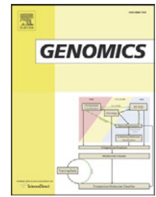
Genomics 96 (2010) 362–368

Contents lists available at ScienceDirect

Genomics

journal homepage: www.elsevier.com/locate/ygeno



Exonic SINE insertion in *STK38L* causes canine early retinal degeneration (*erd*)

Orly Goldstein [a,1], Anna V. Kukekova [a,1], Gustavo D. Aguirre [b], Gregory M. Acland [a,*]

[a] Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Hungerford Hill Road, Ithaca NY 14853, USA
[b] Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey St., Philadelphia, PA 19104, USA

A R T I C L E I N F O

Article history:
Received 16 July 2010
Accepted 22 September 2010
Available online 29 September 2010

Keywords:
Retinal degeneration
Leber Congenital Amaurosis
STK38L
Animal model

A B S T R A C T

Fine mapping followed by candidate gene analysis of *erd* — a canine hereditary retinal degeneration characterized by aberrant photoreceptor development — established that the disease cosegregates with a SINE insertion in exon 4 of the canine *STK38L/NDR2* gene. The mutation removes exon 4 from *STK38L* transcripts and is predicted to remove much of the N terminus from the translated protein, including binding sites for S100B and Mob proteins, part of the protein kinase domain, and a Thr-75 residue critical for autophosphorylation. Although known to have roles in neuronal cell function, the STK38L pathway has not previously been implicated in normal or abnormal photoreceptor development. Loss of *STK38L* function in *erd* provides novel potential insights into the role of the STK38L pathway in neuronal and photoreceptor cell function, and suggests that genes in this pathway need to be considered as candidate genes for hereditary retinal degenerations.

© 2010 Elsevier Inc. All rights reserved.

1. Introduction

Early retinal degeneration (*erd*) is a canine early onset autosomal recessive disease [1] corresponding to Leber Congenital Amaurosis in humans. It is characterized by aberrant functional and structural development of rod photoreceptor inner and outer segments, and of both rod and cone synapses, with subsequent retinal degeneration [1]. The locus was previously mapped to a 30 cM interval on canine chromosome 27 (CFA27), corresponding to a part of human chromosome 12p [2].

In this report, the candidate region was first reduced by fine-scale mapping to a 4.6 cM zero recombination interval on CFA27, a 1.6 Mb region in the canine genome sequence assembly (CanFam2). Positional candidate gene analysis then identified a tRNA-like short interspersed repeat (SINE) insertional mutation in exon 4 of *STK38L* that cosegregates with *erd*.

The gene *STK38L*, also called *NDR2*, codes for serine/threonine kinase 38-like protein — a protein kinase in the nuclear Dbf2-related (NDR) family involved in neuronal cell division and morphology [3–5]. STK38L kinase is activated by S100B (a Ca^{++}-binding protein) and Mob (Mps one binder), enabling STK38L to autophosphorylate at Thr-75, Ser-282, and Thr-444 [5,6]. STK38L and its partners have been implicated in neuronal cytoskeletal development, neurite outgrowth and synaptic remodeling. Despite this, much remains to be determined concerning their exact role and mechanisms of action. A particular gap in this understanding is their role in the retina. Until now, neither *STK38L* nor its partners, has been implicated in any hereditary retinal degeneration, or as having critical roles in photoreceptor development. The *erd*-mutant dog therefore offers potential insights into the role of this pathway in both normal and abnormal photoreceptor and neuronal development.

2. Material and methods

2.1. Animals

The *erd* strain of dogs, maintained at the Retinal Disease Studies Facility (RDSF) in Kennett Square, PA, derives from 2 *erd*-affected Norwegian elkhound dogs, highly inbred siblings, that were outbred to Beagles and other dogs, and their progeny used to generate pedigrees segregating *erd*. Selected dogs from this strain were evaluated by DNA and RNA analysis. Blood samples were also collected from privately owned dogs of several breeds, and DNA extracted as previously described [7]. All procedures involving animals were undertaken according to IACUC approved protocols at Cornell University and the University of Pennsylvania, and in adherence to the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research.

