

MOLECULAR GENETIC MARKERS

A polymorphic (TG)_n microsatellite in an intron of the canine tyrosine transaminase gene

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Accepted 3 November 1997

Source/description: PCR primers 1 and 2 were designed from the published human tyrosine transaminase sequence¹ and used to amplify canine genomic DNA templates. Direct sequencing of the resulting 1.0 kb amplicons revealed 919–929 bp of canine sequence (GenBank accession no. L47165). The coding regions of the canine sequence (88 bp at the 5' end and the 3 bp at the 3' end) had 97% nucleotide sequence identity to corresponding sequences in exons H and I of the human tyrosine transaminase gene¹. The canine coding sequences were separated by an intron of 828–838 bp which contained a polymorphic (TG)_n microsatellite. The canine nucleotide sequence was used to devise PCR primers 3 and 4 which annealed to sites closely flanking the microsatellite.

Primer sequences:

- Primer 1: 5'-AGCACCGATGTCCTCCATCCTGTCTCTG-3'
- Primer 2: 5'-TGA CT CAGCTT CACCAGCCCATCTCG-3'
- Primer 3: 5'-GGCAATGAGGTAAGAGCAATGTGGTG-3'
- Primer 4: 5'-ACAAAAACACCTCCTAACCACTGTG-3'

Genotyping procedure: Prior to amplification, primer 4 was ³²P-labelled with T4 polynucleotide kinase. The 10 µl PCR amplification mixtures contained 10 ng genomic DNA, 0.5 U Taq polymerase, primers 3 and 4 (each 12.5 nM), MgCl₂ (1.5 mM) and dNTPs (each 200 µM). The first cycle was 94 °C for 3 min, 59 °C for 30 s and 72 °C for 30 s followed by 24 cycles of 94 °C (20 s), 59 °C (20 s) and 72 °C (30 s). PCR products were fractionated by electrophoresis into 6% denaturing polyacrylamide gels and bands were visualised by routine autoradiography.

Polymorphism: We surveyed 56 unrelated dogs representing 39 different breeds. Six distinct alleles were detected (Fig. 1, left). Allele frequencies are reported in Table 1. The calculated PIC² was 0.63 and 50% of the surveyed dogs were heterozygous.

Mendelian inheritance: Codominant Mendelian inheritance of alleles was observed in an eight-member of the Golden Retriever family (Fig. 1, right).

Chromosomal location: The location of the canine tyrosine transaminase gene is unknown; the orthologous human gene is on chromosome 16q22.1–22.3³.

Table 1. Allele frequencies

Allele (bp)	Repeat number	Frequency
88	16	0.48
90	17	0.05
92	18	0.08
94	19	0.09
96	20	0.28
98	21	0.02

Acknowledgements: This work was supported by NIH grant 1 R15 H601063 and by a grant from the AKC Canine Health Foundation.

References

- 1 Rettenmeier R. *et al.* (1990) *Nucleic Acids Res* **18**, 3853–61.
- 2 Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- 3 Natt E. *et al.* (1987) *Hum Genet* **77**, 352–8.

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A length polymorphism in an intron of the canine polycystic kidney disease 1 gene

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Accepted 3 November 1997

Source/description: PCR primers 1 and 2 were designed from the published sequences of exons 41 and 42 of the human polycystic kidney disease 1 gene¹, and used to produce a 0.4 kb amplicon from canine genomic DNA. Direct sequencing² revealed 327 bp of canine sequence (GenBank accession no. AF027359). The 58 bp at the 5' end and the 49 bp at the 3' end of canine sequence were 91% identical to corresponding human exon sequences. A 218–220 bp canine intron which separated the exons contained a stretch of six to eight consecutive cytosines starting 19 bp from the 5' exon/intron junction. The canine intron sequence was used to design primers 3 and 4 which closely flank the polymorphic site.

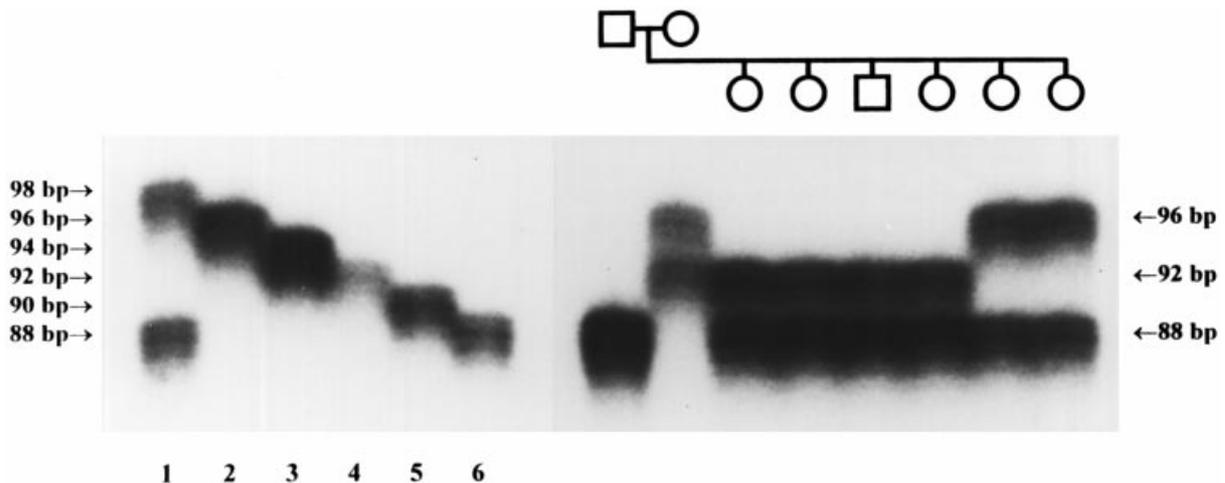


Fig. 1. The left side of the autoradiogram contains examples of the six canine tyrosine transaminase gene alleles. The sample in lane 1 is from a heterozygous dog with TG-repeat numbers of 16 and 21. Marker genotypes of related Golden Retrievers are shown on the right.

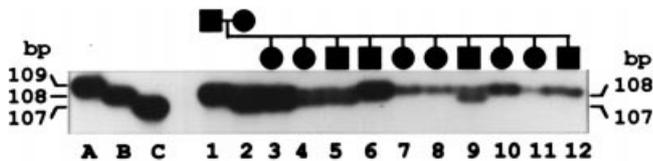


Fig. 1. Autoradiogram containing bands representing three canine polycystic kidney disease 1 gene alleles (left) and genotypes from a family of Pointers (right).

Table 1. Relative frequencies of canine polycystic kidney disease 1 gene alleles

Allele no.	Length (bp)	Repeat no.	Frequency
A	109	8	0.09
B	108	7	0.59
C	107	6	0.32

Primer sequences:

Primer 1: 5'-CTACGTGCAGGAGCTGGGCTGAGCC-3'
 Primer 2: 5'-AGGCGCAGCGTGACGGCGGCGTGAG-3'
 Primer 3: 5'-TGCAGCTGCATAACTGGATCGACAAC-3'
 Primer 4: 5'-CTCGTGGCAGGAGGAGGCTGCTGTG-3'

PCR conditions: Primer 3 was 32P-labelled with T4 polynucleotide kinase. The 10 µl PCR amplification mixture contained 10 ng canine genomic DNA, 0.5 U Taq polymerase, primers 3 and 4 (each 12.5 nM), MgCl₂ (1.5 mM) and dNTPs (200 µM). The first cycle was 94 °C for 2 min, 62 °C for 30 s and 72 °C for 30 s, followed by 27 cycles of 94 °C for 20 s, 62 °C for 20 s and 72 °C for 30 s. PCR products were fractionated by electrophoresis in a 6.0% denaturing polyacrylamide gel. Bands were visualised by routine autoradiography.

Polymorphism: Three distinct alleles with lengths that ranged from 109 to 107 bp were observed (Fig. 1, left) in a survey of 50 unrelated dogs representing 31 breeds (Table 1). The PIC₃ was 0.46 and 50% of the dogs surveyed were heterozygous.

Chromosomal location: The location of the canine polycystic kidney disease 1 gene is unknown; the orthologous human gene is on chromosome 16p13-34.

Mendelian inheritance: Codominant Mendelian inheritance of alleles was observed in a 10-member Pointer family (Fig. 1, right).

Acknowledgements: This work was supported by NIH grant R15 01063, and Grants from the AKC Canine Health Foundation and Chinese Shar Pei Club of America Charitable Trust.

