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Journal
Mammalian Genome, 4(9)

ISSN
0938-8990

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Publication Date
1993-09-01

DOI
10.1007/bf00364792

Peer reviewed

Mammalian Genome 4, 544–548 (1993).

Mammalian Genome

The cat RDS transcript: candidate gene analysis and phylogenetic sequence analysis

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Received: 10 May 1993 / Accepted: 2 July 1993

The RDS gene (human and cat designation, *rds* gene in the mouse) was the first gene to be specifically identified with a mammalian hereditary retinal degeneration (Travis et al. 1989). Also known as peripherin (Connell and Molday 1990), the RDS gene has been investigated for other retinal degenerations, including the progressive rod-cone dysplasia of miniature poodles, and human autosomal dominant retinitis pigmentosa (RP). Recent reports have confirmed linkage to the orthologous RDS locus in several autosomal dominant RP families, and mutations in the gene have been identified (Farrar et al. 1991; Kajiwara et al. 1991). Because of the phenotypic diversity that can occur with mutations of the same gene in different species, we evaluated the RDS transcript for two retinal degenerations that have been identified in cats—the recessive retinal degeneration in Abyssinian cats, rdAC, described by Narfstrom (Narfstrom 1985; Narfstrom and Nilsson 1987) and the dominantly inherited rod-cone dystrophy, Rdy, identified by Curtis (Holmes and Curtis 1990; Leon and Curtis 1990; Leon et al. 1991), that is also present in the Abyssinian breed.

In cats homozygous for the rdAC, ultrastructural alterations can be seen in photoreceptors even prior to retinal maturation (Narfstrom and Nilsson 1987). Visual function and electrophysiology initially appear relatively intact. Ophthalmoscopic abnormalities are not evident until 1.5 years of age, and visual loss progresses to complete blindness in approximately 3 years. ERG changes correlate with the loss of rhodopsin, with no evidence that overall retinal sensitivity is reduced by other mechanisms. In this respect, the ERG changes in rdAC are comparable to those in the diffuse form of human dominantly inherited RP (Narfstrom et al. 1989). The onset and progression of retinal disease are considerably slower than that associated with the murine rd and rds mutations or the rod-cone dysplasias of the Irish setter or collies and comparable to the progressive rod-cone degeneration in miniature poodles (O'Brien and Aguirre 1987). The combination of electrophysiology, visual pigment reflectometry, light and ultrastructural histology all indicate that this recessive retinal disorder is primarily localized to a defect in the photoreceptors, with the initial disturbances being localized to the rods with later degeneration of the cones.

The dominant rod-cone dysplasia, Rdy, exhibits rod and cone abnormalities from the earliest age examined (2 weeks). Degenerative changes become evident at 4.5 weeks as the photoreceptors, which have only rudimentary outer segments, develop pyknotic nuclei and cellular displacement into the subretinal space. The degeneration begins centrally and spreads peripherally with progressive photoreceptor cell loss. Vision is profoundly affected in the early stages of the disease, and blindness becomes total within a few months. Like the rdAC model, both histological and biochemical evidence suggest that the primary defect resides in the photoreceptors.

Candidate gene analysis of the RDS transcript in these two animal models of retinal disease with cross-species RT/PCR can determine whether point mutations in this gene could play a causative role for either of these conditions. The *RDS* is an excellent candidate gene for these feline retinal degenerations because, as discussed above, the rdAC and Rdy degenerations appear to be photoreceptor-specific and the *RDS* gene

The nucleotide sequence data reported in this paper have been assigned GenBank accession number M94047.

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has been implicated for retinal degenerations in three other mammalian species. The lack of an effective genetic map of the cat and the highly inbred nature of the Abyssinian colonies preclude the use of linkage analyses to localize the causative genes.

Cross-species comparisons of nucleotide and amino acid substitutions can help define conserved regions of the RDS peptide. In order to avoid a biased assessment of these differences, these comparisons are founded upon congruence analysis (Bledsoe and Raikow 1990), which employs existing data sets to obtain robust phylogenetic evidence. A subset of the observed nucleotide and amino acid substitutions can be attributed to the evolution of rodentia from a common ancestral sequence. This group of sequence alterations provides the most convincing evidence for the conserved regions of peptide sequence.

