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Organization of the canine gene encoding the E isoform of retinal guanylate cyclase (cGC-E) and exclusion of its involvement in the inherited retinal dystrophy of the Swedish Briard and Briard–Beagle dogs

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Abstract

Intracellular cyclic GMP concentration is known to change in response to a wide variety of agents, including hormones, neurotransmitters or light. In vertebrate photoreceptors, different membrane-bound guanylate cyclase isoforms are responsible for cGMP synthesis and thus directly involved in termination of light signalling via the phototransduction cascade and recovery of the dark state. We have characterized a 4.7 kb long cDNA for the canine retinal guanylate cyclase isoform E (cGC-E) predicting a polypeptide of 1109 amino acids. The genomic structure and the complete sequence of the canine GC-E gene, which consists of 20 exons and spans about 14.5 kb, has also been determined. Northern blot analysis showed that GC-E was expressed in the canine retina as a 4.7 and 6.1 kb large transcript. RT-PCR analysis also detected low expression in cerebrum (occipital lobe). We performed a sequence analysis of the cGC-E gene in animals of a Swedish Briard and Briard–Beagle dog kinship in which an inherited retinal dystrophy is segregating. Several intragenic DNA polymorphisms were identified and used for segregation analysis which excluded cGC-E as a candidate gene for this type of canine retinal dystrophy.

Keywords: Retinal guanylate cyclase; GC-E; Retinal degeneration; Gene (canine)

1. Introduction

In vertebrate photoreceptors, 5′-cyclic guanosine monophosphate (cGMP) plays a major role as intracellular second messenger in phototransduction. The level of cGMP in photoreceptor cells is controlled by the hydrolyzing enzyme, cGMP-specific phosphodiesterase, and the synthesizing enzyme, guanylate cyclase. As resynthesis of cGMP is crucial for the opening of cGMP-gated ion channels and recovery of the dark state, guanylate cyclase is a key enzyme in the quenching step of phototransduction.

Retinal guanylate cyclase (retGC) is a member of the guanylate cyclase superfamily. Two major forms of guanylate cyclases can be distinguished; the soluble and the particulate (membrane) form [1]. All membrane-bound guanylate cyclases, including retGC, are composed of a large single polypeptide with a ligand binding N-terminal domain, a trans-

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membrane domain, an internal protein kinase homology region, and a C-terminal cGMP catalytic domain. Recent studies have demonstrated the existence of several different guanylate cyclase isoforms in mammalian retina. GC-F and ROS-GC2 are expressed only in the retina [2,3], while GC-E is present both in the retina and pineal gland. Natriuretic peptide receptors GC-A [4,5] and GC-B [6] are expressed in many tissues. For the isoforms GC-E [2,7,8], GC-F [2,9] and ROS-GC2 [3], ligands are unknown.

The progressive retinal dystrophy found in Swedish Briard and Briard–Beagle dogs is an autosomal recessive disorder with complete penetrance [10–14]. Night blindness is a constant finding in affected animals. Except for the first two animals identified initially, day vision was also severely affected or the animals became totally blind. The biochemical defect is unknown. Analysis by direct current electroretinography showed signs suggestive of severely delayed phototransduction [11]. Mutations in human genes encoding various proteins involved in the phototransduction cascade have been identified during the past years as the primary genetic cause of different types of retinal degeneration. By analogy, one could hypothesize that canine retinal dystrophies are caused by mutations in the canine equivalents of the genes known to cause retinal disease in other animal species and human. To date, only a single, disease-related mutation has been identified in a canine retina-specific gene; Suber et al. [15] reported a nonsense mutation in the gene encoding the β-subunit of rod photoreceptor cGMP-specific phosphodiesterase in the early-onset rod/cone dysplasia of Irish setters. The importance of GC-E for normal vision has recently been demonstrated by two independent lines of evidence. First, Perrault et al. have demonstrated that mutations in the human GC-E homologue (retGC, encoded by *GUC2D*) result in the earliest and most severe form of inherited retinopathy, Leber congenital amaurosis, in a portion of families of Arab origin [16]. Second, a null mutation in the photoreceptor GC-E gene has recently been identified as the primary genetic defect of the retinal degeneration in *rd* chicken [17]. In order to analyse its possible involvement in the retinal dysplasia observed in a Swedish Briard and Briard–Beagle kindred presenting with some phenotypic similarities to Leber amaurosis in human, we have characterized the cDNA and gene encoding the canine homologue of bovine ROS-GC and human retGC, i.e., the dog retinal guanylate cyclase, cGC-E.

