Molecular Vision 2003; 9:601-5 <http://www.molvis.org/molvis/v9/a73>
Received 17 July 2003 | Accepted 24 October 2003 | Published 11 November 2003

Evaluation of the canine RPE65 gene in affected dogs with generalized progressive retinal atrophy

Gabriele Dekomien, Jörg Thomas Epplen

Molecular Human Genetics, Ruhr-University, 44780 Bochum, Germany

Purpose: The *RPE*65 gene was screened in 26 breeds of dogs in order to identify potential disease-causing mutations in dogs with generalized progressive retinal atrophy (gPRA).
Methods: Intronic sequences were obtained from canine genomic DNA by intron-overlapping polymerase chain reactions (PCRs). Mutation analysis was performed by PCR and demonstration of single strand conformation polymorphisms (SSCP). Genomic variations were verified by sequencing.
Results: A series of exonic and intronic single nucleotide polymorphisms (SNPs) were identified in the investigated breeds, but none of the dogs examined showed the typical RPE deletion for retinal dystrophy in Briards nor any other disease-causing mutation.
Conclusions: The informative SNPs provide evidence allowing indirect exclusion of mutations in the *RPE*65 gene as causing retinal degeneration in 25 of the 26 dog breeds investigated with presumed autosomal recessively transmitted gPRA.

Like retinitis pigmentosa (RP) in man, generalized progressive retinal atrophy (gPRA) represents a genetically heterogeneous disorder [1] in dogs that in most forms is inherited as an autosomal recessive (ar) trait. gPRA shows homologous disease symptoms like RP and is characterized by degeneration of the peripheral retina leading to night blindness and loss of visual fields. The age of onset and the progression rate varies in different dog breeds, but typically the disease progresses to complete blindness [1,2]. Until now, causal mutations for ar gPRA have been identified only in a few dog breeds [3-7]. A number of photoreceptor genes have been excluded as the primary genetic cause of the trait in the many of 26 dog breeds [7-14] investigated here.

The *RPE*65 gene is expressed in the RPE, a monolayer apposed to the outer surface of the retinal photoreceptor cells. Despite studies in *RPE*65-deficient transgenic mice [15], the precise function of the protein remains unknown, but it is required for the regeneration of 11-cis retinal in the visual cycle [16]. Mutations in the *RPE*65 gene are associated with several human inherited retinal degenerations: Lebers congenital amaurosis (LCA), childhood-onset severe retinal dystrophy (CSRD), or autosomal recessive retinitis pigmentosa (arRP) [17-20]. In Swedish briards, a homozygous 4 bp deletion after codon 153 of the *RPE*65 gene leads to congenital stationary blindness (csnb). Gene therapy with a recombinant adeno-associated virus (AAV) carrying wild type *RPE*65 restored the visual function in dogs [21,22].

Correspondence to: Gabriele Dekomien, Molecular Human Genetics, Ruhr-University, 44780 Bochum, Germany; Phone: (+49)234-32-25764; FAX: (+49)234-32-14196; email: gabriele.dekomien@ruhr-uni-bochum.de

Here we investigated the *RPE*65 gene for mutations leading to ar gPRA in several breeds of dogs, including the briard-specific mutation [23,24].

METHODS

Blood of 821 dogs from 26 different breeds including 128 gPRA-affected animals Table 1 was received from the owners in cooperation with the breed clubs (Verband für das Deutsche Hundewesen [VDH]; Nederlandse Vereiniging van Saarlooswolfhonden; Schweizer Kynologischen Gesellschaft [SKG]). The blood of most of the dogs was sent from different regions of Germany. In addition, several Saarloos/Wolfhounds (Sa), Schapendoes (SD), Sloughis (Sl) and Tibetan Terriers (TT) originated from the Netherlands (Sa, SD), Switzerland (Sl, TT), Sweden (Sl) and USA (Sl). Dog breeds with known mutation localisation were included as controls. Breeders have assumed ar inheritance in the aforementioned breeds by observing the cases of gPRA in the pedigrees. Experienced veterinarians confirmed the gPRA status of affected and unaffected dogs by ophthalmoscopy. This was documented in certificates of the eye examinations. Genomic DNA was extracted from peripheral blood according to standard protocols [25].