RNA was purified from retinas of 4-week-old normal and affected cats (Chomczynski and Sacchi 1987). The animals were unrelated, and the normal animal is not from an Abyssinian line. The Abyssinian samples were from two separate colonies. The Rdy cat colony is maintained at the Animal Health Trust in England, and the rdAC animals are in Linkoping, Sweden. Care was taken to keep the retinal samples isolated from one another. The extractions of RNA from the normal and affected cats were carried out on separate days to avoid the possibility of any cross-contamination. A single retinal sample was used for each of the cats—normal (+/+), the rdAC/rdAC, and Rdy/+.

Reverse transcription and PCR were performed according to the Perkin Elmer–Cetus protocols with a magnesium concentration of 1.5 mM. Fifty microliter PCR reactions were routinely performed with 30 cycles consisting of the following parameters: initial denaturation, 90°C, 3 min; annealing, 50°C, 2 min; synthesis, 72°C, 3 min; cycle denaturation, 90°C, 1 min. For some primer sets, the annealing temperature was raised to 60°C to reduce nonspecific synthesis. The 3′ end of the coding region was amplified by the 3′ RACE method (BRL/Gibco) while the 5′ end of the coding region was amplified by RT/PCR with a 5′ primer from the untranslated region of the transcript.

The major amplified segments were cloned with the Bluescript vector. After the PCR segments were demonstrated to lack internal *Eco*RI and *Hin*dIII sites, a new set of primers were made with additional sequences for the asymmetric cloning of the fragments. A series of 10 or more clones were selected, screened for the appropriately sized insert, and then pooled for dideoxynucleotide sequencing of the mixtures. All of these steps were performed for the PCR products derived from each of the three retinal sources—the normal cat, the rdAC/rdAC cat, and the Rdy/+ animal.

Double-stranded nucleotide sequencing was performed with plasmid-based primers as well as PCR and internal primers. All sequencing reactions were done with standard protocols (Sequenase), and each fragment was sequenced on both strands from at least two different start positions. DeazaGTP was used in several sequencing runs to resolve a sequence compression at the 3′ end of the cloned coding region. For obtaining the nucleotide sequences of the 5′ and 3′ ends of the coding region, direct dideoxynucleotide sequencing of PCR products was done with nested primers on both strands (Gibbs et al. 1989).

The majority of the sequencing was done after subcloning the two larger PCR fragments in pBluescript and using pools of 10 or more clones as templates for double-stranded dideoxynucleotide sequencing (Snyder et al. 1990). The 4% of the cat coding region not covered by the cloned PCR fragments were amplified by 3′ RACE and with a 5′ primer that was a composite of the 5′ untranslated region of the human, mouse, and rat RDS transcript. The coding regions for the orthologous RDS coding region from the normal cat and the two retinal degenerations in the Abyssinian cat were completely and independently sequenced. A silent polymorphism was identified within the transcript of the normal cat retina, but no other nucleotide differences among the transcripts of normal, rdAC/rdAC, and Rdy/+ cats were found (Fig. 1).

The RT/PCR analysis confirmed that the normal processed transcript is present in the retina of all three animals. It is possible that RT/PCR might not detect an aberrantly spliced transcript that would be detected by Northern hybridization. Such a transcript would be unlikely to cause dysfunction in a recessive mutation such as rdAC, but it could be a mechanism for the dominant mutation, Rdy. Northern analysis of RNA was not performed because of the limited amount of RNA that was available.

A comparison of the cat RDS nucleotide sequence with those of other mammals demonstrates that the gene product is highly conserved. Most models of mammalian phylogeny are unable to distinguish the evolutionary points of bifurcation of carnivora, artiodactyla (cow), and primates (human) along the phylogenetic tree (McKenna 1987; Novacek and Wyss 1986; Shoshani 1986). The minimum nucleotide and amino acid substitutions along the RDS coding region are based upon the simplest, consensus phylogeny—rodentia, carnivora, artiodactyla, and primates arise independently and simultaneously from an ancestral line with a later bifurcation of rodentia to give rise to the rat and mouse lineages.