2. Materials and methods

2.1. Animals

The dogs examined in the present study were bred at the Swedish University of Agricultural Sciences, Faculty of Veterinary Medicine, Department of Medicine and Surgery in Uppsala.

2.2. Purification of retinal mRNA and synthesis of cDNA

Poly(A) + RNA was prepared from frozen (-70°C) tissues of a cross-bred and an affected Briard dog using Dynabeads® Oligo $(dT)_{25}$ (Dynal) according to the manufacturer's recommendations. Reverse transcription reaction was carried out using 1 μl of mRNA (~ 1 μg) in 50 μl volume at 37°C for 1 h with SuperScript™ (Gibco, Life Technologies) and oligoT_{22} or random hexamers as synthesis primers. The cDNA pool was used subsequently for further PCR reactions. Double-stranded cDNA (ds cDNA) for RACE (rapid amplification of cDNA ends)-PCR [18,19] was generated from retinal mRNA of normal and affected animals using the Marathon™ cDNA amplification Kit (Clontech). Adaptors (Clontech) for the RACE-PCR were ligated to the ends of the ds cDNA and the construct was used for RACE-PCR.

2.3. Amplification of the canine GC-E cDNA with RACE-PCR

For the RACE-PCR we used the Marathon™ cDNA Amplification Kit following the manufacturer's instructions. The 3′-end of the canine retinal GC-E cDNA was amplified from the canine retinal cDNA pool using 3′-RACE with primer GC3 (5′TTCACCACCATCTCAGCCAT3′) and adaptor-specific AP1. A second, nested PCR was carried out using 1:100 diluted first-round PCR products as template with nested primer GC3N (5′CAACAAACCCATCCCCAAAC3′), deduced from the consensus sequence

of the conserved catalytic domain of bovine, human, and mouse analogues, and with the adaptor-specific primer AP2. The amplicon, containing the 3′-end of the cGC-E cDNA from exon 18 to the start of the polyA signal, was sequenced and used to design primers for further experiments. Another primer pair (sequence available on request), based on the homology among mammalian retinal guanylate cyclases in exon 2, was designed and used with canine-specific primers in exon 19 to amplify the middle region of the cDNA. Primers for 5′-RACE were adaptor-specific, AP1, and canine GC-E exon 2-specific, GCW1 (5′CACAGGTCCTGGGGCGCGGTGATTAG3′). Amplified PCR products were used for subsequent nested PCR with adaptor-specific nested primer AP2 and gene-specific primer GCW2 (5′GCGCGGAACAGGGCGTACAGAGCATC3′). PCR reactions were carried out in a final volume of 25 μl with 1 μl of template (cDNA or diluted PCR product) and 0.5 U Taq polymerase. We used a touch-down PCR procedure to increase both fidelity and yield of PCR [20]. After an initial denaturation step (3 min at 95°C) amplification was performed for 3′-RACE as three cycles of denaturation (10 s at 95°C), annealing (10 s at 60°C), and elongation (30 s at 72°C), followed by three cycles of annealing at 58°C, 30 cycles of annealing at 56°C (the other parameters were unaltered), and a final elongation step (5 min at 72°C). Nested 3′-RACE-PCR was performed at annealing temperatures 62–60–58°C (other parameters were the same). For 5′-RACE first-round PCR was performed with the addition of 5% of DMSO in the PCR mixture, at annealing temperatures 67–65–63°C, and nested PCR with annealing temperatures 66–64–62°C. PCR products were purified by QIAquick PCR Purification Kit (Qiagen) and both strands from an unaffected animal were sequenced using primers (5 pmol) specific to the amplicon and dRhodamine Terminator Ready Reaction Sequencing Kit (Applied Biosystems) on an automatic flurometric DNA sequencer (Applied Biosystems DNA Sequencing System, Model 373A).