Exon/intron boundaries were analysed by comparison with the mRNA sequence of the canine *RPE*65 gene ([24]; EMBL accession number AF084537) with the genomic sequences of chromosome 1 (EMBL accession number AL139413). Sequences of intron 1, 4-9, and 11-13 of the *RPE*65 gene were amplified (Table 2) by overlapping PCRs including neighboring exons in a thermocycler (Biometra, Goettingen, Germany). PCRs were performed under standard PCR conditions [11-13] with *Taq* Polymerase (Genecraft, Münster, Germany) and varying concentrations of $MgCl_2$ (Table 3). For SSCP analy-

sis, 0.06 µl of [α^{32}P] dCTP (10 mCi/ml) was included in the PCR.

Amplified fragments were extracted from 1% agarose gels using the Easy Pure extraction kit (Biozym, Hess. Oldendorf, Germany) and sequenced with intron-overlapping primers (Table 3). Sequencing reactions were carried out by the dideoxy-chain termination method using the Big Dye Terminator kit (BDT; Perkin-Elmer, Norwalk, CT) according to the manufacturer's instructions in order to identify the intronic sequences. All sequencing reactions were run on an automated DNA sequencer (Applied Biosystems 373 XL, Foster City, USA) and analysed using the ABI Prism™ 373XL Collection and Sequencing Analysis 3.0 software.

Primer pairs for mutation screening were designed after DNA sequence analysis of the intron-overlapping *RPE*65 fragments (Table 3). PCR products were digested dependent on the lengths of the fragments with different restriction enzymes (Table 3) to optimize the mutation screening by SSCP analysis [26]. SSCP samples were treated according to [7-9]. PCR products were separated through two sets of 6% polyacrylamide (acrylamide / bisacrylamide: 19/1) gels, one set containing 10% glycerol, another containing 5% glycerol and 1 M urea. Autoradiographs were examined for band alterations, which were confirmed after purification and cycle sequencing as described above.

TABLE 1. CHARACTERISTICS OF 26 DOG BREEDS

Breed (abbreviation)	Number of dogs	Diagnosis	Onset forms of gPRA	Age distribution (year)
Afghan Hound (AW)	1	gPRA-affected	mid-onset	5
	4	normal		5-6
Am. Cocker Spaniel (ACS)	1	gPRA-affected	late	10
	1	normal		12
Australian Cattle dog (AC)	2	gPRA-affected	late	10
	19	normal		2-4
Berger des Pyrénées	1	gPRA-affected	mid-onset	5
(Pyrenean Shepherd; BDP)	42	normal		1-10
Bichon Bolognese (Bo)	1	gPRA-affected	late	9
Curly Coated Retriever (CCR)	1	gPRA-affected	late	6
Engl. Cocker Spaniel (ECS)	6	gPRA-affected	late	3-11
	6	normal		6-14
English Springer Spaniel (SP)	1	gPRA-affected	late	7
Entlebucher Mountain Dog (EM)	17	gPRA-affected	late	5-13
	10	normal		1-7
Giant Schnauzer (GS)	1	gPRA affected	late	11
Golden Retriever (GR)	2	gPRA-affected	late	5-10
	8	normal		3-6
Irish Setter (IRS)	2	gPRA-affected	early/late	0.6-7
	1	normal		3-13
Labrador Retriever (LR)	5	gPRA-affected	late	8-12
	140	normal		3-13
Miniature Poodle (MP)	35	gPRA-affected	late	5-12
	10	normal		1-12
Newfoundland (NF)	1	gPRA-affected	mid-onset	3
Polish Lowland Sheepdog (PON)	1	gPRA-affected	late	9
Rottweiler (Ro)	1	gPRA-affected	late	3
Saarloos/Wolfhound (Sa)	7	gPRA-affected	late	7-9
	118	normal		2-10
Saluki (Persian Greyhound; Sal)	1	gPRA-affected	late	8
	2	normal		8
Scottish Collie (Co)	3	gPRA-affected	early	4-8
	1	normal		8
Scottish Terrier (ScT)	1	gPRA-affected	late	6
Schapendoes	3	gPRA-affected	early	2-6
(Dutch Sheepdog; SD)	7	normal		3-6
Sloughi (Arabian Greyhound; Sl)	5	gPRA-affected	mid-onset	2
	183	normal		0.1-12
Yorkshire Terrier (Y)	1	gPRA-affected	late	8
	2	normal		9-10
Tibetan Terrier (TT)	3	gPRA-affected	mid-onset	7-8
	93	normal		2-10
Wire-haired Dachshund (D)	20	gPRA-affected	variable	1-13
	49	normal		6-13

