cDNA cloning and mRNA expression of cat and dog Cdkal1

Ichiro Yamamoto
Shingo Ishikawa
Li Gebin
Hiroshi Takemitsu
Megumi Fujiwara
Nobuko Mori
Yutaka Hatano
Tomoko Suzuki
Akihiro Mori
Megumi Fujiwara
Koh Kawasumi
Toshinori Sako
Toshiro Arai

Laboratory of Veterinary Biochemistry, Nippon Veterinary and Life Science University, Tokyo, Japan

Abstract: The cyclin-dependent kinase 5 regulatory subunit–associated protein 1–like 1 (CDKAL1) gene encodes methylthiotransferase, and the gene contains risk variants for type 2 diabetes in humans. In this study, we performed complementary DNA cloning for Cdkal1 in the cat and dog and characterized the tissue expression profiles of its messenger RNA. Cat and dog Cdkal1 complementary DNA encoded 576 and 578 amino acids, showing very high sequence homology to mammalian CDKAL1 (>88.4%). Real-time polymerase chain reaction analyses revealed that Cdkal1 messenger RNA is highly expressed in smooth muscle and that tissue distribution of Cdkal1 is similar in cats and dogs. Genotyping analysis of single-nucleotide polymorphism for cat Cdkal1 revealed that obese cats had different tendencies from normal cats. These findings suggest that the cat and dog Cdkal1 gene is highly conserved among mammals and that cat Cdkal1 may be a candidate marker for genetic diagnosis of obesity.

Keywords: cat, dog, Cdkal1, obese, cDNA cloning, Q-PCR

Introduction

Genome-wide association studies (GWAS) have identified many novel susceptibility genes for type 2 diabetes since 2007. The gene for cyclin-dependent kinase 5 regulatory subunit–associated protein 1–like 1 (CDKAL1) has been identified as homologous to the gene for CDK5 regulatory subunit–associated protein 1 (CDK5RAP1), an inhibitor of CDK5 activation. Single-nucleotide polymorphisms (SNPs) of CDKAL1 were correlated with body mass index in East Asian populations. GWAS also showed correlations between CDKAL1 variants and important physiological functions. CDKAL1 variants were associated with decreases in beta-cell glucose sensitivity, reductions in first-phase insulin secretion, and lower birth weight. CDKAL1 is not only correlated with obesity but is also a risk loci for Crohn’s disease. Functional analysis recently identified that human CDKAL1 belongs to the e-MtaB subfamily of methylthiotransferase. The first mammalian methylthiotransferase identified that biosynthesizes 2-methylthio-N6-threonylcarbamoyladenosine (ms2t6A) in tRNA (Lys) (UUA) and is required for accurate translation of AAA and AAG codons.

Type 1 diabetes (>50% of diabetes cases) appears to be the most common form of diabetes in dogs, whereas type 2 diabetes prevails in cats (80%–95% of diabetes cases). However, very little information is available about Cdkal1 in cats and dogs because the cDNA sequences, gene structure, and mRNA expression mechanisms have not been determined. Therefore, the aim of this study was to determine the cDNA sequence of Cdkal1 and examine its mRNA expression profiles in cats and dogs.
Materials and methods

Cdkal1 cDNA cloning in a cat and a dog

Total RNA from tissues of a cat (3-year-old male) and a dog (2-year-old male) were purchased from Zyagen (San Diego, CA). The amount of RNA was measured by spectrophotometry. A cDNA library was prepared from total liver RNA using the SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, CA). We referred to the human CDKAL1 cDNA sequence (GenBank accession number NM_017774) and the cat genome DNA sequence (GenBank accession number ti:914360954 and ti:728752993) to design specific primers for cat Cdkal1. We designed primers 1 and 2 to obtain the partial cat cDNA sequence (Table 1). The polymerase chain reaction (PCR fragment) was cloned and the cDNA sequence was determined using the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Primer 3 was used for amplification of the 3′ end of the cat Cdkal1 cDNA sequence, and primer 4 was used for 5′ rapid amplification of cDNA ends (RACE-PCR). Following cDNA cloning of cat Cdkal1, we obtained a partial Cdkal1 cDNA sequence from the dog using primers 5 and 6. Primers 7 and 8 were used for 3′ and 5′ RACE-PCR.