2.4. Characterization of the cGC-E gene

Genomic DNA from an unaffected cross-bred dog was isolated from peripheral blood by conventional methods. To amplify genomic DNA, overlapping exonic primers were designed based on the dog cDNA sequence. For amplification of the long introns 7 and 8 we used the TaqPlus Precision™ PCR system (Stratagene). Large introns were sequenced by the primer walking method. To determine the sequence of the longest intron (#3), for which direct amplification was not successful, we used the method of rapid amplification of genomic DNA ends (RAGE) [21] and the Universal GenomeWalker™Kit (Clontech). Based on the canine cDNA data and the genomic organization of the human (*GUC2D*) [16] and mouse [22] GC-E genes, we predicted the exon–intron boundaries of the canine gene. Data analysis was done with LASERGENE software for Windows 95 (DNASTAR, Madison), BLAST [23], GENSCAN software [24], and RepeatMasker2 Web.

2.5. Northern blot and RT-PCR analysis

mRNA (5 μg) from various dog tissues was separated on 1% denaturing formaldehyde-agarose gels, transferred to positively charged nylon membranes (Boehringer) by capillary transfer, and hybridized overnight at 42°C with a ^{32}P-labeled 1321-bp long PCR-amplified cDNA fragment (cDNA nucleotides 3180–4500) of cGC-E. Blots were washed for 5 min at 42°C in 2 × SSC/0.1% SDS and twice for 10 min at 42°C with 0.1 × SSC/0.1% SDS. Autoradiography was performed at −70°C for 2–5 days. RT-PCR was done using the cDNA pool prepared from various tissues. PCR was performed using forward primer GC391 (5′CCAGCCAGCCCTTGGATAAC3′) in combination with reverse primer GC1970 (5′ACACGACCTCCTGCATGATG3′) to amplify a 594-bp long fragment spanning exons 7–11 (nucleotides 1822–2415). Touch-down PCR annealing temperatures were 60°–58°–56°C.

2.6. PCR-SSCP analysis of the GC-E gene

Single-strand conformation analysis (SSCP) [25] and silver staining of the gels were performed as described elsewhere [26,27]. PCR fragment size used for SSCP was 120–420 bp. All DNA samples with band shifts in the SSCP electrophoresis were purified and cycle sequenced as described before.

3. Results

3.1. Characterization of the canine GC-E cDNA

Canine retinal GC-E cDNA was amplified using 5′- and 3′-RACE techniques and both strands of the overlapping PCR amplicons were sequenced directly. The full-length canine GC-E cDNA obtained is 4687 bp long and contains an open reading frame between nt 183 and 3512 (see GenBank accession number Y15483). The transcription initiation site was not determined directly. However, sequencing of 5′-RACE-PCR products detected a 112-bp long first exon, which is four nucleotides larger than the bovine homologue [28]. The 3′-UTR is 1174-bp long (without the polyA tail) and contains a variant polyadenylation signal, ATTAAA, 34 bp before the polyA tract. Amino acid alignment of various vertebrate GC-E homologues is shown in Fig. 1.

3.2. Structure of the canine GC-E gene

The complete exon and intron sequence was determined and, for the coding part, confirmed by comparison to the cDNA sequence (see GenBank accession number Y15484). The canine GC-E gene is composed of 20 exons, the first and last one are non-coding. The exons are interrupted by 19 introns, and the gene spans over 14 kb genomic DNA (Fig. 2). The exon/intron structure is similar to the human and bovine homologues.

A search for repetitive elements with RepeatMasker2 revealed a LINE element long terminal repeat and a retroviral long terminal repeat element in intron 3 of the dog GC-E gene (Fig. 2). Three short interspersed nucleotide elements (Can SINE, GenBank accession no. X57357), which represent most likely tRNA-derived retroposons specific to the Canoidea superfamily [29], are located in intron 7 and 8. In addition, there is evidence for a medium reiteration element MER45B in intron 7, and for medium reiteration elements MER5A in intron 19 and exon 20.