From the total number of 821 animals, all 123 gPRA-affected and some representative unaffected dogs from the pedigrees were investigated. Characteristics included age of onset forms and age distribution of normal and gPRA affected dogs during the time of DNA isolation of the *RPE*65 gene in 26 breeds. The ages of onset were classified according to [29] for AC; according to the owners' report/certificate of eye examination for Bo, CCR, D, GR, GS, IRS (late form) NF, PON, Ro, Sa, ScT, SD and Sl; according to reviews [1,30] and online information from gPRA Today for Co, ECS, IRS (early form) LR, MP and TT; and according to [31] for EM.

RESULTS & DISCUSSION

Intronic sequences of canine *RPE*65 gene have not yet been published. The alignment of mRNA sequences of the dog with

TABLE 2. EXON-/INTRON ORGANIZATION OF THE HUMAN AND CANINE *RPE*65 GENES

	Exon			Intron	
	#	Length (bp)	Exon-/Intron boundaries	#	Length (bp)
Dog	1	65	TGTCCATCCAgtgagtatgc	1	925
Human		69	TATCCAGTAAgtatctctgg		1184
Dog			accttctcagAGTGGAGCAT		
Human			accatttcagGGTTGAGCAT		
Dog	2	83	CACGTGACAG..........	2	?
Human		83	CATGTAACAGgttggtctcg		1763
Dog			..........GCAGGATCCC		
Human			ttcatcacagGCAGGATCCC		
Dog	3	151	ATCACAGAAG..........	3	?
Human		151	ACCACAGAAGgtaaagcagc		1826
Dog			..........GTTCATCCGC		
Human			tgtccttcagGTTCATCCCG		
Dog	4	108	TATTTTCCAGgttattgaaa	4	104
Human		108	TATTTTCCAGgttactgaac		103
Dog			gcttctgtagGTTTTTTTCT		
Human			gtttctacagGTTTTTTTCT		
Dog	5	142	AATTAAGCAGgtaggacgaa	5	~2800
Human		142	AATTAAGCAGgtgggacaca		3530
Dog			cctcccgcagGTTGATCTCT		
Human			acttccgtagGTTGATCTTT		
Dog	6	148	CTCCAAGCAGgtcagtttac	6	~2000
Human		148	CTGCAAGCAGgtgagtttac		1210
Dog			tttatttcagACAAGGAAGA		
Human			tttatttcagACAAGGAAGA		
Dog	7	82	ACGTCCATAGgtaacttgaa	7	227
Human		82	ACGTTCATAGgtaacttgaa		237
Dog			ctctaaacagTTTTGGTTTG		
Human			ttctgaacagTTTTGGTCTG		
Dog	8	133	AACCATGGGGgtaagtcggg	8	123
Human		133	ATGGGGGTAAgtcttagata		105
Dog			atttttcaagGTTTGGCTTC		
Human			atttttcaagGTTTGGCTTC		
Dog	9	140	GCTGGAAAGGgtaagagagg	9	~1700
Human		140	GCTGGAAAGGgtaagaaagg		625
Dog			atttttgcagATTTGAATTC		
Human			atttttgcagATTTGAGTTT		
Dog	10	130	TATCGACAAG..........	10	?
Human		130	TATTGACAAGgtaacctgct		6601
Dog			..........GCCGACACAG		
Human			tctctcctagGCTGACACAG		
Dog	11	115	CCTCGTCAAGgtgaggtgat	11	330
Human		115	CCTCGTCAAGgtgagatgat		94
Dog			tattttgtagCCTTTGAGTT		
Human			tatttcgtagCATTTGAGTT		
Dog	12	95	TCCGGACAGGgtaccgctcc	12	320
Human		95	TCCAGATAGGgtaattaatc		105
Dog			tcacaagcagCTCTGCAAGC		
Human			tcacaaacagCTCTGTAAGC		
Dog	13	112	GAAGATGATGgtaatgaaag	13	~1500
Human		112	GAAGATGATGgtaatgaaag		1137
Dog	14	152/734	tgattaacagGTGTAGTTCT		
Human		152/1023	tgataaacagGTGTAGTTCT		