References

- Hughes J. *et al.* (1995) *Nature Genet* **10**, 151-60.
- Shibuya H. *et al.* (1993) *Anim Genet* **24**, 345-8.
- Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314-31.
- Somlo S. *et al.* (1992) *Genomics* **13**, 152-8.

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KRN1 maps to bovine chromosome 29

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Accepted 20 November 1997

Source/description: KRN1 encodes ultra high-sulphur keratin protein, one of the three proteins found in hair keratin¹. PCR primers were used to amplify a 395 bp region the bovine homologue of the sheep and human genes coding this protein. The primers were designed from an ovine sequence with MAC VECTOR4-1.4 sequence analysis software (International Biotechnologies, Inc., New Haven,

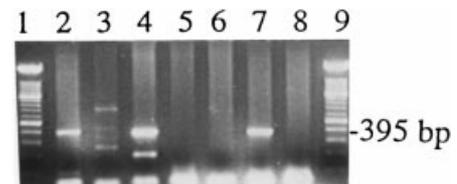


Fig. 1. Lane 1 and 9, 100 bp DNA ladder (Boehringer Mannheim). Lane 2, PCR product in bovine. Lane 3, PCR product in mouse. Lanes 4 and 5, PCR products in mouse/bovine somatic cell lines. Lane 6, PCR product in hamster. Lanes 7 and 8, PCR products in hamster/bovine somatic cell lines.

Table 1. Information on the marker

Locus	GenBank accession no.	Origin	Reference
KRN1	X55294	Sheep mRNA	McKinnon <i>et al.</i> (1990) ⁴

CT). Forward and reverse primers were in adjacent exons so that the amplified fragment contains an intron.

PCR primers:

F: 5'-GGT CCC TCA TGT TTG GAG TCT TAT C-3'
 R: 5'-GAC CAG AAA TAC ACA TCT CCC TGG-3'

PCR conditions: Amplification was carried out in a 10 µl reaction, 10 mM Tris-HCl 1.5 mM MgCl₂, 50 mM KCl, pH 8.3, 100 mM of each dNTP, 100 ng genomic DNA and 0.5 U Taq polymerase (Ampli Taq Gold; Perkin Elmer). The PCR cycled for 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, for 40 cycles, preceded by 95 °C for 10 min.

Chromosome assignment: This gene was mapped by synteny analysis using a panel of 31 hamster/bovine and mouse/bovine somatic hybrid cell lines previously described². KRN1 was 97% concordant with OCAM, a previously mapped marker of bovine chromosome 29³ (Fig. 1; Table 1).

Acknowledgements: We are grateful to CAPES (Brazil) grant number 2679/95-5 which has supported M. R. V. Amarante as USDA NRI Grant 95-37205-2190 and TAES Project 6718 to J. E. Womack.

References

- Fraser R.D.B. *et al.* (1973) *Comp Biochem Physiol* **44B**, 943-7.
- Womack J.E., Moll, Y.D. (1986) *J Hered* **77**, 2-7.
- Dietz A.B. (1992) PhD thesis, Texas A&M University, TX.
- McKinnon P.J. *et al.* (1990) *J Cell Biol* **111**, 2587-600.

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Dinucleotide repeat polymorphisms in waterfowl (family Anatidae): characterization of a sex-linked (Z-specific) and 14 autosomal loci

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Accepted 27 November 1997

Source/description: Canada goose (*Branta canadensis*) and harlequin duck (*Histrionicus histrionicus*) DNAs were digested with Sau3AI, and size selected (300-700 bp) fragments were ligated into BamHI-digested pBluescriptII KS⁺. The enrichment protocol of Ostrander *et al.*¹ was followed. The resulting libraries were screened using a [³²P]ATP end-labelled (CA)₂₀ oligonucleotide as a hybridization probe. Positive clones were sequenced using cycle-sequencing protocols (Epicentre Technologies, Madison, WI) and

Table 1. Characterization of Canada goose (*Branta canadensis*) and harlequin duck (*Histrionicus histrionicus*) microsatellite loci

Locus	Primer sequences	Repeat motif	Annealing temperature (°C) ^a	Product size (bp) ^b	No. of alleles	H _{obs} ^{c,d}	PIC ^d	GenBank accession no.	
Bcaμ1	fwd	TgCTTTTTACCCCAgTgTTCT	(TA) ₁₅ (CA) ₁₀	56	138	6	0.6	0.79	AF025889
	rev	AgAATCTgCTATATTTCCAgCTC							
Bcaμ2	fwd	CAACCAGTAGTAATgCTgAAgATg	(CA) ₁₁	51	130	1	0.0	0.00	AF025890
	rev	TgTgTgCTTgTATgTATATATCATA							
Bcaμ3	fwd	ATACCACACAgCCACCCACAT	(CA) ₁₄	56	167	9	0.9	0.85	AF025891
	rev	CCTTCCTgTCCTTCCAAATCTT							
Bcaμ4	fwd	ACACAACCTCAAAGTCAATCCAAT	(CA) ₁₂	60	173	1	0.0	0.00	AF025892
	rev	TCCTgACgCTCTCgAgT							
Bcaμ5	fwd	AgTgTTTCTTTTCATCTCCACAAGC	(CA) ₉	60	199	1	0.0	0.00	AF025893
	rev	AgACCACAATCgACCACATATTC							
Bcaμ6	fwd	TTTAACCCAgTAGCCTATCATgTCA	(CA) ₁₀	60	151	3	0.7	0.51	AF025894
	rev	gTCTgAAgATAATgCTgCATgTgTT							
Bcaμ7	fwd	TAgTTTCTATTTgCACCCAATgAg	(CA) ₆ 5nt	60	171	4	0.4	0.63	AF025895
	rev	CggTCCTgTCCTTgCTgTAA							
Bcaμ8	fwd	CCCAGACTCACAAACAgAAAT	(CA) ₈	52	159	1	0.0	0.00	AF025896
	rev	ATgAAAgAAgAgTTAAACgTgTgCAA							
Bcaμ9	fwd	CCCAGTTCTCTCATTCTCCTT	(CA) ₉	56	109	6	0.7	0.72	AF025897
	rev	AAACAgggAggTgAAAgTgCTT							
Bcaμ10	fwd	ATgTAGCCATgAAAATTAAAAATg	(CA) ₉	55	110	1	0.0	0.00	AF025898
	rev	CCAgTATTAgCCgAAAATgA							
Bcaμ11	fwd	TAgAAAAGgCTgAAgAgTggC	(CA) ₈	55	134	4	0.7	0.54	AF025899
	rev	TgAggAAgCAACTgTAAATAggAgA							
Hhiμ1	fwd	ggATCAAAGgCACAAATgTgAAAT	(CA) ₁₃	51	134	1	0.0	0.00	AF025900
	rev	gCCACATgCTTTCCCATC							
Hhiμ2	fwd	gCTTgAgAAATACTAgAAACATCT	(CA) ₁₁	58	96	4	0.6	0.68	AF025901
	rev	gggATCTTgAggTCTCTCCAA							
Hhiμ3	fwd	CAggTTgAgTgTgTTCATg	(CA) ₇ 8nt	56	110	1	0.0	0.00	AF025902
	rev	CgTAgTAAACCAAgCTgCTAAACAT							
Hhiμ5	fwd	CTCTCCTTTACTACAAATCCCTT	(CA) ₁₃	55	150	3	0.5	0.54	AF025903
	rev	ATAAAgTAggTgACCCAATCCT							

Values are based on sample sizes of 10 unrelated individuals per species.

^aAnnealing temperatures are for the species from which the loci were cloned (Bca, *Branta canadensis*; Hhi, *Histrionicus histrionicus*).

^bPCR fragment sizes determined from cloned alleles.

^cObserved heterozygosity.

^dValues for species from which loci were cloned (non-parenthetical values). Parenthetical values were derived from the species from the other library (i.e. *H. histrionicus* for the Bca loci and *B. canadensis* for the Hhi loci).

^eProducts were amplified in other species (see Table 2).