A separate analysis was done with only those substitutions that could be unambiguously placed within rodentia (Begy and Bridges 1990; Travis et al. 1991a). At these positions, the nucleotides from the cat, human, and cow are identical, and there is a high degree of certainty that the substitutions in the mouse and/or rat sequences reflect a change from an established ancestral condition. The cumulative distribution of all nucleotide substitutions along the RDS coding region, as well as the cumulative amino acid substitutions, are shown in Fig. 2. Of the 294 nucleotide substitutions, 101 could be specifically localized to the rodential branch. This is consistent with the reportedly higher rate of mutations that has been observed in the evolution of rodentia (Brownell 1983; Wu and Li 1985). The distribution of this selected group of nucleotide substitutions is not shown, but it is virtually identical to the distribution of all of the nucleotide substitutions.

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-155        ACCTG  CTCCC  TTCCC  AAGGC  CCTGA  ATCTT  TGGAG  CCCCT  GGGCT CACTA      -51
-55  AGGTC  AGGAG  TGGTT  GCTGT  GTCCT  GGGAA  GCTGC  CTAGA  CTGCA  CCCAG CAAGT      -1

                                    1    ATG GCG CTG CTG AAA GTC AAA TTT GAC CAG     30
                                    1    Met Ala Leu Leu Lys Val Lys Phe Asp Gln     10

AAG AAG CGG GTC AAG TTG GCC CAA GGG CTC TGG CTG ATG AAC TGG CTC TCC GTG TTG GCT     90
Lys Lys Arg Val Lys Leu Ala Gln Gly Leu Trp Leu Met Asn Trp Leu Ser Val Leu Ala     30

GGC ATC GTC ATC TTC AGC CTA GGG CTG TTC CTT AAG ATC GAG CTG CGG AAG AGG AGT GAT    150
Gly Ile Val Ile Phe Ser Leu Gly Leu Phe Leu Lys Ile Glu Leu Arg Lys Arg Ser Asp     50

GTG ATG AAT AAT TCA GAG AGC CAT TTT GTG CCC AAC TCC TTG ATA GGG ATG GGG GTG CTG    210
Val Met Asn Asn Ser Glu Ser His Phe Val Pro Asn Ser Leu Ile Gly Met Gly Val Leu     70

TCC TGC GTC TTC AAC TCT CTG GCT GGC AAG ATC TGC TAT GAC GCC CTG GAC CCC TCC AAG    270
Ser Cys Val Phe Asn Ser Leu Ala Gly Lys Ile Cys Tyr Asp Ala Leu Asp Pro Ser Lys     90

TAT GCC AAG TGG AAG CCC TGG CTG AAG TCG TAT CTG GTC GTC TGT GTC CTC TTC AAC ATT    330
Tyr Ala Lys Trp Lys Pro Trp Leu Lys Ser Tyr Leu Val Val Cys Val Leu Phe Asn Ile    110

GTC CTC TTC CTG GTG GCC CTC TGC TGC TTC CTA ATG CGG GGC TCC CTG GAG AGC ACC CTG    390
Val Leu Phe Leu Val Ala Leu Cys Cys Phe Leu Met Arg Gly Ser Leu Glu Ser Thr Leu    130

GCC CAG GGG CTC AAG AAT GGC ATG AAG TAC TAC CGA GAC ACA GAC ACC CCC GGC CGG TGT    450
Ala Gln Gly Leu Lys Asn Gly Met Lys Tyr Tyr Arg Asp Thr Asp Thr Pro Gly Arg Cys    150

TTC ATG AAG AAG ACC ATC GAC CTG CTA CAG ATC GAG TTC AAA TGC TGC GGC AAC AAT GGC    510
Phe Met Lys Lys Thr Ile Asp Leu Leu Gln Ile Glu Phe Lys Cys Cys Gly Asn Asn Gly    170

TTT CGA GAC TGG TTT GAG ATT CAG TGG ATC AGC AAT CGT TAC CTG GAC TTC TCC TCC AAA    570
Phe Arg Asp Trp Phe Glu Ile Gln Trp Ile Ser Asn Arg Tyr Leu Asp Phe Ser Ser Lys    190