Comparison of the exon and intron lengths and homologous sequences of the splice sites of man and dog. Canine introns 2, 3, and 10 could not be amplified. Exon sequences are depicted in upper case, intron sequences in lower case (canine EMBL accession numbers see above; human DNA sequences from chromosome 1: EMBL accessions number AL139413).

genomic sequences of chromosome 1 of the human retinal *RPE*65 gene shows 14 exons with a size between 65 and 149 bp and introns mostly under 2 kb in the human gene. The canine intron/exon organisation corresponds to that of the human gene including high homologies in the exon-intron borders (Table 2). But after sequencing of the overlapping PCR products, introns 6, 8, 9, 11, 12, and 13 turned out to be longer than the human counterparts. This is in contrast according to our previous experience with other gPRA candidate genes, e.g. *RCV*1 and *PDC* ([12,13]; Table 2).

Introns 2, 3, and 10 could not be identified, probably because of excessive size (or special sequence conformation). Nevertheless we detected many informative SNPs of the *RPE*65 gene that allowed indirect gene analysis in the breeds. Alignment of the canine sequences with the BLAST search program pointed to short repeat areas in introns 6 (position 4-66 EMBL accession number AJ506756) and 12 (position 580-704 EMBL accession number AJ251207) corresponding to a canine tRNA-derived short interspersed nucleotide element (SINE; [27]). In addition, two fragmented long interspersed nucleotide elements (LINE; position 139-224 and 312-359 EMBL accession number AJ251207; [28]) were localized in intron 11.

For PCR-SSCP analysis, primers were designed flanking the exons including whenever possible the conserved splice sites (Table 3). Thirteen of 14 exons of the canine *RPE*65 gene were investigated for disease-causing mutations in all gPRA affected and some unaffected dogs. The 4 bp deletion that leads to CSNB in Briards was not found in the gPRA affected dogs. Many novel SNPs were observed. The published SNPs (exons 5 (T459C) and 6; [23]) were identified in 20 and 8 different dog breeds, respectively (Table 4). Additionally, in the ORF single nucleotide substitutions in exons 9 (C900T) and 10 (A1026T), lack of amino acid exchanges were verified in 4 breeds (Table 4). Further SNPs were identified within different introns: deletions within introns 1, 8, 11, and the 3'UTR,