Table 2. Amplification and partial characterization of microsatellite loci in waterfowl species of varying degrees of taxonomic relatedness

Locus	Species ^a	Anserini tribe			Anatini tribe		Aythiini tribe	Mergini tribe
		TUSW	WFGO	BLBR	NOPI	GRSC	SPEI	
Bcaμ1	Alleles (n)	1	2	3	NP ^b	4	2	
	H _{obs}	0.0	0.1	0.1		0.3	0.1	
Bcaμ2	Alleles (n)	NP	2	NP	NP	NP	NP	
	H _{obs}		0.0					
Bcaμ3	Alleles (n)	NP	1	NP	1	1	1	
	H _{obs}		0.0		0.0	0.0	0.0	
Bcaμ5	Alleles (n)	5	2	1	1	4	1	
	H _{obs}	0.0	0.3	0.0	0.0	0.0	0.0	
Bcaμ6	Alleles (n)	1	2	1	2	4	5	
	H _{obs}	0.0	0.2	0.0	0.1	0.4	0.5	
Bcaμ7	Alleles (n)	3	3	3	NP	NP	NP	
	H _{obs}	0.2	0.7	0.2				
Bcaμ8	Alleles (n)	3	4	2	NP	NP	NP	
	H _{obs}	0.5	0.7	0.1				
Bcaμ9	Alleles (n)	2	3	NP	1	NP	1	
	H _{obs}	0.5	0.8		0.0		0.0	
Bcaμ10	Alleles (n)	1	2	1	2	6	1	
	H _{obs}	0.0	0.1	0.0	0.2	0.6	0.0	
Bcaμ11	Alleles (n)	1	3	2	2	3	1	
	H _{obs}	0.0	0.5	0.1	0.6	0.3	0.0	
Hhiμ1	Alleles (n)	1	8	NP	1	2	1	
	H _{obs}	0.0	0.7		0.0	0.0	0.0	
Hhiμ2	Alleles (n)	NP	NP	NP	NP	NP	NP	
	H _{obs}							

Table 2. Continued

Locus	Species ^a	Anserini tribe			Anatini tribe		Aythini tribe	Mergini tribe
		TUSW	WFGO	BLBR	NOPI	GRSC	SPEI	
Hhi μ 3	Alleles (<i>n</i>)	2	5	2	NP	1	NP	
	H _{obs}	0·6	0·4	0·4		0·0		
Hhi μ 5	Alleles (<i>n</i>)	1	1	1	3	3	NP	
	H _{obs}	0·0	0·0	0·0	0·5	0·1		

Values are based on sample sizes of 10 unrelated individuals.

^aTUSW, *Cygnus columbianus*; WFGO, *Anser albifrons*; BLBR, *Branta bernicla*; NOPI, *Anas acuta*; GRSC, *Aythya marila*; SPEI, *Somateria fischeri*.

^bNP, no product was detected using the annealing temperatures given in Table 1 or 5 °C lower than that value.

primers flanking the inserts. PCR primers were designed to amplify the repeat and yield amplification products of \approx 100–200 bp. DNA samples were screened for variation at these loci using [γ -³²P]ATP end-labelled primers. The products were resolved using 6% denaturing polyacrylamide gels and autoradiography.

Mendelian inheritance: Segregation of markers was confirmed by analysis of full-sib families. Data for all loci, except Bca μ 4, are consistent with codominant inheritance of alleles. Analyses of individuals of known sex indicate that Bca μ 4 is Z chromosome-linked. Female *Cygnus columbianus* (*n* = 6), *B. canadensis* (*n* = 49), *Branta bernicla* (*n* = 5), *Somateria fischeri* (*n* = 33) and *H. histrionicus* (*n* = 10) had four, four, three, four and eight alleles, respectively, with observed heterozygosity (H_{obs}) = 0. In contrast, male *C. columbianus* (*n* = 4), *B. canadensis* (*n* = 16), *B. bernicla* (*n* = 2), *S. fischeri* (*n* = 12) and *H. histrionicus* (*n* = 10) had four, six, three, three, and 10 alleles, respectively, with H_{obs} values of 1·0, 0·4, 0·5, 0·2 and 0·9, respectively.

Polymorphism: For each locus, the number of alleles, H_{obs} and polymorphic information content (PIC)² values for the species from which the markers were cloned (Bca, *B. canadensis*; Hhi, *H. histrionicus*) are given in Table 1. The number of alleles and H_{obs} for other waterfowl of varying taxonomic affinity are given in Table 2.

PCR conditions: PCR reactions were carried out in a total volume of 25 μ l with final concentrations of 10 mM Tris (pH 8·5), 1·5 mM MgCl₂, 50 mM KCl, 10 μ g/ml BSA, 0·0025% Tween 20 (Bio-Rad Laboratories, Hercules, CA), 200 μ M each dNTP, 0·36 μ M unlabelled and 0·04 μ M ³²P-labelled forward primer, 0·4 μ M reverse primer, 0·25 units *Taq* polymerase (Perkin Elmer, Foster City, CA) and \approx 100 ng genomic DNA. The thermocycler regime was: one cycle of 94 °C, 2 min followed by 30 cycles of 94 °C, 1 min; annealing temperature, 1 min; 72 °C, 1 min and one cycle of annealing temperature, 1 min and elongation at 72 °C for 5 min.

Acknowledgements: We thank A. Fowler, P. Svete, C. Ely, and M. Brown for providing samples of various waterfowl tissue and full-sib families and R. Lanctot for laboratory assistance. This research was supported by the Biological Resources Division of the US Geological Survey and by a grant from the Exxon Valdez Oil Spill Trustee Council.

References

- Ostrander E. *et al.* (1992) *Proc Natl Acad Sci (USA)* **89**, 3419–23.
- Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.

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A PCR-RSP *Csp6I* site in the canine ornithine aminotransferase (*OAT*) gene

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Accepted 27 November 1997

Source/description: Universal mammalian sequence-tagged site (UM-STS) primers for the ornithine aminotransferase (*OAT*) gene

have been previously described¹. Primers are designed to conserved regions contained within human exons 7 and 8^{2,3}. GenBank contains a portion of the gene sequence derived from the dog using one of these primers (GenBank accession no. AF034781). The amplified product is 860 bp in length. This band is cut into fragments that are 620 bp and 240 bp in length for the restriction site polymorphism (RSP) allele in which the *Csp6I* site is present.

Primer sequences: Primer sequences have been previously described¹. They are:

HOATEX7D: CGTGCTCTTCAGGATCCAAA
HOATEX8U: GCCAGCCATCTACCAGTTCT

PCR/RSP conditions: Amplifications were performed in 25 μ l volumes containing: 10 mM Tris–HCl (pH 8·3 at 20 °C), 50 mM KCl, 1·5 mM MgCl₂, 0·2 mM dNTPs, 0·1 μ g of each primer, and 1 U *Taq* DNA polymerase. Target DNA was derived from blood or buccal cells containing 50–200 ng DNA. Amplification was accomplished with 50 cycles of the following steps; 94 °C, 0·5 min; 57 °C, 1·5 min; and 72 °C, 1·5 min. Amplified products were digested with 10 U *Csp6I* for 60 min at 37 °C in the original PCR buffer to which 3 μ l of 50 mM MgCl₂ had been added. Eight microlitres of digested products were run on a 1·5% agarose gel.

Allele frequency: The frequency of the allele containing the *Csp6I* site was determined using 10 unrelated animals for four breeds; Doberman pinscher, 0·75; Labrador retriever, 0·90; Beagle, 0·50; and Pointer, 0·95. In addition, three unrelated animals were also tested in each of six additional breeds (Cairn terrier, Golden retriever, Scottish terrier, Alaskan malamute, Shetland sheepdog, and Rhodesian ridgeback); the frequency for this group of dogs was found to be 0·56. The overall frequencies for this set of data were found to be 0·62 with the site, and 0·38 without the site (Fig. 1).

Chromosomal location: The chromosomal location of the *OAT* gene within the canine genome is unknown. Its location within the human genome is 10q26.

Mendelian inheritance: Codominant Mendelian inheritance was observed in a six-member Cairn terrier pedigree and five-member Scottish terrier pedigree.

Acknowledgements: This work was supported by the Glassen Foundation and the Doberman Pinscher Foundation of America.

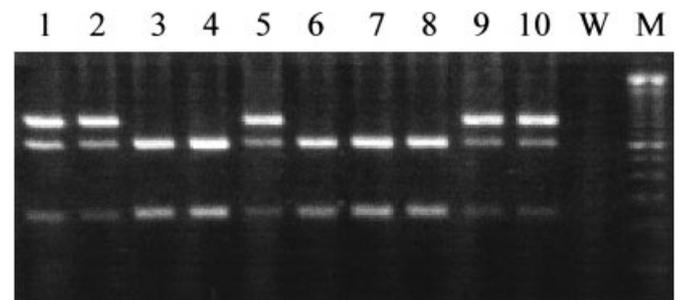


Fig. 1. Representative gel showing the results for 10 unrelated Doberman pinschers. W shows a water-blank control and M is a 100 bp ladder.

