative levels of Tfam transcript were much lower in RPE tissue than in retinal tissue.

Mitochondrial Morphology

Electron micrographs of representative neutrophils from a normal and an affected miniature Schnauzer are shown in Figure 4. Mitochondria from skeletal muscle (Fig. 5), from epithelial cells in semen (Fig. 6) and from the midpiece of a spermatozoa from an affected dog (Fig. 7) are also shown. The mitochondria from the affected dog were reduced in number and size and had abnormal structure in every tissue examined. The number of cristae within each mitochondrion was reduced, and there was poor definition of the characteristic double cristal membrane. Unusual electron-dense inclusions were apparent in neutrophil, and epithelial cells in ejaculate samples and in midpiece mitochondria from different affected dogs. These striking differences in mitochondrial morphology were observed in all cells examined in every affected dog.

DISCUSSION

Various forms of visual impairment have been associated with mutations in mitochondrial DNA. Leber’s hereditary optic neuropathy (LHON); mitochondrial myopathy, encephalopathy, lactic acid, and stroke-like episodes (MELAS); and neuropathy, ataxia, and retinitis pigmentosum (NARP), are conditions with various degrees of maternal inheritance, mitochondrial defect expression between different tissues, and problems with vision. The mitochondrial genes affected in these disorders vary from leucyl-tRNA in MELAS20 to subunit 6 of the F1FO-ATPase in NARP,21 and at least three separate gene loci in LHON that may affect cytochrome b and other proteins and tRNA molecules transcribed from the mitochondrial DNA.22 This evidence implies that mutations that reduce mitochondrial function put eye tissues at risk.

The molecular effect seen in miniature Schnauzers affected with retinal dysplasia could arise from the introduction of a base change or a short in-frame deletion that has parallel effects on function of some critical gene product, and on hybridization of the mutated gene with the wild-type sequence. Alternatively, the effect could be due to the loss or gain of expression of a critical gene in the affected dogs. The direction of assignment of driver and tester should not affect the ability to detect a base change or a deletion. However, detection of loss of expression of a critical gene in affected dogs requires that

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**FIGURE 4.** Transmission electron micrographs of a neutrophil from a 4-year-old female miniature Schnauzer with no detectable abnormalities in the retina (a) and a 4-year-old female affected with inherited retinal dysplasia (b). Arrows: mitochondria. Note the reduced number and size, the disorganized cristae, and increased electron density of the mitochondria in the neutrophil from the affected dog. Magnification, ×18,000.

**FIGURE 5.** Transmission electron micrographs of skeletal muscle from a 1-year-old female miniature Schnauzer with no detectable abnormalities in the retina (a) and a 1-year-old female affected with inherited retinal dysplasia (b). Arrowheads: mitochondria. Note the abnormal cristae in the affected dog. Magnification, ×18,000.
the “driver” hybridization pool be assigned to the affected subject, while the “tester” pool represents the normal.

The strong evidence provided for an autosomal recessive mode of inheritance of the retinal dysplasia condition appears to be at odds with the identification of mitochondrial gene expression as the basis of the problem. A defect residing in the mitochondrial genome should show a maternal inheritance pattern with partial penetrance rather than following an autosomal recessive mode of transmission. However, the identification of three differentially expressed mitochondrial genes by RDA and the finding of an additional affected mitochondrial gene by quantitative PCR and the abnormal leukocyte mitochondrial morphology suggest a general problem in mitochondrial gene expression.

As for other mammalian species, there are only two promoters in the complete 16,728-bp dog mitochondrial genome. The RNA transcript from each promoter is polycistrionic and is processed into separate mRNA and tRNA molecules after transcription. This accounts for parallel effects of a single mutation in a promoter or in a mitochondrial transcription factor on several mitochondrial-encoded genes. Again, promoter mutations in the mitochondrial DNA should show a maternal inheritance pattern that is inconsistent with our observations, but genomic mutations affecting the abundance or the activity of mitochondrial transcription factors would be expected to have the observed autosomal recessive inheritance pattern and pleiotropic effects on mitochondrial gene expression.

Transcription factor A mitochondria has been identified as a major mitochondrial transcription factor controlling both mitochondrial DNA copy number and transcription activity. There are several reports of natural or artificial cre-lox tissue-specific reductions in nuclear Tfam expression associated with myopathic conditions, but we have not found literature reports of reduced Tfam expression related to retinal disorders. Evidence of a reduced mitochondrial DNA content (~25% of normal) from white blood cells of affected dogs suggested a systemic problem that would be anticipated to be similar to what others have found with reduced Tfam expression in mice. Using Southern blot quantification, Ekstrand et al. have reported that Tfam reduction to approximately 30% of normal expression levels caused a marked decrease in COX1 expression, and that embryonic mice with these low expression levels did not survive. It may be difficult to make direct comparisons with this study considering the difference in analytical methods. However, our results are in agreement that, in tissues such as retina, there was a disproportionate reduction between Tfam expression and mitochondrial gene expression. In contrast, the much more severe reduction in Tfam expression in RPE was not associated with a further decline in mitochondrial gene expression.

The two other less-studied, but still major, mitochondrial transcription factors also participate in transcription initiation. McCulloch and Shadel reported that mtTFB1 interacts with the C terminus of Tfam to promote binding and initiation of transcription of mtDNA by mtRNA polymerase. Apparently, mtTFB2 is also dependent on Tfam for activation of transcription from either the light- or heavy-strand promoters. Current data do not permit identification of a unique mutated gene in affected dogs. However, the evidence supports the central involvement of mitochondrial transcription factors in the genesis of retinal dysplasia in the miniature Schnauzer.

Mitochondria from each tissue examined from miniature Schnauzers affected with inherited retinal dysplasia had fewer
cristae, poor definition of the membranes of existing cristae, and a tendency to increased electron density of the mitochondrial matrix. Mitochondrial morphologic anomalies in each tissue examined support the molecular data obtained from eye tissue and support the hypothesis that there is a systemic inherited condition in these dogs. Mutations that cause moderate reductions in mitochondrial gene expression could be expected to produce prominent phenotypic effects in tissues with high energy requirements during embryogenesis and high oxidative energy requirements in the differentiated state. In his interesting review of mitochondrial disease Schon proposes that the highest energy requirements occur in tissues with the largest ion transport responsibilities. Thus the “dogs that do bark” are tissues including the retina and RPE with very large energy requirements that exceed any possible ATP supply from glycolysis. Given that the dysplastic condition originates in embryogenesis at a time when the retina differentiates from a single cell layer to multiple cell layers with discrete but intensive ion transport potential, it is possible that a deficiency in energy supply could affect cell organization and the cell–cell contacts necessary to establish normal retinal structure. This could explain why an apparently systemic mitochondrial abnormality has no overt systemic effects, yet induces an inherited retinal dysplasia in miniature Schnauzers.

References