

## Cloning of canine galactokinase (*GALK1*) and evaluation as a candidate gene for hereditary cataracts in Labrador retrievers

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### Source/description

We identified a pedigree of Labrador retrievers (LR) that develop hereditary cataracts between 6 and 18 months of age. In humans, galactokinase deficiency is an autosomal recessive disorder characterized by juvenile onset of cataracts.<sup>1</sup> In order to evaluate *GALK1* as a candidate gene, we cloned and sequenced the canine *GALK1* gene and tested a single nucleotide polymorphism (SNP) in the gene for segregation with cataracts in the LR pedigree.

### Pedigree

Progeny from the LR pedigree were examined at 8 weeks of age and again between 14 and 20 months. At the time of the progeny eye exam, the parents were also evaluated for cataracts. Mydriasis was induced using 1% tropicamide, and the corneas, anterior segments, lenses and ocular fundi were examined using indirect ophthalmoscopy and slit lamp bio-microscopy. The cataracts developed between 6 and 18 months of age, and initially appear as posterior subcapsular triangular cataracts. Progression was characterized by very slight linear opacification of the posterior cortical fibers originating from the sides of the triangle. The hereditary cataracts in golden retrievers and LR have been proposed to be a dominant trait with incomplete penetrance.<sup>4,5</sup> However, based on planned matings and subsequent pedigree analyses, we established an autosomal recessive mode of inheritance for the cataracts in both breeds (G. D. Aguirre, unpublished results).

### PCR conditions

All PCRs were performed using 0.4  $\mu\text{M}$  of each primer, 1x PCR buffer, 2 mM  $\text{MgCl}_2$ , 0.2 mM each dNTP and 2.5 U AmpliTaq (Applied Biosystems, Foster City, CA, USA). The PCR conditions were: 94 °C for 3 min followed by 25 cycles of 94 °C for 30 s, annealing temperature as indicated in Table 1 for 30 s, 72 °C for 30 s and then 72 °C for 7 min. All PCR products were electrophoresed, cleaned by QIAquick PCR purification kit (Qiagen Inc., Chatsworth, CA, USA) and sequenced.

## Sequence analysis

The initial fragment of canine *GALK1* cDNA was obtained by PCR screening the canine retinal cDNA library (Stratagene, La Jolla, CA, USA) with primers GALK-8 and GALK-12 designed based on human *GALK1* sequence (GenBank accession no. NM\_000154). The 5'-end of the gene was cloned with canine specific 5'-reverse primer GALK-9 in combination with the vector primer PBK-III(f). To obtain the 3'-end, canine-specific GALK-17 primer was used in combination with the vector primer PBK-VI(r). The canine *GALK1* cDNA (NM\_001003104) contains 1179 bp of coding sequence showing similarity with human (NM\_000154) and mouse (NM\_016905) *GALK1* cDNAs of 87.9 and 85.8% respectively.

Canine BAC 298M24 (AY178787) containing the *GALK1* gene<sup>2</sup> was further analysed as described previously.<sup>2</sup> Analysis of genomic sequence shows that *GALK1* gene has eight exons interrupted by seven introns (AF454963). The sizes of all exons are identical for dog, human and mouse *GALK1*.<sup>1,6</sup> For evaluation of *GALK1* as a candidate gene in the LR New Pedigree, genomic DNA was extracted from blood samples<sup>3</sup> collected from two cataract affected and two normal progeny. All eight *GALK1* exons were amplified utilizing primers outlined in Table 1. Sequence analysis of *GALK1* exons and intron/exon junctions did not identify any sequence differences between cataract affected and wild-type progeny.

However, we did identify a G/A SNP at nucleotide 1583 (AF454963) in intron 2. For the SNP analysis a 560-bp fragment was PCR amplified with GALK-8 and GALK-6 primers, digested with *Bpi*I and electrophoresed. In the presence of the G allele the 560-bp product is cleaved with *Bpi*I into 345 and 215-bp fragments. The A allele disrupts the *Bpi*I site and consequently DNA is not cleaved. The relative frequency of the alleles was estimated using two dogs from 10 different breeds (LR, Chesapeake Bay Retriever, English Cocker Spaniel, Miniature Poodle, Greyhound, Briard, English Springier Spaniel, Nova Scotia Duck Tolling Retriever, Papillon, Portuguese Water Dog). Only one of 20 dogs, a LR, was heterozygous for the allele (G/A). We tested an additional 22 unrelated LR. The results show that out of 24 LR tested, the A allele was detected at the frequency of 0.14. This polymorphism was tested in our cataract affected LR pedigree (Fig. 1) and it was not informative.