References

- 1 Venta P.J. *et al.* (1996) *Biochem Genet* **34**, 321–41.
 2 Muekler M.M. & Pitot H.C. (1985) *J Biol Chem* **260**, 12993–97.
 3 Mitchell G.A. *et al.* (1988) *J Biol Chem* **263**, 14288–95.

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Assignment of the canine microsatellite ZuBeCa3 to canine chromosome 9q21–q22

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Accepted 27 November 1997

Source/description: Positive clones were isolated from a genomic canine cosmid¹ library after screening with a mixture of the end-labelled oligonucleotide probes (AAAG)₂₀, (GGAT)₂₀ and (GACA)₂₀. After *Sau3A* subcloning into a pUC19 plasmid vector (Boehringer Mannheim) and rescreening a positive clone ZuBeCa3 was isolated and sequenced (EMBL accession no. AJ002396). Primers flanking the sequence CATA(CA)₁₃TA(CA)₃GG(CA)₂ were designed using the OLIGO 5.0 program (National Biosciences).

Table 1. Sizes and frequencies of alleles observed

Allele (bp)	200	204
Frequency	0.95	0.05

Primer sequences:

5' TCAGGCCTTTGATGATTTCA 3'
 5' CAGGGCTGGCATTATGTAAG 3'

PCR conditions: Amplifications were carried out as previously described¹. Thermocycling was performed using the following touch-down program²: initial denaturation for 3 min at 95 °C, two cycles each of 30 s at 95 °C, 30 s at 63 °C to 55 °C and 30 s at 72 °C, followed by 14 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C and 30 min final extension at 72 °C. Sizes of the alleles were determined on 6% denaturing polyacrylamide gels using a LI-COR DNA sequencer model 4000 L.

Polymorphism: The sizes and frequencies of alleles observed in a panel of 52 dogs, representing 52 different breeds, are given in Table 1.

PIC: The calculated heterozygosity and PIC³ were 0.09 and 0.09, respectively.

Chromosomal location: ZuBeCa3 was localized by FISH⁴ to chromosome 9q21–q22 (Fig. 1) according to the partial canine standard karyotype⁵.

Mendelian inheritance: Codominant inheritance was observed in

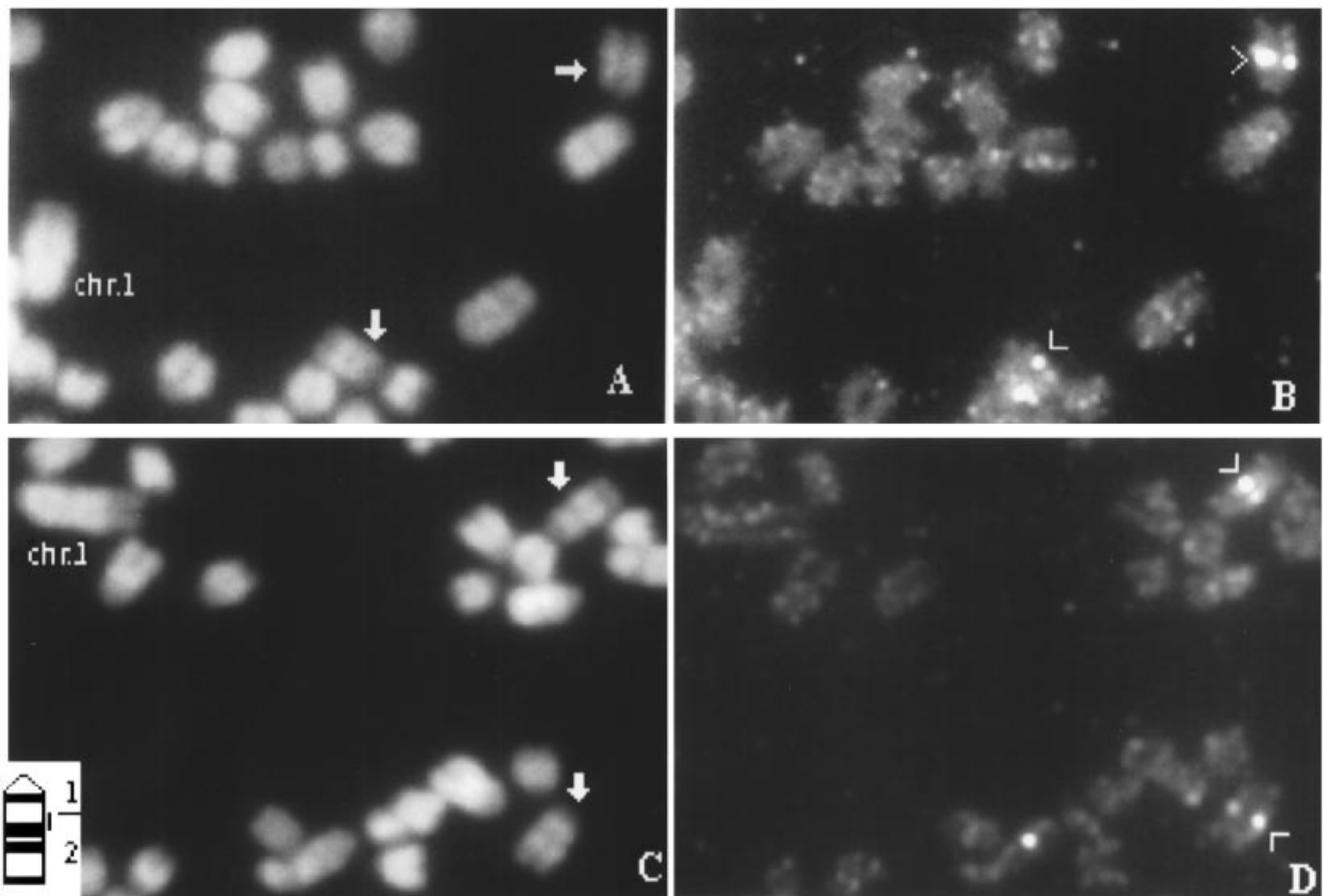


Fig. 1. Two partial metaphases, QFQ-banded (A and C) prior to FISH (Band D) with the cosmid ZuBeCa3. QFQ-banded chromosomes 9 (arrows) and the hybridization signals (arrowheads) are indicated. The length of the chromosomes showing a hybridization signal is about 60% of the length of chromosome 1 (chr.1). Inset is a schematic representation of chromosome 9 adapted to the GTG banding system.

two Beagle families with three and nine offspring, respectively, displaying alleles of 198 and 200 bp. The first allele was not observed in the 52 unrelated dogs.

Other comments: ZuBeCa3 amplifies in wolf and red fox.

Acknowledgements: We thank E. Garbely, M. Holub, B. Colomb and U. Sattler for their technical assistance and P. Schawalder for providing dog blood samples. This work was supported by the Albert-Heim Stiftung, Switzerland.

References

- 1 Dolf G. *et al.* (1997) *Anim Genet* **28**, 156–7.
- 2 Don R.H. *et al.* (1991) *Nucl Acid Res* **19**, 4008.
- 3 Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- 4 Solinas-Toldo S. *et al.* (1993) *Mamm Genome* **4**, 720–7.
- 5 Switonski M. *et al.* (1996) *Chrom Res* **4**, 306–9.

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Length polymorphism in a CT-rich microsatellite in an intron of the canine tyrosinase-related protein-2 gene

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Accepted 27 November 1997

Source/description: PCR primers 1 and 2 were designed from the published sequences of exons 2 and 3 of the human and mouse tyrosinase-related protein-2 genes^{1,2} and used to produce a 2.9 kb amplicon from canine genomic DNA. Direct sequencing³ of the amplicons from several dogs revealed 2780–2850 bp of canine sequence (GenBank AF029683). The 98 bp at the 5' end and the 77 bp at the 3' end of canine sequences were 89% identical to corresponding human exon sequences. A 2605–2675 bp canine intron separated the exons and contained a CT-rich compound microsatellite with the following structure: (CTTTT)_W(CTTT)_X(CTTTT)_Y(CTTT)_Z. The canine intron sequence was used to design primers 3 and 4 which closely flank the microsatellite.