3.3. Expression analysis

Northern analysis of mRNA from various dog tissues demonstrated two prominent transcripts of about 4.7 kb and 6.2 kb, both of them only in retinal tissue (Fig. 3). The predominant band (4.7 kb) corresponds to the dog cDNA size determined here.

In contrast to the results of Northern blot analysis, which demonstrate expression of dog GC-E only in retina among all tissues examined, low level of expression in dog brain (occipital lobe) was also detected by RT-PCR. No amplification products were observed for the other tissues studied.

3.4. Sequence variants and segregation data

SSCP analysis and subsequent direct sequencing of the coding part and, at a limited extent, of intronic sequences of the dog GC-E gene revealed several polymorphic changes. In intron 13, we found a single base pair variant, 11207T/C, while, in intron 18, a GTGCTCC heptamer (nucleotides 12 582–12 588) duplication was identified (numbers according to the sequence deposited in GenBank under the entry Y15484). We have studied 36 unrelated Briard and foxhounds dogs to determine the allele frequency of the 11207T/C and GTGCTCC-duplication polymorphisms. The heterozygosity value for the first polymorphism is $H = 0.491$, with allele frequencies of 0.434 and 0.566 for T and C, respectively. The heterozygosity value for the second polymorphism is $H = 0.413$ with allele frequencies 0.709 for one and 0.291 for two repeats.

Furthermore we found four missense mutations in the coding region of the canine retinal guanylate cyclase gene. One unaffected dog is heterozygous for the 2795G/A change in exon 14. This nucleotide variant predicts a nonconservative amino acid change,

Fig. 1. Amino acid alignment of canine retinal GC-E with other vertebrate retinal guanylate cyclases (human retGC [7]; cow ROS-GC [8]; mouse GC-E [22], and rat GC-E [7]). Amino acid sequences were aligned for maximal homology using the Clustal algorithm of the DNASTAR® software (gap penalty 10). Only differences from canine retinal GC-E sequence are indicated. Amino acid residue numbers are given on the right. The four polymorphic amino acid substitutions detected in the canine GC-E are underlined and shown above the wild-type sequence. The canine GC-E cDNA sequence has been deposited in the GenBank database under the accession number Y15483.

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                                                R
dog     MSACALLAGGLPDPRLCAPARWARSPPGVPGAPPWPQPRLR-LLLLLLLLPPSALSAVFTVGVLGPWACDPIFARARPDL     79
cow     .T..TF.....R..G..G.T...P....L.PI..R.RL...PP........R.V.........................     80
human   .T...RR.......G..G..W..P.L.RL.R.L-----...P-.........Q.P.....................S......     75
mouse   ...WL.P.....GA.F.V.----Q..SSFSRVLR..R.G.PG.......PS........K...................     78
rat     ...WL.P...F.GAGF.I..W--Q.RSSLSRVLR..G.G.PG.......PS...F....K...................     78

dog     AARLAAARLNRDAALEDGPRFEVTLLPEPCRTPGSLGAVSSALGRVSGLVGPVNPAACRPAELLAQEAGVALVPWSCPGT    159
cow     ......S...HA....G......A..................T.................................G....    160
human   ............PG.AG......A..................A.....................E...I.....G..W.    155
mouse   ......N.....FG.DG......A......L...........S.............G...................G....    158
rat     .....TD.....L..DG..W..........L...........T.................................G....    158

dog     RAGGTTAPAGTPAADALYALLRAFRWARVALITAPQDLWVEAGRALSAALRARGLPVALVTTMEPSDLSGAREALRRVQD    239
cow     ..A.....VV.................H...V...........H...T.............S.................    240
human   Q.E......V..............G......V............S..T..........S..S...L..........K.R.    235
mouse   ..A......V.........v.............................T.............S..T..R.......G.IR.    238
rat     ..A......V.........V..K.........................T.............S.V.............IR.    238