The mRNA expression profile of cat Cdkal1 in tissues

Total RNA (1 µg) was reverse-transcribed using the Quantitect Reverse Transcription Kit (Qiagen, Hilden, Germany). The genomic DNA was removed by DNase treatment, and cDNA was synthesized. One µL of the cDNA product was subjected to quantitative PCR (Q-PCR) according to the user’s instructions for the Real-Time PCR System 7300 (Applied Biosystems). PCR was performed at 95°C for 5 seconds and 60°C for 35 seconds in 20 µL of buffer containing 1 × Premix EX Taq II (Takara, Otsu, Japan), 1 × ROX reference dye, and 0.4 µM each of primers 9 and 10 for cat and dog Cdkal1. Primers 11 and 12 derived from the cat and dog beta-actin cDNA sequence were used for beta-actin mRNA. Following real-time PCR, the fragment was subjected to dissociation-curve analysis to avoid nonspecific PCR amplification. Quantitative measurements were performed by establishing a linear amplification curve from serial dilutions of a plasmid containing dog Cdkal1 and beta-actin cDNA fragments.

Detection of SNP in the cat genome

Seventy-four client-owned cats (37 females and 37 males, 4–17 years old) from five veterinary clinics were used. All cats were subjected to medical examinations at their veterinary clinics between March 2008 and May 2011 and were not being clinically treated for any disease. Cats were assessed by a body condition score (BCS, 1–5) and thus divided as follows: normal healthy control and obese (BCS > 4) cats. Genomic DNA was extracted from 100 µL of whole blood from obese and normal cats using a DNA Extractor WB Kit (Wako, Osaka, Japan). The quantity of the extracted genomic DNA was measured by spectrophotometry. PCR was performed with 1 × Ex Taq buffer and 0.2 µM of primers 13 and 14. The amplified PCR products were cleaned up and sequenced using primer 15.

Statistical analysis

Data are presented as mean ± standard error of the mean and were analyzed using Tukey’s test following analysis of variance. All analyses were performed using GraphPad Prism (GraphPad Software, San Diego, CA).

Results and discussion

We cloned the Cdkal1 cDNA from the cat and dog. The cat cDNA sequence consisted of a 160-bp 5′ untranslated region (UTR), a 1728-bp open reading frame (ORF), and a 953-bp 3′ UTR. The dog Cdkal1 cDNA sequence consisted of a 128-bp 5′ UTR, a 1734-bp ORF, and a 906-bp 3′ UTR. The predicted cat and dog Cdkal1 amino acid sequences were compared with those of other animals, which revealed high sequence similarity (Figure 1) (cat vs dog [92.1%], vs human [91.3%], vs cow [91.1%], vs mouse [88.4%], vs frog [82.6%], vs chicken [80.9%], vs salmon [77.2%]). The predicted amino acid sequences of cat and dog Cdkal1 showed several conserved domains, including UPF0004, radical
<table>
<thead>
<tr>
<th>Species</th>
<th>Amino Acid Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cat</td>
<td>MPSASCDS1LDDIEDIVSQED1LPQDQRHFSRKhVPVKVRSTQKYLQDE-NSPPSNTPIGQKTHMrRTWGCISHNNSDGEYMAGQLAA 89</td>
</tr>
<tr>
<td>Dog</td>
<td>1-T.....D.....G.....A.....A.....N.....E.....D.....I..... 89</td>
</tr>
<tr>
<td>Human</td>
<td>1-T.....D.....S.....V.....D.....R.....N.....E.....D.....I..... 89</td>
</tr>
<tr>
<td>Cow</td>
<td>1-T.....F.....N.....S.....R.....E.....D.....I.....E..... 89</td>
</tr>
<tr>
<td>Mouse</td>
<td>1-V.....I.....S.....Q.....F.....R.....N.....E.....D.....I..... 89</td>
</tr>
<tr>
<td>Frog</td>
<td>1-A.....E.....AT.....P.....H.....QNA.....QNI.....RA.....NKR.....IDEE.....AD.....I..... 86</td>
</tr>
<tr>
<td>Chicken</td>
<td>1-A.....V.....E.....AQ.....R.....W.....N.....F.....S.....QRA.....TD.....DD.....ND.....V.....I..... 88</td>
</tr>
<tr>
<td>Salmon</td>
<td>1-S.....I.....M.....AD.....PT.....E.....QIA.....SII.....RA.....N.....QTEVELL.....HAD.....V.....M.....S..... 85</td>
</tr>
</tbody>
</table>

**Figure 1** Comparison of the cyclin-dependent kinase 5 regulatory subunit–associated protein 1–like 1 (Cdkal1) amino acid sequence of cats with those of other animals.

**Notes:** The amino acid sequence of cat Cdkal1 (AB720722) was aligned with those of dog Cdkal1 (AB720723), human CDKAL1 (NP_060244), cow Cdkal1 (NP_001179620), mouse Cdkal1 (NP_653119), frog Cdkal1 (NP_989194), chicken Cdkal1 (XP_418914), and salmon Cdkal1 (NP_001167148). Dots matched to cat Cdkal1. Single underline indicates UPF0004 domain, double underline indicates radical S-adenosylmethionine domain, and broken underline indicates tRNA-binding domain.
S-adenosylmethionine (SAM), and tRNA-binding (TRAM) domain. The UPF0004 domain has not been characterized structurally, but it contains three highly conserved cysteine residues. The radical SAM domain is a catalytic domain with suggested importance in tRNA modification, and the TRAM domain is involved in substrate (tRNA or protein) recognition. Our findings suggest that the Cdkal1 gene may be functionally conserved in dogs and cats in the same manner as the human CDKAL1 gene.