Primer sequences:

- Primer 1: 5'-CTACGTGATCACCACACAACACTGGC-3'
 Primer 2: 5'-ACCTGGAGATCTCTTTCCAGACACAA-3'
 Primer 3: 5'-TACAGCCAAAGCTTCAGAACTGCACC-3'
 Primer 4: 5'-CAACCCTGAGCCACCGAGGCATACC-3'

PCR conditions: Primer 3 was ³²P-labelled with T4 polynucleotide kinase. The 10 µl PCR amplification mixture contained 10 ng canine genomic DNA, 0.5 U Taq polymerase, primers 3 and 4

Table 1. Relative frequencies of canine tyrosinase-related protein-2 gene alleles

Allele no.	Length (bp)	Structure (W,X,Y,Z)*	Frequency
1	257	20,2,3,12	0.026
2	ND	ND	0.018
3	ND	ND	0.018
4	ND	ND	0.044
5	ND	ND	0.018
6	ND	ND	0.009
7	215	16,1,1,10	0.123
8	211	16,1,1,9	0.079
9	210	15,1,1,10	0.096
10	ND	ND	0.105
11	202	15,1,1,8	0.096
12	197	15,9,0,0	0.167
13	191	13,10,0,0	0.132
14	187	13,9,0,0	0.070

* (CTTTT)_W(CTTT)_X(CTTTT)_Y(CTTT)_Z.

ND, not determined.

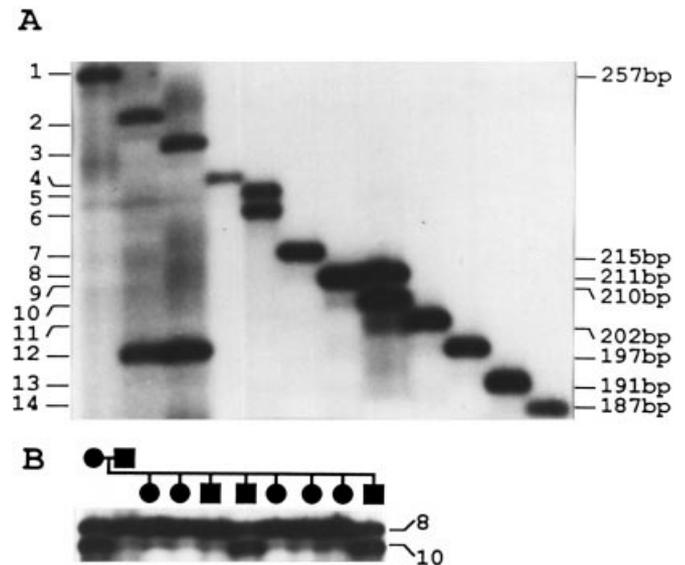


Fig. 1. (A) Autoradiogram contains bands representing 14 canine tyrosinase-related protein-2 gene alleles. (B) Genotypes from a 10-member English setter family.

(each 12.5 nM), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and dNTPs (each 200 µM). The first cycle was 94 °C for 2 min, 62 °C for 30 s and 72 °C for 30 s followed by 27 cycles of 94 °C for 20 s, 62 °C for 20 s and 72 °C for 30 s. PCR products were fractionated by electrophoresis in a 4.0% denaturing polyacrylamide gel. Bands were visualized by routine autoradiography.

Polymorphism: A survey of 57 unrelated dogs representing 37 breeds revealed at least 14 distinct alleles with lengths that ranged from 187 to 257 bp (Table 1; Fig. 1A). The PIC⁴ was 0.89 and 68% of the dogs surveyed were heterozygous.

Mendelian inheritance: Codominant Mendelian inheritance of alleles was observed in a 10-member English setter family (Fig. 1B).

Chromosomal location: The location of the canine tyrosinase-related protein-2 gene is unknown; the orthologous human gene is on chromosome 13q31–q32⁵.

Acknowledgements: This work was supported by grants from the AKC Canine Health Foundation and the Batten Disease Support and Research Association and by NIH grant R01 NS30155.

References

- 1 Bouchard B. *et al.* (1994) *Eur J Biochem* **219**, 121–34.
- 2 Jackson I.J. *et al.* (1992) *EMBO J* **11** (2), 527–35.
- 3 Shibuya H. *et al.* (1993) *Anim Genet* **24**, 345–8.
- 4 Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- 5 Sturm R.A. *et al.* (1994) *Genomics* **21**, 293–6.

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A *Bs*II PCR/RFLP in the renin binding protein (RnBP) gene on canine chromosome X

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Accepted 27 November 1997

Source/description: PCR primers 1 and 2 were designed from published human and rat renin binding protein (RnBP) cDNA sequences¹ and used to amplify canine genomic DNA producing a 0.9 kb amplicon. Direct sequencing produced 842 bp of canine sequence (GenBank accession no. AF033014) including a 735 bp intron which separated 104 bp of the 5' exon from 3 bp of the 3' exon. The canine exonic sequences were 88% identical to the

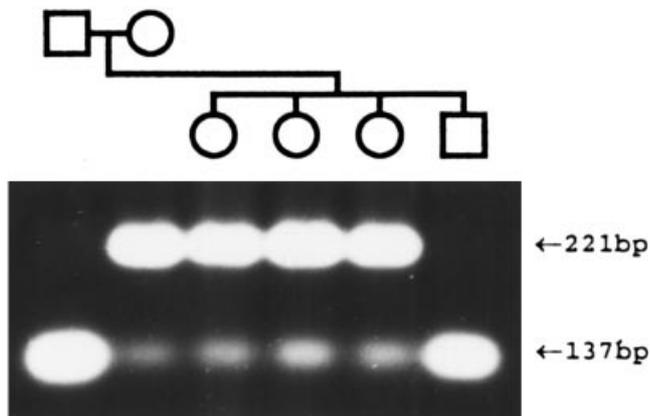


Fig. 1. RnBP *BstI* genotypes from Portuguese water dogs.

corresponding human coding sequences. Comparison of intron sequences from several dogs revealed an A or G polymorphism at nucleotide 631 of the canine intron. The G allele contained a *BstI* restriction site not present in the A allele. Primer 3 was designed from the canine intron sequence to be used with primer 1 in the *BstI* PCR/RFLP assay.

Primer sequences:

Primer 1: 5'-GAGCCCATGGCAGTGCCCATGATGC-3'

Primer 2: 5'-ACATTCTCCAGCACAGCTTGCCATC-3'

Primer 3: 5'-AGGGTCAGAACTCAACCAGGTCCTCG-3'

PCR/RFLP assay: The 20 μ l PCR-amplification mixtures contained the following: primers 2 and 3, each 1 μ M; dNTPs, each 200 μ M; 10 mM Tris-HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl₂; and 500 ng canine genomic DNA. The first cycle was 94 °C for 2 min. This was followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 60 s. PCR amplicons which contained the polymorphic site were digested with restriction enzyme *BstI*, producing 84 and 137 bp fragments from the G allele, while the A allele remained intact. The digests were fractionated by electrophoresis on 3.6% NuSieve agarose gels and the bands were visualized with ethidium bromide.

Polymorphism: In a survey of 56 unrelated dogs (40 males and 16 females) representing 36 breeds, 74% of the chromosomes had the G allele and 26% had the A allele.

Chromosomal location: The human RnBP gene has been mapped to chromosome Xq28². The finding that four of the 16 female dogs were heterozygous while all 40 of the male dogs were homozygous is consistent with an X chromosome location for the canine RnBP gene.

Mendelian inheritance: Codominant Mendelian inheritance of alleles was observed in a six-member Portuguese water dog family (Fig. 1).

Acknowledgements: This work was supported by a grant from the AKC Canine Health Foundation and by NIH grant NS32956.

References

- Inoue H. *et al.* (1991) *J Biochem* **110**, 493–500.
- van den Ouweland A.M.W. *et al.* (1994) *Genomics* **21**, 279–81.

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A polymorphic (CA)_n microsatellite in the canine lecithin:cholesterol acyltransferase gene

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Accepted 27 November 1997

Source/description: A series of PCR primers designed from the

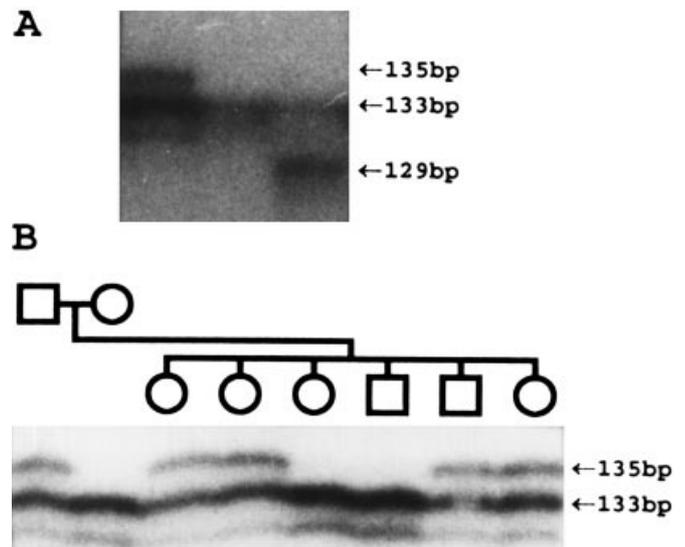


Fig. 1. (A) Autoradiogram showing examples of all three LCAT gene alleles; (B) LCAT marker genotypes of related golden retrievers.