dog     GPRVRAVIMVMHSVLLGGEEQRCLLQAAEELGLADGSLVFLPFDTLHYALSPGPEALAVLANSSQLRRAHDAVLILTRHC    319
cow     ...........................E.........................D............K.......T.....    320
human   ....T.................Y..E.......T...........I............A..............T.....    315
mouse   .....V.................Y..E.....A.T.......................AFV.............T...R.    318
rat     .....V.................Y..E.......T.......................AFV...K.........T...R.    318
                                                                    DS
dog     PPGGSVMDNLRRAQEHQELPSDLDLQQVSPFFGTIYDAVLLLAGGVARARAAAGGGWVSGATVAHHIPDAQVPGFCGTLG    399
cow     .L....R.S.......R...L..N......L......S.F..........V..........A..R..R..R......A..    400
human   .SE...L.S......RR......N......L........F...R...E.......R.....A..R..R.........D..    395
mouse   ......Q.S...........L..N.K....L........F......K...T.V........S..RQVRE...S....V..    398
rat     ......Q.S...........L....K....L........F......T.....V........S..RQMRE...F....I..    398

dog     GAQEPPFVLLDTDAAGDRLFATYMLDPTRGSLLSAGTPVHFPRGGGTPGSDPSCWFEPGVICNGGVEPGLVFLGFLLVVG    479
cow     ..E..S........T..Q.....V....Q.FFH.........K..RG..P......D.DT........SV..I.......    480
human   .DE.......................A...F.....RM......SA..P......D.NN..G..L..............    475
mouse   RTE..S........S.EQ....HL...VL...R.....M......PA..P......D.D.............V.....I.    478
rat     RTE..S..........E...T.HL...VL...R............APA..P......D.D.............V.....IV    478

dog     MGLTGAFLAHYLRHRLLHIQMVSGPNKIILTLDDVTFLHPHGGSTRKVVQGSRSSLAARSTSDIRSVPSQPLDNSNIGLF    559
cow     ...A........C.....................I........NS...A....T......I..V..IH..LP.YT....Y    560
human   ...A........V......M.............V..I........TS...A.......G...M.....G...H..SP...VY    555
mouse   .................M..A..........E.......P...S............T..A..........QEST.V..Y    558
rat     V.................M.............E.......Q...S...A........T.............QEST....Y    558

dog     EGDWVWLKKFPGDQHIAIRPATKTAFSKLRELRHENVVLYLGLFLGSGGAGGSAAGEGVLAVVSEHCARGSLHDLLAQRD    639
cow     .............R.........M....I.........A.......AG.AG.PA.P.................Q.......    640
human   ...R.........................Q........A.......AR.AE.PA.LW..N.........T....Q......E    635
mouse   ............EH.M.....................A.......AGTADSPATP...I.....................E    638
rat     ............EH.M.......M.............A.......AGTADSPATP...I.....................    638

dog     IKLDWMFKSSLLLDLIKGMRYLHHRGVAHGRLKSRNCVVDGRFVLKVTDHGHARLMEAQRVLLEPPSAEDQLWTAPELLR    719
cow     ..................I.................................G..L......P.................    720
human   ..................I..................I........I.....G..L...K..P...R.............    715
mouse   ...................................................G..L......P.................    718
rat     ...................................................G..L......P.................    718

dog     DPALERRGTLPGDVFSLGIIMQEVVCRSAPYAMLELTPEEVVERVRSPPPLCRPSVSMDQAPVECIQLMKQCWAEHPDLR    799
cow     ..V.......A.............................K..Q............I....M.............Q.E..    800
human   ..........A......A.......................Q............L...........L.........Q.E..    795
mouse   ..S.......A......A..........T...........IQ............L.......M......T.......E..    798
rat     ..........A......A..........T...........IQ............L.......M......A.......E..    798
                                                                                        I
dog     PSLGHIFDQFKSINKGRKTNIIDSMLRMLEQYSSNLEDLIRERTEELELEKQKTDRLLTQMLPPSVAEALKMGTPVEPEY    879
cow     ..MDRT.EL.........M.....................................................................    880
human   ..MD.T..L..N.....................................................T.........    875
mouse   ..MDLT..L.....................................Q.........................S.....    878
rat     ..MDLT..L..G..................................Q.........................S.....    878