2.2. Gene-associated markers for linkage mapping

When fine mapping of *erd* began, the canine genome assembly was not available. The *erd* locus had previously been mapped to the CFA27 interval flanked by genes PFKM, LALBA, PTHLH, and IAPP [2], homologously located on HSA12p. From comparison of the canine and human genomes [8], 7 genes (*BICD1*, *PTHLH*, *ITPR2*, *SSPN*, *SHARP1*,

* Corresponding author. Fax: +1 607 256 5608.
E-mail address: gma2@cornell.edu (G.M. Acland).
[1] These 2 authors contributed equally to the work described in this manuscript.

0888-7543/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.ygeno.2010.09.003

LRMP, and *SIAT8A*) were selected from HSA12p between *LALBA* and *IAPP*, for fine mapping *erd*.

The RPC181 canine 8.1-fold BAC library from Roswell Park Cancer Institute (http:www.chori.org/bacpac/mcanine81.htm) was screened to identify BAC clones specific for *SIAT8A*, *BICD1*, and *SHARP1* as previously described [9]. Gene-associated polymorphic markers were developed from identified BAC clones (Supplementary Table 1A) as previously described [9].

For *ITPR2*, *PTHLH* and *SSPN*, partial canine genomic sequences were obtained from the Institute for Genome Research (TIGR, E. Kirkness, pers. comm.) and used to develop gene-specific polymorphic markers (Supplementary Table 1A). A polymorphic (CA)n repeat was identified in the *LRMP* gene by amplifying and sequencing canine intron 1 using primers designed from human sequences.

When the canine genome assembly (CanFam2) became available the position of all markers was identified in CanFam2 using UCSC Bioinformatics tools (electronic PCR, Blat; <http://genome.ucsc.edu/cgi-bin/hgGateway >).

2.3. Genotyping and linkage analyses

Pedigrees segregating *erd* were genotyped for informative markers (Supplementary Table 1). Markers were amplified from 50 ng of genomic DNA as per: 96 °C for 2 min; 30 cycles of 96 °C (20 s), 58 °C (20 s), and 72 °C (20 s); and a final extension at 72 °C for 5 min. PCR products were analyzed either in 12% polyacrylamide gel or on an ABI310 Genetic Analyzer (Applied Biosystems, Foster City, CA). For the latter method, forward primers had a 5′-end fluorescent label 6Fam, Hex, or Tet (GIBCO® Invitrogen, Carlsbad, CA).

Marker genotypes were checked for Mendelian segregation and analyzed to establish linkage and map order using MultiMap [10] as previously described [11]. A sex-averaged framework map was constructed with LOD 3.0 support starting with the most informative markers (*BICD1* and *SIAT8A*). Further markers were added with LOD 2.0 support (Fig. 1). Haplotype analysis of *erd* pedigrees was performed manually following “the minimal recombination number” rule.

2.4. CFA27 radiation hybrid mapping

To refine marker order on CFA27 and saturate the map with gene-specific markers, an RH Map of CFA27 was built using a commercially available 3000 rd canine radiation hybrid panel (RH3000) (Research Genetics, Huntsville, Al). The 22 markers selected for RH mapping, comprised 3 microsatellites linked meiotically to *erd* (FH2289, LEI002, REN277K09), 12 markers selected from the CFA27 RH Map 5000 cR [8], and 7 gene-specific markers (*BICD1, ITPR2, ARG99, SHARP1/BHLHE41, BCAT1, LRMP, SIAT8A/ST8SIA1*) derived from sequence of gene-specific canine BAC clones or the 3′UTR and intron sequences of the canine genes (Fig. 1; Supplementary Table 1A). Markers were amplified and an RH map constructed as described previously [9]. When the canine genome assembly CanFam2 became available, the position of all markers was identified using UCSC Bioinformatics tools (electronic PCR, Blat; <http://genome.ucsc.edu/cgi-bin/hgGateway >).

2.5. Candidate gene selection

Within the final LD region, 15 *Refseq* genes were identified. Initial candidate gene screening was limited to genes expressed in retina or eye (either mRNA or ESTs deposited in database) and mapped to HSA12p12.1-11.22, which exhibits conserved synteny with CFA27. The most proximal gene (*KLHDC5*) and the most distal two genes (*SSPN* and *ITPR2*) were also excluded from initial screening because of their proximity to the ends of the interval. As none of the remaining 7 genes had previously been implicated in an hereditary retinal degeneration, or otherwise identified as critical for photoreceptor development, all were considered equally viable candidates (Supplementary Tables 1B and Ci).