Table 3. Primers and conditions for PCR amplification

PCR-system	Location	Primer sequenceA (5'->3')	Fragment length (bp)	Annealing T/MgCl2/ formamide [(°C)/(mM)/%]	Restriction enzymes for SSCP-analyses
RPE65UTR 1-F	Exon 1	CGACCGTCTGTCCTGCCC	186	56/1.5/5	-
RPE65 I-1-R	Intron 1	GGGTATTTGCTTCAATCCATG			
RPE65UTR21F	Exon 1	CTGGGAGACAATGTCCATC	1124	51/1.5	-
RPE65 E2-2R	Exon 2	CGACGACAGCTCTTCCAC			
RPE65 E4 F	Exon 4	AGGTTCATCCGCACCGATG	357	54/1.5	AluI/HincII
RPE65 E5 R	Exon 5	ACCTGCTTAATTGTCTCCAG			
RPE65 I 4 F	Intron 4	CACAGCTTGAAGGTTACTGGAC	286	54/1.5	HincII
RPE65 I 5 R	Intron 5	TCTATTTGGCCCTCATGAGC			
RPE65 E5 F	Exon 5	AGGTTTTTTTCTTACTTCCGA	~2800	60/1.5	-
RPE65 E6 R	Exon 6	TGCTTGGAGTGGAGGGATC			
RPE65 E-6-F	Exon 6	AGGTTGATCTCTGCAACTAC	377	56/1.5	DdeI
RPE65 I 6 R	Intron 6	CTGTGGAATACGACTTGGC			
RPE65 E-6-F	Exon 6	AGGTTGATCTCTGCAACTAC	~2000	60/1.0	-
RPE65 E-7-R	Exon 7	CTATGGACGTACGATGGC			
RPE65 7EF	Exon 7	AGACAAGGAAGATCCAATAAGC	443	54/1.5	TruI
RPE65 8ER	Exon 8	CCCATGGTTTCATTGGACTC			
RPE65 8EF	Exon 8	TTTTGTGGAGACGCCAGTC	369	60/2.5	BsiHKAI
RPE65 9ER	Exon 9	CCTTTCCAGCAGCAGAGATC			
RPE65 E-9-F	Exon 9	AGGTTTGGCTTCACATCGCTG	~2000	60/1.0	-
RPE65 E-10-R	Exon 10	TTGTCGATATTCAGAGGAAGC			
RPE65 I-9 F	Intron 9	ACACCCCAGAGCAAGCAGG	229	59/1.5	EcoRI
RPE65 E-10-R	Exon 10	TTGTCGATATTCAGAGGAAGC			
RPE65 E11-F	Exon 11	GCCGACACAGGCAAGAAC	542	55/1.5	DdeI
RPE65 E12-R	Exon 12	ACCCTGTCCGGAACGAAG			
RPE65 E-12-F	Exon 12	CCTCAAATCAACTATCAGAAGTC	580	58/1.0	-
RPE65 I-13-R	Intron 13	AACACACTAACATAGAGAACTC			
RPE65 I-12 F	Intron 12	AAGAGAAAAGTAGTTTGAGTCAC	261	57/1.5	HphI
RPE65 I-13-R	Intron 13	AACACACTAACATAGAGAACTC			
RPE65 E13-F	Exon 13	CTCTGCAAGCTGAACGTC	~1800	55/1.0	-
RPE65 E/I14R	Exon 14	CCATGTAATACACAGCAGGCTAA			
RPE65 E 14-F	Exon 14	AGGTGTAGTTCTGAGTGTG	249	55/1.0	RsaI
RPE65 E 14-R	Exon 14	CCGTATACAGCAGGCTAAA			
RPE65 UTR14F	Exon 14	CAAAGTCAAGAAAAAGTGAGGT	705	53/2.0	MseI
RPE65 UTR14R	Exon 14	GCTTTGATGTTATGTAAGCTTTT			
RPE65 UTR14F	Exon 14	CAAAGTCAAGAAAAAGTGAGGT	277	55/1.5	MnlI
RPE65 UTR214R	Exon 14	CACTTTCATAATAGGAACAAGAA			

PCR protocols used to characterize the canine *RPE*65 gene including mutation screening. For *RPE*65 sequences, see EMBL accession entries AJ506753, AJ506754, AJ506755, AJ506756, AJ506757, AJ506758, AJ506759, and AJ251207.