GAA GTC AAA GAT CGC ATC AAG AGC AAT GTG GAC GGG CGA TAC CTG GTG GAT GGA GTT CCC    630
Glu Val Lys Asp Arg Ile Lys Ser Arg Val Asp Gly Arg Tyr Leu Val Asp Gly Val Pro    210

TTC AGC TGC TGC AAC CCC AAC TCG CCA CGG CCC TGC ATT CAG TAC CAG CTC ACC AAC AAT    690
Phe Ser Cys Cys Asn Pro Asn Ser Pro Arg Pro Cys Ile Gln Tyr Gln Leu Thr Asn Asn    230

TCG GCT CAC TAC AGC TAC GAC CAC CAG ACA GAG GAG CTC AAC CTG TGG GTG CGT GGC TGC    750
Ser Ala His Tyr Ser Tyr Asp His Gln Thr Glu Glu Leu Asn Leu Trp Val Arg Gly Cys    250

AGG GCC GCC CTG CTA AGC TAC TAC GGC AGC CTC ATG AAC TCC ATG GGC GCC GTC ACA CTC    810
Arg Ala Ala Leu Leu Ser Tyr Tyr Gly Ser Leu Met Asn Ser Met Gly Ala Val Thr Leu    270

CTC GTC TGG CTC TTT GAG GTG TCC ATC ACA ATT GGC CTA CGC TAC CTG CAC ACA GCA CTG    870
Leu Val Trp Leu Phe Glu Val Ser Ile Thr Ile Gly Leu Arg Tyr Leu His Thr Ala Leu    290

GAA GGT GTG TCG AAC CCC GAA GAC CTT GAG TGC GAG AGT GAG GGC TGG CTT CTG GAA AAG    930
Glu Ser Val Ser Asn Pro Glu Asp Pro Glu Cys Glu Ser Glu Gly Trp Leu Leu Glu Lys    310

AGC GTG TCG GAG ACT TGG AAG GCC TTT CTG GAG AGC TTG AAA AAG CTG GGC AAG AGT AAC    990
Ser Val Ser Glu Thr Trp Lys Ala Phe Leu Glu Ser Phe Lys Lys Leu Gly Lys Ser Asn    330

CAG GTG GAA GCC GAG GGT GCA GAC GCA GGC CAG GCC CCA GAG GCT GGC TGA              1038
Gln Val Glu Ala Glu Gly Ala Asp Ala Gly Gln Ala Pro Glu Ala Gly ***              346

   TGCCC  TGGGC  CCCCT  CCCCT  CCTGA  ACACT  GAGAA  GTAGC  AGACT  CCAAG  AAAGC      1093
   GGATA  CCCCA  CTTA                                                          1107
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Fig. 1. Nucleotide and amino acid sequence of the cat RDS transcript. The nucleotide sequence was independently determined from retinal RNA templates from +/+, rdAC/rdAC, and Rdy/+ animals. The adenosine at position 21 is **underlined** to indicate the location of the silent polymorphism (A → G substitution) that was heterozygous in the +/+ transcript. No other nucleotide differences were found among the different cat RDS transcripts. The 5′ primer sequence derived from the mouse, rat, and human transcripts and used for RT/PCR of the untranslated region is not included in the nucleotide sequence of the cat RDS transcript.

The 17 amino acid substitutions within the rodentia branch of the phylogenetic tree are shown in Fig. 2, line B. The distribution of these substitutions provides strong confirmatory evidence for three major conserved regions of the RDS peptide—amino acids 1–32, 37–90, and 141–255—that are seen when all amino acid substitutions are compiled (Fig. 2, line A).

A phylogeny that places carnivora closer to rodentia reduces the total number of nucleic acid substitutions by 3.4% and the minimum number of amino acid substitutions by 5.5%. The overall distributions of the substitutions along the coding region were unaffected by these changes. No other arrangement of the mammalian phylogeny resulted in reductions of the minimum number of nucleic acid substitutions.