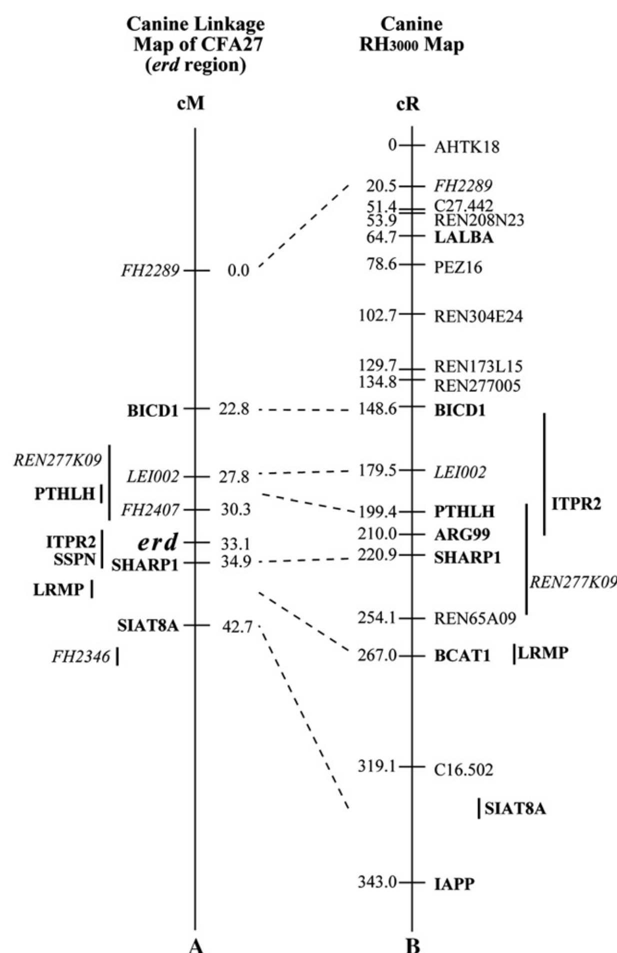


Fig. 1. Fine mapping of the *erd* interval on canine chromosome 27. A. Meiotic linkage map of the *erd* interval on canine chromosome 27 (CFA27) established by multipoint linkage analysis. The *erd* locus lies in a 4.6 cM zero recombination interval between microsatellite FH2407 and a gene-specific polymorphism in *SHARP1*. The gene-specific polymorphism in *PTHLH* cosegregates with FH2407. B. Radiation hybrid map of the corresponding interval. Marker order is in agreement with the linkage map (A) for markers mapped both ways. The *erd* interval corresponds to the region between *PTHLH* and *SHARP1*, a distance of 1.5 cR_{3000}.

2.6. PCR amplification and sequencing

Primers were designed to amplify coding sequences or genomic fragments within genes under standardized amplification conditions (Tm between 56 °C and 63 °C). Products were sequenced using the Applied Biosystems Automated 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Sequences were then analyzed and compared using Sequencher® 4.2.2 Software (Gene Codes Corporation, Ann Arbor, MI).

2.7. Population screening for the 4 bp deletion in intron 3

PCR screening of the 4-base deletion was done using three primers: two gene-specific primers flanking the 4 bp polymorphism (Supplementary Table 1Cii, primer pair 7) and a fluorescent-labeled FAM-M13(-21) universal primer: 5′-FAM-TGTAAAACGACGGCCAGT-3′. Unlabeled M13(-21) sequence (18 bp) was fused to the 5′ end of the forward primer. The reaction used: 2 pmol FAM-M13(-21) forward primer; 4 pmol reverse primer; and 1 pmol M13-tailed forward primer, made to a final volume of 11 µl with master mix (Invitrogen standard PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 U of Taq

Download English Version:

https://daneshyari.com/en/article/2821132

Download Persian Version:

https://daneshyari.com/article/2821132

Daneshyari.com