insertions in introns 6 and 8 as well as nucleotide substitutions within introns 6, 7, 8, 11, and 13 (Table 4). After sequencing of intron 11, breed-specific haplotypes (CAA or ATG) could be defined. The SNPs were present in heterozygous state in affected dogs in 25 of the 26 investigated breeds and are therefore informative markers for the exclusion of the *RPE*65 gene in these breeds with assumed ar transmitted gPRA. The Rottweiler investigated shows SNPs exclusively in homozygous state in introns 7 and 8. This fact does not allow to exclude the *RPE*65 gene as a cause for gPRA in this breed.

The founder effect in connection with inbreeding (i.e., effective genetic separation) leads to breed-specific gPRA mutations. Therefore, identical mutations are not likely to have happened independently in different breeds. Heteroallelism is an unlikely cause of ar gPRA within a single breed, as one would expect such disease to result from a single founder mutation. Therefore, we excluded the *RPE*65 gene as a cause for gPRA in Afghan Hounds, American Cocker Spaniels, Australian Cattle dogs, Berger des Pyrénées, Bolognese, Curly Coated Retrievers, English Cocker Spaniels, English Springers, Entlebucher Mountain Dogs, Giant Schnauzers, Golden Retrievers, Irish Setters, Labrador Retrievers, Miniature poodles, Newfoundlanddogs, Polish Lowland Sheepdogs, Saarloos, Salukis, Scottish Collies, Scottish Terriers, Schapendoes, Sloughis, Yorkshire Terrier, Tibetan Terriers and Wire-haired Dachshunds.

ACKNOWLEDGEMENTS

We thank the owners of the dogs for blood samples, the veterinarians of the Dortmunder Ophthalmologenkreis (DOK) for the ophthalmologic investigations of the dogs and for the support of different breeding clubs. These studies were supported in part by the Gesellschaft für kynologische Forschung, Bonn (Germany).

REFERENCES

1. Clements PJ, Sargan DR, Gould DJ, Petersen-Jones SM. Recent advances in understanding the spectrum of canine generalised progressive retinal atrophy. J Small Anim Pract 1996; 37:155-62.
2. Petersen-Jones SM. A review of research to elucidate the causes of the generalized progressive retinal atrophies. Vet J 1998; 155:5-18.
3. Suber ML, Pittler SJ, Qin N, Wright GC, Holcombe V, Lee RH, Craft CM, Lolley RN, Baehr W, Hurwitz RL. Irish setter dogs affected with rod/cone dysplasia contain a nonsense mutation in the rod cGMP phosphodiesterase beta-subunit gene. Proc Natl Acad Sci U S A 1993; 90:3968-72.
4. Petersen-Jones SM, Entz DD, Sargan DR. cGMP phosphodiesterase-alpha mutation causes progressive retinal atrophy in the Cardigan Welsh corgi dog. Invest Ophthalmol Vis Sci 1999; 40:1637-44.
5. Aguirre G. Genes and diseases in man and models. Prog Brain Res 2001; 131:663-78.