Table 1. Allele frequencies

Allele (bp)	Repeat number	Frequency
129	13	0.02
133	15	0.87
135	16	0.11

sequences of exons 3, 5 and 6 from the human lecithin:cholesterol acyltransferase (LCAT) gene¹ were used with canine genomic DNA template to amplify and sequence segments of the corresponding canine exons. PCR primers 1 and 2 were designed from these canine sequences and produced a 1.2 kb canine amplicon. Direct sequencing revealed 1113–1119 bp of canine sequence (GenBank accession no. AF031490). The coding regions of the canine sequence (57 bp at the 5' end and 113 bp at the 3' end) had 89% nucleotide sequence identity to corresponding sequences in exons 5 and 6 of the human LCAT gene. The canine coding sequences were separated by an intron of 943–949 bp which contained a polymorphic (CA)_n microsatellite. A microsatellite assay was performed with PCR primers 3 and 4 which anneal to sites closely flanking the microsatellite.

Primer sequences:

Primer 1: 5'-AAGGACCACTTCATCGATGGGTTC-3'

Primer 2: 5'-TGAAGCTGGCGTGGAAATGAACAC-3'

Primer 3: 5'-CAGGGGCCAGCAGACACCTGGCTGA-3'

Primer 4: 5'-CATGTCTGTGGAGCAGACTAAAGA-3'

Genotyping procedure: For genotyping, primer 3 was ³²P-labelled with T4 polynucleotide kinase. The PCR amplifications were performed in 10 μ l containing 10 ng genomic DNA, 0.5 U Taq polymerase, primers 3 and 4 (each 12.5 nM), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂ and dNTPs (each 200 μ M). The first cycle was 94 °C for 3 min, 59 °C for 30 s and 72 °C for 30 s followed by 27 cycles of 94 °C for 20 s, 59 °C for 20 s and 72 °C for 30 s. PCR products were fractionated on a 6% denaturing polyacrylamide gel; bands were visualized by routine autoradiography.

Polymorphism: We surveyed 51 unrelated dogs representing 34 different breeds. Three distinct alleles were detected (Fig. 1A). Allele frequencies are reported in Table 1. The calculated PIC² was 0.21 and the observed heterozygosity was 22%.

Mendelian inheritance: Codominant Mendelian inheritance of

alleles was observed in an eight-member of golden retriever family (Fig. 1B).

Chromosomal location: The location of the canine lecithin:cholesterol acyltransferase gene is unknown; the orthologous human gene is on chromosome 16q22·1³.

Acknowledgements: This work was supported by a grant from the AKC Canine Health Foundation and NIH grant R15 HG01063.

References

- McLean J. *et al.* (1986) *Nucl Acids Res* **14**, 9397–406.
- Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- Azoulay M. *et al.* (1987) *Ann Hum Genet* **51**, 129–36.

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A *Bse*RI PCR/RFLP in an intron of the canine phenol sulfotransferase gene

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Accepted 4 December 1997

Source/description: PCR primers 1 and 2 were designed from the published sequence of human phenol sulfotransferase cDNA¹ and used to produce a 0·3 kb amplicon from canine genomic DNA. Direct sequencing revealed 264 bp of canine sequence (GenBank accession no. AF034534). The canine sequence shared 83% identity with 139 bp of human exon sequences. A 125 bp canine intron separated the exon sequences and contained a C or T single-base substitution polymorphism. The T allele contains an allele-specific *Bse*RI restriction site. To avoid a *Bse*RI restriction site on primer 1, primer 3 was designed from the canine sequence to produce a 302 bp PCR amplicon when paired with primer 2.

Primer sequences:

- Primer 1: 5'-CGCTCCCTGCCAGAGGACCATGGA-3' (forward)
 Primer 2: 5'-AAGCGCTCATTCTGCGCCACGGTGAA-3' (reverse)
 Primer 3: 5'-GGAGACTGTGGATCTCATTGTCCAGC-3' (forward)

PCR/RFLP assay: The 20 µl PCR-amplification mixtures contained the following: primers 2 and 3, each 1 µM; dNTPs, each 200 µM; 10 mM Tris-HCl, pH 8·3; 50 mM KCl; 1·5 mM MgCl₂; and 500 ng canine genomic DNA. The first cycle was 94 °C for 2 min. This was followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s and 72 °C for 60 s. When digested with *Bse*RI, PCR amplicons from the T allele produced 202 and 100 bp fragments, while C allele amplicons remained intact. The digests were fractionated by electrophoresis on 3·3% NuSieve agarose gels and the bands were visualized with ethidium bromide.

Polymorphism: In a survey of 57 unrelated dogs representing 39 breeds, 36% of the chromosomes had the C allele and 64% of the chromosomes had the T allele. Thirty-three per cent of the dogs surveyed were heterozygous.

Chromosomal location: The location of the canine phenol sulfotransferase gene is unknown; the orthologous human gene is on chromosome 16p12·1–11·2².

Mendelian inheritance: Codominant Mendelian inheritance was observed in a 10-member English setter family (Fig. 1).



Fig. 1. Canine phenol sulfotransferase PCR/RFLP genotypes in a 10-member English setter family.

Acknowledgements: This work was supported by NIH grants R15 HG01063 and R01 NS30155 and by grants from the AKC Canine Health Foundation and the Batten Disease Support and Research Association.

References

- Wilborn T.W. *et al.* (1993) *Mol Pharm* **43**, 70–7.
- Dooley, T.P. *et al.* (1993) *Genomics* **18**, 440–3.

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The highly polymorphic canine microsatellite ZuBeCa2 is localized on canine chromosome 1q210–q211

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Accepted 4 December 1997

Source/description: Positive clones were isolated from a genomic canine cosmid¹ library after screening with a mixture of the end-

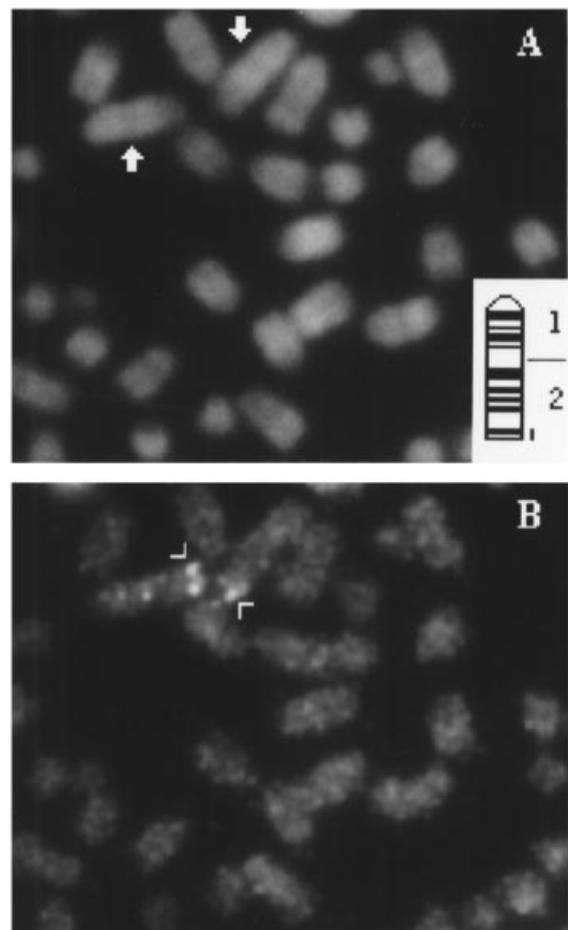


Fig. 1. QFQ-banded partial metaphases (A) prior to FISH (B) with the cosmid ZuBeCa2. (A) Chromosomes 1 (arrows) are indicated. Inset is a schematic representation of chromosome 1 adapted to the GTG banding system. (B) Hybridization signals on the chromosomes 1 are indicated by arrowheads.