dog     FEEVTLYFSDIVGFTTISAMSEPIEVVDLLNDLYTLFDAIIGSHDVYKVETIGDAYMVASGLPQRNGQRHAAEIANMALD    959
cow     ...........................................................................H.....    960
human   ..Q.....................................................................S..    955
mouse   .....................................A.....................................S..    958
rat     .....................................................................S..    958

dog     ILSAVGSFRMRHMPEVPVRIRIGLHSGPCVAGVVGLTMPRYCLFGDTVNTASRMESTGLPYRIHVNMSTVRILHALDEGF   1039
cow     ......T.....................................A.............R...Q..S..N...   1040
human   ......T.....................................................L...G..R...S.Y   1035
mouse   ...........................................................RS..Q..          1038
rat     ...........................................................R...Q..          1038

dog     QTEVRGRTELKGKGAEDTYWLVGRRGFNKPIPKPPDLQPGASNHGISLQEIPLDRRWKLEKARPGQFSGK            1109
cow     L..............E.........................H...P...Q.............               1110
human   .V.L.............F.....................S...........PE..R..........               1103
mouse   .M.C.........I.........L.....................PE..K.........T..                  1108
rat     .M.C.........V.........V.....................PE..K.........T..                  1108
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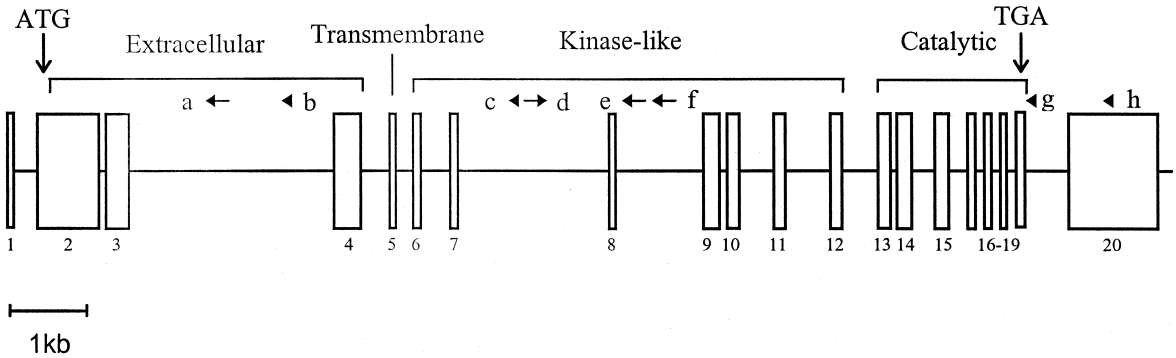

Fig. 2. Structure of the dog GC-E gene. Exons are indicated as numbered boxes. The extracellular domain is encoded by exons 2–4, while exon 5 corresponds to the transmembrane domain. The kinase-like and cyclase catalytic domains are encoded by exons 6–12 and 13–19, respectively. The start (ATG) and stop (TGA) codons are indicated by vertical arrows. Horizontal arrows represent repeat elements; a, LINE element long terminal repeat; b, retroviral long terminal repeat; c, medium reiteration element MER45B; d–f, short interspersed nucleotide element (Can SINE); g–h, medium reiteration elements MER5A.