TABLE 4. SEQUENCE VARIATIONS IN THE *RPE*65 GENE IN DIFFERENT DOG BREEDS

Gene	Localization	Sequence variation	Amino acid exchange	Breed
RPE65	Intron 1	IVS1 +76DelG	-	AC, AW, CCR, ECS, GR, LR, NF, Sal, MP
RPE65	Exon 5	T459C	Y144Y	AC, AW, ECS, Co, EM, GR, LR, GS, BDP, Sa, Sal, ScT, Sl, D, TT, Y, MP
RPE65	Exon 6	G541A	V172I	ACS, IRS, D, ECS, LR, TT, Sa, GR
RPE65	Intron 6	IVS6+23T->C	-	AW, SP, GS
		IVS6+31C->G	-	GS, Sl
		IVS6+71-72Ins.GG	-	MP
		IVS6+116G->C	-	MP
		IVS6+164G->A	-	SP, Sl
RPE65	Intron 7	IVS7-69T->A	-	ECS, EM, Ro, GS, Sa, Sl, D, TT, MP, BDP
		IVS7-18C->A	-	ECS, EM, Ro, GS, Sa, Sl, D, TT, MP, BDP
RPE65	Intron 8	IVS8+24T->C	-	AC, Bo, EM, GR, IRS, SD, D, PON, Sa, MP, BDP
		IVS8+109TIns/Del	-	AW, Bo, ECS, EM, IRS, GR, PON, Ro, GS, BDP, Sa, SD, Sp, D, MP
RPE65	Exon 9	C900T	I291I	ECS, Sa, D, BDP
RPE65	Exon 10	A1026T	G333G	SD, GS, TT, MP
RPE65	Intron 11C	IVS11+110C->A	-	AC, Co, ECS, EM, LR, Sa, ScT, Sl,
		IVS11+138A->T		D, TT, Y, MP
		IVS11+143A->G		
		IVS11+203G->A	-	ECS, EM, IRS, NF, ScT, SD, Sa, D, TT, MP, BDP
		IVS11-61Del.T	-	AW, D, TT, MP
RPE65	Intron 13	IVS13+36 C->T	-	AW, EM, IRS, ScT, Sp, D, NF, GS, Sa, TT, MP, BDP
RPE65	Exon 14	c1856 Del.A	-	AC, ECS, EM, GR, LR, Sa, Sl, D, TT, Y, MP

gPRA-affected breeds (abbreviations see Table 1) with sequence variation patterns in heterozygous state are shown in red type. Haplotypes in intron 11 were either CAA or ATG in the respective dog breeds. Positions of the sequence variations in the cDNA: EMBL accession number AF084537.