Table 1. Sizes and frequencies of alleles observed

Allele (bp)	306	344	348	350	352	354	356	358	362	364
Frequency	0.010	0.029	0.038	0.077	0.019	0.019	0.106	0.038	0.010	0.019
Allele (bp)	366	368	370	372	374	376	380	382	386	390
Frequency	0.048	0.010	0.029	0.029	0.010	0.029	0.038	0.029	0.038	0.029
Allele (bp)	400	404	406	410	414	418	420	424	436	440
Frequency	0.019	0.048	0.038	0.038	0.048	0.058	0.058	0.019	0.010	0.010

labelled oligonucleotide probes (AAAG)₂₀, (GGAT)₂₀ and (GACA)₂₀. After *Sau*3A subcloning into a pUC19 plasmid vector (Boehringer Mannheim, Mannheim, Germany) and rescreening a positive clone ZuBeCa2 was isolated and sequenced (EMBL accession no. AJ003059). Primers flanking the sequence (CTTT)₁ CTT(CTTT)₁₈ CTT(CTTT)₂ CTT(CTTT)₁ CT(CTTT)₂ CTT(CTTT)₂ TTT(CTTT)₂ T(CTTT)₆ CTT(CTTT)₆ CTT(CTTT)₃ TTATATTTATTTATTCATGATAGT(AG)₁ AC(AG)₅ were designed using the OLIGO 5.0 program (National Biosciences, Plymouth, MN).

Primer sequences:

5' TGGGTGGCTCAGCAGTTTAG 3'
5' TTGCTAGTGGACCCGAGAG 3'

PCR conditions: Amplifications were carried out as previously described¹. Thermocycling was performed using the following touch-down program²: initial denaturation for 3 min at 95 °C, two cycles each of 30 s at 95 °C, 30 s at 63 °C to 55 °C and 30 s at 72 °C, followed by 19 cycles of 30 s at 95 °C, 30 s at 55 °C and 30 s at 72 °C and 30 min final extension at 72 °C. Sizes of the alleles were determined on 8% denaturing polyacrylamide gels using a LI-COR DNA sequencer model 4000 L (LI-COR, Lincoln, NE).

Polymorphism: The sizes and frequencies of alleles observed in a panel of 52 dogs, representing 52 different breeds, are given in Table 1.

PIC: The calculated heterozygosity and PIC³ were 0.95 and 0.95, respectively.

Chromosomal location: ZuBeCa2 was localized by FISH⁴ to chromosome 1q210–q211 (Fig. 1) according to the partial canine standard karyotype⁵.

Mendelian inheritance: Codominant inheritance was observed in five Beagle families with seven, seven, three, 13 and nine offspring, respectively.

Other comments: ZuBeCa2 is difficult to score due to the large number and wide range of allele sizes. ZuBeCa2 amplifies in wolf.

Acknowledgements: We thank E. Garbely, M. Holub and B. Colomb for their technical assistance and P. Schawalder for providing dog blood samples. This work was supported by the Albert-Heim Stiftung, Switzerland.

References

- 1 Dolf G. *et al.* (1997) *Anim Genet* **28**, 156–7.
- 2 Don R.H. *et al.* (1991) *Nucl Acid Res* **19**, 4008.
- 3 Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- 4 Solinas-Toldo S. *et al.* (1993) *Mamm Genome* **4**, 720–7.
- 5 Switonski M. *et al.* (1996) *Chrom Res* **4**, 306–9.

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A *Nla*III PCR/RFLP in an intron of the retinitis pigmentosa GTPase regulator gene (*RPGR*) on the canine X chromosome

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Accepted 4 December 1997

Source/description: The published nucleotide sequences of exons 14 and 15 of human *RPGR*¹ were used to design PCR primers 1 and 2, respectively. Amplification of canine genomic DNA with these primers yielded a 0.77 kb amplicon. Direct sequencing of this amplicon produced 721 bp of canine sequence (GenBank accession no. AF030561). The coding sequences (71 bp at the 5' end of the amplicon plus 32 bp at the 3' end) were 85% identical to sequences from exons 14 and 15 of human *RPGR*¹. The canine 5' and 3' coding sequences were interrupted by a 618 bp intron.

Comparisons of amplicon sequences from several dogs revealed an A or G polymorphism at nucleotide 285 of the intron. The A allele contained a *Nla*III site not present in the G allele. PCR primers 3 and 4 were designed from the canine sequence and used to produce a 305 bp amplicon that contained the polymorphic site.

Primer sequences:

Primer 1 5'-ATGAAAGAAGGGAAAGCATGTAAACA-3'
Primer 2 5'-TGCTCCTCTATTCATTTCCTTTTGA-3'
Primer 3 5'-TCTCTGAGAAGGAAAACGAGCTGGCG-3'
Primer 4 5'-CTCTCCACTACTAAATCTACTGACT-3'

PCR/RFLP assay conditions: The 20 µl PCR amplification mixtures contained primers 3 and 4 (each 1 µM), dNTPs (each 200 µM), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.5 U Taq polymerase, and 100 ng canine genomic DNA. Thirty-five thermal cycles of 94 °C (0.5 min), 59 °C (0.5 min) and 72 °C (1.0 min) were used for amplification. Digestion with *Nla*III was performed as recommended by the supplier, New England Biolabs (Beverly, MA). Digests were electrophoresed into 3.0% NuSieve agarose gels and the restriction bands were visualized with ethidium bromide fluorescence. Restriction fragments of 189 and 116 bp were present in digests of amplicons from samples containing the A allele. No digestion was detected in amplicons from G allele homozygotes.

Mendelian inheritance: Codominant Mendelian inheritance was demonstrated in a 12-member family of pointers (Fig. 1).

Distributions of alleles: Allele frequencies were estimated by genotyping 51 unrelated dogs from 44 different breeds. This sample included 33 females and 18 males. Of the 84 chromosomes examined, 51 chromosomes (61%) had the G allele and 33 chromosomes (39%) had the A allele. Seven (21%) of the 33 females were G/A heterozygous, while none of the 18 males were heterozygotes.

Chromosomal location: Human *RPGR* has been mapped to chromosome Xp21.1². The finding that all dogs heterozygous for the *RPGR* marker were females is consistent with location of canine *RPGR* on chromosome X, as predicted by Ohno's law³.

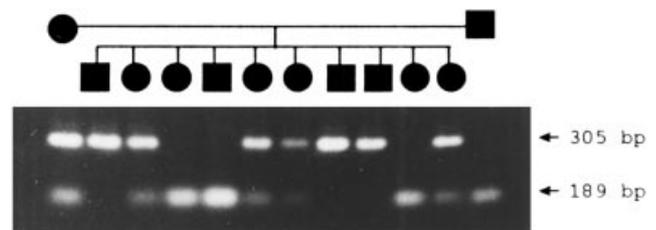


Fig. 1. *RPGR Nla*III-PCR/RFLP genotypes in a pointer family.

Acknowledgements: This work was supported by a grant from the AKC Canine Health Foundation and by NIH grant R15 NS32956.

References

- 1 Meindl A. *et al.* (1996) *Nat Genet* **13**, 35–42.
- 2 Roepman R. *et al.* (1996) *Hum Mol Genet* **5**, 1035–41.
- 3 Ohno S. (1967) *Chromosomes and Sex-linked Genes*. Springer, Berlin.

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Ostrich microsatellite polymorphisms at the VIAS-OS4, VIAS-OS8, VIAS-OS14, VIAS-OS22, and VIAS-OS29 loci

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Accepted 4 December 1997

Source/description: Five AC repeat microsatellites were isolated from an ostrich (*Struthio camelus*) genomic DNA library constructed in pUC18. *Sau3A* size selected fragments (between 220 and 460 bp) were screened with a (CA)_n(GT)_n probe and polymerase chain reaction (PCR) primers were designed from the sequence of positive clones. These repeats were in the form of (GTGTAT)₂(GT)₆(GCCGT)₄(GT)₁₇ for VIAS-OS4 (EMBL AJ002877), (AC)₁₁ for VIAS-OS8 (GenBank U22053), (AC)₂₁ for VIAS-OS14 (GenBank U22435), (TG)₂TA(TG)₇TTTA(TG)₄CA(TG)₄ for VIAS-OS22 (EMBL AJ002878), and (AC)₁₃GG(AC)₆GG(AC)₄ for VIAS-OS29 (EMBL AJ003011).