Travis and colleagues recently published a comparison of the amino acid sequences for the RDS peptide that have been deduced from mouse, rat, human, and bovine cDNA clones (Travis et al. 1991a). The addition of the cat sequences confirms and extends those observations. The scoring of the cumulative nucleotide and the amino acid substitutions on the basis of the phylogenetic tree provides an effective strategy of cross-species comparisons and highlights the major conserved regions of the peptide. Both putative membrane-bound, cytosolic, and extracellular segments of

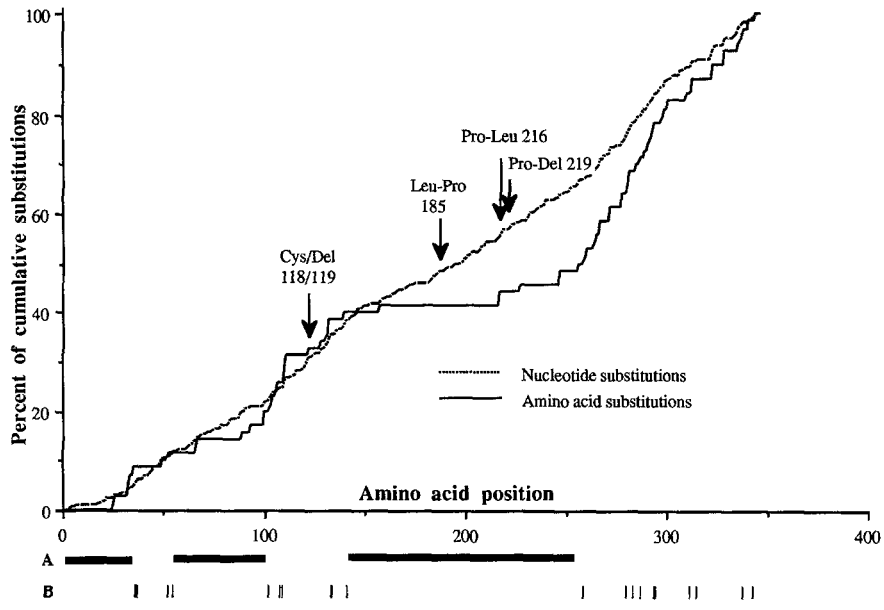

Fig. 2. The distribution of nucleotide and amino acid substitutions along the RDS coding region. The graph demonstrates the cumulative occurrences of nucleotide **(broken line)** and amino acid **(solid line)** along the RDS coding region. The curves have been standardized to 100%, based upon a total of 294 nucleotide substitutions and 70 amino acid substitutions. The locations of the reported rds mutations responsible for human autosomal dominant RP are indicated by **downward arrows.** The **dark bars in row A** indicate the most conserved regions of the peptide, while the **vertical lines in row B** mark the position of the 17 amino acid changes that are unambiguously assigned to the rodentia branch of the phylogenetic tree.

the peptide are contained within these conserved regions (Travis et al. 1991b). The C-terminal portion of the protein, which is thought to be exposed to the intracellular compartment, is the least conserved region of the entire peptide. Three of the four mutations that have been reported for the human RDS gene as causative for autosomal dominant RP (Pro 219-del, Pro 216-Leu, and Leu185-Pro; Kajiwara et al. 1991) are localized within the central conserved region of the peptide. The fourth mutation (Farrar et al. 1991), Cys 118 or 119-del, is located within a region that has accumulated a relatively high number of conservative amino acid substitutions. If the major determinants of the secondary structure in this region are disulfide linkages, then one would expect that a number of neutral amino acid substitutions might be well tolerated, whereas a mutation in one of the highly conserved cysteine residues would have a major impact on protein structure and function.

This analysis of the RDS transcript in the cat retina indicates that a point mutation within the coding region of this gene is not responsible for the two hereditary retinal disorders that have been identified in Abyssinian cats. As we acquire nucleotide sequence data for genes from a variety of species, we can identify regions of amino acid conservation and integrate cladistic analyses and estimates of phylogeny into these comparative studies.

Acknowledgments. The authors express their appreciation to Dr. Anthony Bledsoe, Department of Biological Sciences, University of Pittsburgh, for his proposal to use phylogenetic data to identify regions of protein conservation. We also wish to thank Dr. Gabriel Travis, who graciously made the human RDS nucleotide sequence available to us prior to its publication. This work was supported in part by a grant from National Eye Institute, EY09192-01, and by The Eye & Ear Institute of Pittsburgh. A portion of this work was previously presented at the Association of Research in Vision and Ophthalmology, Sarasota, Florida, 1990.

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