We determined the structure of the dog Cdkal1 gene using the cDNA sequence and the BLAST Genome database (http://blast.ncbi.nlm.nih.gov). BLAST genome analysis revealed that the dog Cdkal1 gene consists of 15 exons and approximately 630-kb introns on chromosome 35. Human CDKAL1, which consists of 16 exons, is an approximately 700-kb gene located on chromosome 6. Bovine Cdkal1, which consists of 15 exons, is an approximately 600-kb gene located on chromosome 23. The cat Cdkal1 gene consists of 16 exons and is approximately 730 kb in length. The discrepancies in the total exon numbers were caused by the differences in 5' UTR. Although the start codon is located on exon 3 in humans and the cat, it is located on exon 2 in other animals.

We determined the Cdkal1 mRNA expression profiles by Q-PCR to examine the tissue-specific mRNA expression mechanism using cat and dog specific primers (Figure 2). The expression levels of cat and dog Cdkal1 mRNA were normalized to those of beta-actin mRNA. Cdkal1 mRNA was expressed in all examined tissues of a 3-year-old male cat and a 2-year-old male dog. The highest level of Cdkal1 mRNA expression was observed in muscle, and it matched with the tissue distribution of human CDKAL1 mRNA.

A comparison of the Cdkal1 expression profiles in cats and dogs showed a similar tendency, suggesting that the cat and dog Cdkal1 genes play a role in these tissues. The Cdkal1 mRNA expression profiles of dogs and cats also revealed the conservation of regulation of this expression.

We attempted to detect SNPs in obese cats on the basis of the Cdkal1 genomic structure because type 2 diabetes prevails in cats. The frequencies of the SNP genotypes in normal and obese cats are shown in Table 2. The following two SNPs were observed: the T-allele frequency for rs44089532 of normal and obese cats was lower than the C-allele frequency, but the T-allele frequency for rs44089531 of normal cats was higher than the C-allele frequency and that of obese cats was equal to the C-allele frequency. These two SNPs were located on intron 11 of the cat Cdkal1 gene. Many human GWAS have revealed four SNPs on intron 5 of the human CDKAL1 gene. Our findings suggest that rs44089531 may play an important role.

Table 2 Genotypes and allele frequencies in introns of Cdkal1 in normal and obese cat

<table>
<thead>
<tr>
<th>Genotype and allele frequency</th>
<th>Genotype</th>
<th>C/C</th>
<th>C/T</th>
<th>T/T</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs44089532 normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>obesi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs440895321 normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>obesi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 2 Tissue distribution profile of cyclin-dependent kinase 5 regulatory subunit–associated protein 1–like 1 (Cdkal1) mRNA in the dog (left; open box bars) and cat (right; closed box bars).

Notes: The expression levels of Cdkal1 mRNA in tissues of a 2-year-old male dog and a 3-year-old male cat were determined by real-time polymerase chain reaction. Each value of Cdkal1 mRNA was normalized to that of beta-actin mRNA and is the mean ± standard error of mean (n = 3) of triplicate experiments for an individual RNA sample. The statistical analysis was performed using Tukey’s test following analysis of variance. Values with different letters are significantly different (P < 0.05) in each dog and cat.
in the pathogenesis of type 2 diabetes in cats. Because cats are thought to be good animal models of type 2 diabetes, functional SNP marker information will contribute to translational studies of humans and other animals. We did not complete SNP genotyping in cats, thus further analysis is required to determine whether SNPs found in this study would be helpful in diabetes diagnosis in obese cats.

Acknowledgments
This study was supported by a Grant-in-Aid for Young Scientists (B 22780270) and a Grant-in-Aid for Scientific Research (21380195 to Arai) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

These sequence data have been submitted to DDBJ/EMBL/GenBank database under accession No AB720722 (cat Cdkal1) and AB720723 (dog Cdkal1).

Disclosure
The authors report no conflicts of interest in this work.

References

Veterinary Medicine: Research and Reports
Publish your work in this journal
Veterinary Medicine: Research and Reports is an international, peer-reviewed, open access journal publishing original research, case reports, editorials, reviews and commentaries on all areas of veterinary medicine. The manuscript management system is completely online and includes a very quick and fair peer-review system.

Submit your manuscript here: http://www.dovepress.com/veterinary-medicine-research-and-reports-journal

Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.