Primer sequences:

VIAS-OS4 F-CTCCTGGATGTTCTAGCAGT
R-CTCCTTGTCAGCCATATAC
VIAS-OS8 F-ACTAAACTCCTCGCTGCTGG
R-CTAAGATGCAAGGGTGAATTGAG
VIAS-OS14 F-CACTTCTCCGAATTTTAAAAGG
R-AGGAAGAGATGTGGAGTCCC
VIAS-OS22 F-AAGTAGGAGAATGGTTCTGC
R-TCATACACACATGCACAC
VIAS-OS29 F-TTTTCGTCTTCCACCCACTG
R-CTGCTTCTCCGTGTGTGTC

Allele frequencies: Alleles were named according to their size (in bp) which was determined by comparison to an M13 sequence ladder. Table 1 shows the allele frequency data for each locus. Mendelian inheritance was demonstrated.

PCR conditions: Loci were amplified from 100 ng of ostrich genomic DNA using the oligonucleotide primers above. The 5 µl PCR mixture contained 1 U of AmpliTaq Gold thermostable DNA polymerase (Perkin Elmer), 200 µM of each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.001% Gelatin, 1.5 mM MgCl₂. Primer concentrations were 200 nM each. The forward primer was end-labelled with [γ -³²P]dATP. The amplifications were performed using a Hybaid Omnigene temperature cycler with the following conditions: one cycle of 95 °C for 10 min; 30 cycles of 95 °C for 45 s; 55 °C for 45 s; one cycle of 72 °C for 10 min for VIAS-OS14 and VIAS-OS29; and one cycle of 95 °C for 10 min; 30 cycles of 95 °C for 45 s; 60 °C for 45 s; one cycle of 72 °C for 10 min for VIAS-OS4, VIAS-OS8, and VIAS-OS22. PCR products were run on a 6% denaturing polyacrylamide sequencing gel and visualized by exposing the gel to X-ray film.

Chromosomal location: Unassigned.

References

- 1 Botstein D. *et al.* (1980) *Am J Hum Genet* **32**, 314–31.
- 2 Morgan G.R. & Cox K.J. (1995) *Allele and Genotype Analysis: Statistical Package (version 2.23)*.

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Table 1. Numbers of unrelated animals used (*n*) to calculate allele frequency, *PIC*¹, heterozygosity (*H*), and average power of exclusion *Av(PrEx)*² for each locus

Locus name and details	Alleles (bp)	Frequency
VIAS-OS4	216	0.01
<i>n</i> = 41	230	0.04
<i>PIC</i> = 0.782	232	0.06
<i>H</i> = 0.8169	240	0.02
<i>Av(PrEx)</i> = 64.12%	244	0.07
	246	0.04
	248	0.18
	250	0.36
	252	0.01
	262	0.01
	264	0.14
	268	0.06
VIAS-OS8	124	0.02
<i>n</i> = 49	128	0.64
<i>PIC</i> = 0.475	130	0.24
<i>H</i> = 0.5312	134	0.04
<i>Av(PrEx)</i> = 29.38%	140	0.06
VIAS-OS14	209	0.06
<i>n</i> = 50	211	0.01
<i>PIC</i> = 0.906	213	0.02
<i>H</i> = 0.9214	215	0.07
<i>Av(PrEx)</i> = 82.23%	217	0.07
	219	0.10
	221	0.13
	223	0.05
	225	0.08
	227	0.06
	229	0.17
	231	0.04
	233	0.04
	235	0.01
	237	0.03
	239	0.03
	241	0.02
	245	0.01
VIAS-OS22	156	0.13
<i>n</i> = 49	158	0.40
<i>PIC</i> = 0.676	160	0.06
<i>H</i> = 0.8746	162	0.29
<i>Av(PrEx)</i> = 48.93%	164	0.12
VIAS-OS29	123	0.25
<i>n</i> = 47	125	0.01
<i>PIC</i> = 0.826	127	0.01
<i>H</i> = 0.8572	129	0.12
<i>Av(PrEx)</i> = 70.63%	133	0.01
	135	0.01
	137	0.01
	141	0.04
	143	0.25
	145	0.03
	147	0.09
	149	0.05
	153	0.06
	155	0.01
	157	0.01
	161	0.01
	167	0.02
	173	0.01

Detection of an SSCP within intronic sequence of the bovine TIMP-2 gene

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Accepted 15 December 1997

Source/description: Bovine sequence for the tissue inhibitor of metalloproteinases-2 (TIMP-2) gene was obtained by designing oligonucleotide primers (MacVector 6.0, International Biotechnologies) to span intron 4 from a TIMP-2 consensus sequence of human¹, mouse², and rat³ mRNA sequences (GenBank accession nos. J05593, X62622 and S82718, respectively). The gene was verified as bovine TIMP-2 from sequence analysis of a pUC19 (Promega) entire bovine intronic insert using the available peripheral exon sequence to demonstrate 89% identity to human sequence¹. A fragment of 155 bp in bovine TIMP-2 gene, at a position about 300–455 bp downstream from the exon 4/intron splice site, was amplified for SSCP analysis.

Primer sequences:

5'-CAT CCC AGG TGT TAT GGT TTT GC-3' (forward primer)

5'-TTA GCA CTG CTG TGA CTG TGC CTG-3' (reverse primer)

PCR conditions: Approximately 50 ng of bovine genomic DNA was used as a template for PCR in a total volume of 25 µl. PCR mix: 1× PCR Buffer (Biotech International) containing dNTPs (5 nmol dATP, dGTP, dTTP and 0.5 nmol dCTP), 2.5 mM MgCl₂, 15 ng of each primer, 0.4 U *Tth Plus* DNA polymerase (Biotech International), and 0.6 µCi [α -³²P]dCTP (3000 Ci/mmol). The SSCP was amplified in 96-well microtitre plates using a MJ Research thermal cycler. The PCR program comprised of a predenaturation step of 3 min at 95 °C followed by 30 cycles each consisting of 45 s at 95 °C, 1 min at 58 °C, and 1 min 30 s at 72 °C, and a final extension for 10 min at 72 °C. PCR products were diluted 1:1.4 with load dye (95% formamide, 5% bromophenol blue/xylene cyanol), denatured for 5 min then cooled on ice. Samples were analyzed by electrophoresis in 6% non-denaturing gels (59:1 acrylamide/bis) with 2.5% glycerol, and run at 4 W for 16 h before drying and autoradiography.

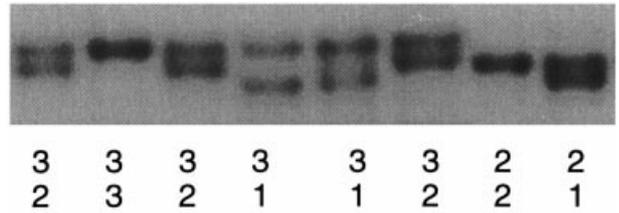


Fig. 1. Segregation of alleles at the TIMP-2 locus in three half-sib families consisting of an equal proportion of African, Brahman, Hereford and Shorthorn breeds. Genotypes are noted below each SSCP pattern.

Polymorphism and Mendelian inheritance: SSCP analysis identified a three-allele polymorphism in 553 individuals from three half-sib families of a *Bos indicus/Bos taurus* composite herd comprising an equal proportion of African, Brahman, Hereford and Shorthorn breeds (Fig. 1). The frequency of allele one was 0.17, allele two 0.18, and allele three 0.65. Codominant inheritance of the alleles was observed with no deviation from Mendelian inheritance.

Chromosomal location: Two-point linkage analysis revealed significant linkage to BMS501 (recombination frequency = 0.07, LOD score = 33.86), and DIK42 (recombination frequency = 0.01, LOD score = 25.88). Maximum likelihood position of TIMP-2 on chromosome 19 was estimated at CSSM65 – 8.6 cM – TIMP-2 – 1.0 cM – DIK42 (CRI-MAP⁴ version 2.4).

Acknowledgements: The authors thank Blair Harrison for technical assistance, Gerard Davis for statistical advice, and Jay Hetzel for helpful comments. This work was supported by The Co-operative Research Centre for the Cattle and Beef Industry (Meat Quality).

References

- 1 Stetler-Stevenson W.G. *et al.* (1990) *Biol Chem* **265**, 13933–8.
- 2 Shimizu S. *et al.* (1992) *Gene* **114**, 291–2.
- 3 Grima J. *et al.* (1996) *J Androl* **17**, 263–75.
- 4 Green *et al.* (1990) *Documentation for CRI-MAP, version 2.4*.

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