6. Kijas JW, Cideciyan AV, Aleman TS, Pianta MJ, Pearce-Kelling SE, Miller BJ, Jacobson SG, Aguirre GD, Acland GM. Naturally occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa. Proc Natl Acad Sci U S A 2002; 99:6328-33.
7. Dekomien G, Runte M, Godde R, Epplen JT. Generalized progressive retinal atrophy of Sloughi dogs is due to an 8-bp insertion in exon 21 of the PDE6B gene. Cytogenet Cell Genet 2000; 90:261-7.
8. Dekomien G, Klein W, Epplen JT. Polymorphisms in the canine rod transducin gene and exclusion as cause for generalised progressive retinal atrophy (gPRA). J Exp Anim Sci 1998; 39:86-90.
9. Dekomien G, Epplen JT. Exclusion of the PDE6A gene for generalised progressive retinal atrophy in 11 breeds of dog. Anim Genet 2000; 31:135-39.
10. Runte M, Dekomien G, Epplen JT. Evaluation of RDS/Peripherin and ROM1 as candidate genes in generalised progressive retinal atrophy and exclusion of digenic inheritance. Anim Genet 2000; 31:223-37.
11. Dekomien G, Epplen JT. Screening of the arrestin gene in dogs afflicted with generalized progressive retinal atrophy. BMC Genetics 2002; 3:12.
12. Dekomien G, Epplen JT. The canine Recoverin (RCV1) gene: a candidate gene for generalized progressive retinal atrophy. Mol Vis 2002; 8:436-41 .
13. Dekomien G, Epplen JT. The canine Phosducin gene: characterization of the exon-intron structure and exclusion as a candidate gene for generalized retinal atrophy in 11 dog breeds. Mol Vis 2002; 8:138-42 .
14. Dekomien G, Epplen JT. Analysis of PDE6D and PDE6G genes for generalised progressive retinal atrophy (gPRA) mutations in dogs. Genet Sel Evol 2003; 35:445-56.
15. Redmond TM, Yu S, Lee E, Bok D, Hamasaki D, Chen N, Goletz P, Ma JX, Crouch RK, Pfeifer K. Rpe65 is necessary for production of 11-cis-vitamin A in the retinal visual cycle. Nat Genet 1998; 20:344-51.
16. Saari JC. Retinoids in photosensitive systems. In: Sporn MB, Roberts AB, Goodman DS, editors. The retinoids: biology chemistry and medicine. 2nd ed. New York: Raven; 1994. p. 351-85.
17. Marlhens F, Bareil C, Griffoin JM, Zrenner E, Amalric P, Eliaou C, Liu SY, Harris E, Redmond TM, Arnaud B, Claustres M, Hamel CP. Mutations in RPE65 cause Leber's congenital amaurosis. Nat Genet 1997; 17:139-41.
18. Gu SM, Thompson DA, Srikumari CR, Lorenz B, Finckh U, Nicoletti A, Murthy KR, Rathmann M, Kumaramanickavel G, Denton MJ, Gal A. Mutations in RPE65 cause autosomal recessive childhood-onset severe retinal dystrophy. Nat Genet 1997; 17:194-7.
19. Morimura H, Fishman GA, Grover SA, Fulton AB, Berson EL, Dryja TP. Mutations in the RPE65 gene in patients with autosomal recessive retinitis pigmentosa or Leber congenital amaurosis. Proc Natl Acad Sci U S A 1998; 95:3088-93.
20. Thompson DA, Gyurus P, Fleischer LL, Bingham EL, McHenry CL, Apfelstedt-Sylla E, Zrenner E, Lorenz B, Richards JE, Jacobson SG, Sieving PA, Gal A. Genetics and phenotypes of RPE65 mutations in inherited retinal degeneration. Invest Ophthalmol Vis Sci 2000; 41:4293-9.
21. Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet 2001; 28:92-5.
22. Narfstrom K, Katz ML, Ford M, Redmond TM, Rakoczy E, Bragadottir R. In vivo gene therapy in young and adult RPE65-/- dogs produces long-term visual improvement. J Hered 2003; 94:31-7.
23. Veske A, Nilsson SE, Narfstrom K, Gal A. Retinal dystrophy of Swedish briard/briard-beagle dogs is due to a 4-bp deletion in RPE65. Genomics 1999; 57:57-61.
24. Aguirre GD, Baldwin V, Pearce-Kelling S, Narfstrom K, Ray K, Acland GM. Congenital stationary night blindness in the dog: common mutation in the RPE65 gene indicates founder effect. Mol Vis 1998; 4:23 .
25. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16:1215.
26. Jaeckel S, Epplen JT, Kauth M, Miterski B, Tschentscher F, Epplen C. Polymerase chain reaction-single strand conformation polymorphism or how to detect reliably and efficiently each sequence variation in many samples and many genes. Electrophoresis 1998; 19:3055-61.
27. Bentolila S, Bach JM, Kessler JL, Bordelais I, Cruaud C, Weissenbach J, Panthier JJ. Analysis of major repetitive DNA sequences in the dog (Canis familiaris) genome. Mamm Genome 1999; 10:699-705.
28. Choi Y, Ishiguro N, Shinagawa M, Kim CJ, Okamoto Y, Minami S, Ogihara K. Molecular structure of canine LINE-1 elements in canine transmissible venereal tumor. Anim Genet 1999; 30:51-3.
29. Laratta LJ, Sims MH, Brooks DE. Progressive retinal degeneration in the Australian cattle dog. Proceedings of the American College of Veterinary Ophthalmology 1988; 19:9.
30. Gropp KE, Huang JC, Aguirre GD. Differential expression of photoreceptor-specific proteins during disease and degeneration in the progressive rod-cone degeneration (prcd) retina. Exp Eye Res 1997; 64:875-86.
31. Spiess BM. [Inherited ocular diseases in the Entlebucher mountain dog]. Schweiz Arch Tierheilkd 1994; 136:105-10.

The print version of this article was created on 11 Nov 2003. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.