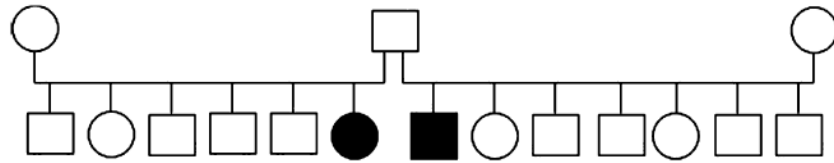
Results from this study did not identify any mutations in canine *GALK1* exons and intron/exon junctions in cataract affected LR from our pedigree. However, a possibility exists that a mutation may be outside of the *GALK1* exons that would affect *GALK1* expression. Canine *GALK1* is a candidate gene for hereditary cataracts in other breeds of dogs.

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### References

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**Figure 1.**

A pedigree of Labrador retrievers affected with posterior cortical subcapsular cataracts. Squares and circles represent male and female animals respectively. Solid symbols represent cataract affected dogs; open symbols represent non-affected animals.

**Table 1**  
Primer sequences and PCR conditions for amplification of canine *GALK1*.

Target	Primer	Sequence (5'-3')	Template	PCR annealing temperature (LC)
Partial cDNA	GALK-8 (f)	AGTTTCCACTGCCTACAGCC	cDNA library	58
	GALK-12 (r)	ATCGGCTGCTTAGAGAGGTAGA		
5'UTR	PBK-III (f)	GGTCGACACTAGTGGATCCAAAC	cDNA library	58
	GALK-9 (r)	TGAATCACTCCCTTGACATAGTT		
3'UTR	GALK-17 (f)	TGCTGGAAGCCTCCTTCACTTC	cDNA library	58
	PBK-VI (r)	CGGCCGCTCTAGAAGTACTCTCG		
Polymorphism in intron 2	GALK-8 (f)	AGTTTCCACTGCCTACAGCC	Genomic DNA	63
	GALK-6 (r)	CCACCACTGCACTGAAGCCA		
Exon 1	GALK-EX1F	CCGCGTTCCAGGAAATGCGC	Genomic DNA	64
	GALK-EX1R	GCCTTGAAGAGCCCGAAGT		
Exon 2	GALK-EX2F	CCCCTGTTCCTTTGTTGTGIGC	Genomic DNA	58
	GALK-EX2R	TTCCCTGCAAGCTATCCCGTGGTGG		
Exon 3	GALK-EX3F	GGCTCCAGCGCTGTAAACCA	Genomic DNA	58
	GALK-EX3R	CAGCAGCAGCTCCAAACAACACTCC		
Exon 4	GALK-EX4F	ATCCAGCCCAATCCAAC	Genomic DNA	58
	GALK-EX4R	GGCATGCTCCACCAGAAACTC		
Exon 5	GALK-EX5F	GCAAGCGGACACTGTCTCA	Genomic DNA	58
	GALK-EX5R	ACCTTCAGTCTGCAGTGTGG		
Exon 6	GALK-EX6F	GGAGTTACCCTGCTGCTGATGG	Genomic DNA	58
	GALK-EX6R	CGCCAAAGCCACCACCAGTCA		
Exon 7	GALK-EX7F	GTCCCGGGAGACTACAGAGCC	Genomic DNA	58
	GALK-EX7R	GCCCGGGAGCTTCGTGACTTG		
Exon 8	GALK-EX8F	ATCACTGGTGGTGTGGG	Genomic DNA	58
	GALK-EX8R	CCCCAGCCCAATTTGTGATAACCA		

The direction of the primers (f = forward; r = reverse) is indicated in parentheses.