871 Met(ATG) to Ile(ATA), in the catalytic domain. The M871I allele seems to be rare, as we have not found it in 36 control animals. In exon 4, we found two nucleotide changes on the same allele, 1319C/A and 1321A/T, which lead to two amino acid substitutions, 380-Ala(GCC) to Asp(GAC) and 381-Thr(ACG) to Ser(TCG), at the same time. The double nucleotide substitution creates a novel restriction site for *Xmn*I, which gives rise to restriction fragments of 93 and 305 bp following restriction digestion of the 398-bp large amplicon. In the Swedish Briard and Briard–Beagle dogs, all but one of the unaffected animals were homozygous for this latter allele (Fig. 4). Two animals from the control group also carried the variant allele in heterozygous form. Using two further allelic nucleotide changes in exon 2, 264G/A

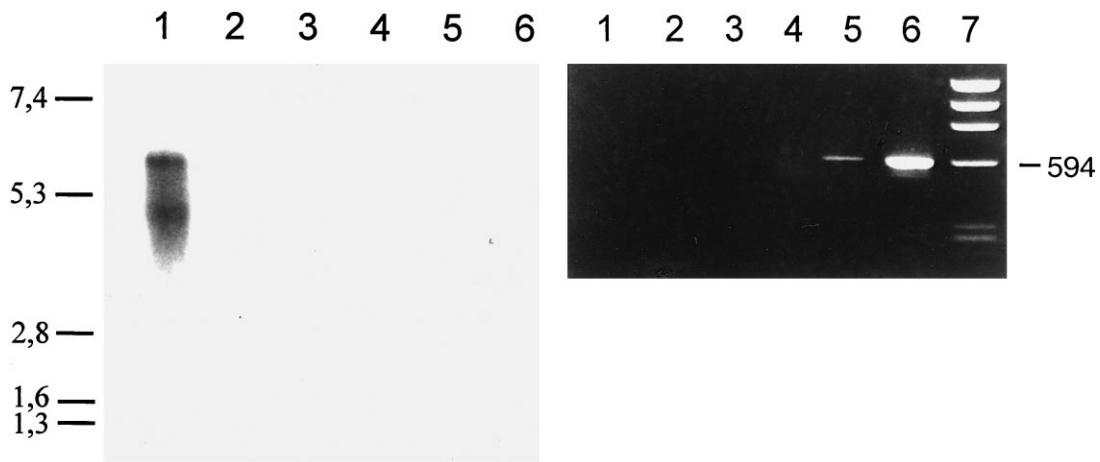

Fig. 3. Expression pattern of the canine GC-E. Left panel: Northern blot analysis of canine GC-E mRNA in different dog tissues. Lane 1: retina, 2: cerebrum, 3: liver, 4: kidney, 5: cardiac muscle, and 6: skeletal muscle. Estimated molecular weight (kb) is shown on the left and has been calculated by using RNA molecular weight markers (Boehringer Mannheim) visualized by ethidium bromide staining. Right panel: RT-PCR analysis of GC-E expression in various tissues. A 594-bp long fragment spanning exons 7 to 11 was amplified and separated on an 1.5% agarose gel. Lane 1: skeletal muscle, 2: cardiac muscle, 3: kidney, 4: liver, 5: cerebrum (occipital lobe), 6: retina, and 7: molecular weight marker (φX174 RF DNA/*Hae*III fragments).

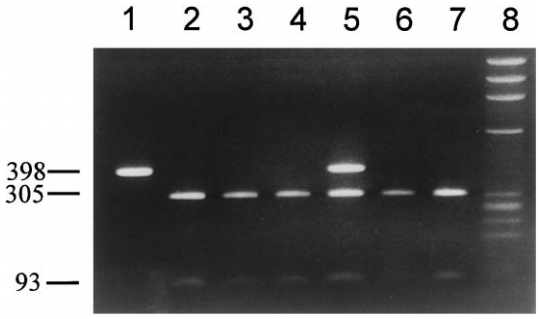

Fig. 4. Detection of the sequence polymorphisms 1319C/A and 1321A/T in exon 4 of the canine GC-E gene by restriction enzyme analysis. The PCR-amplified 398 bp fragment was digested with Hinf I and separated on 2% SeaKem agarose gel. Lane 1: homozygous pattern of 1319C and 1321A (unaffected control); lanes 2, 4, and 7: homozygous pattern of 1319A and 1321T (affected animals); lanes 3, 6: homozygous pattern of 1319A and 1321T (unaffected animals); lane 5: double heterozygous sample 1319C/A and 1321A/T (unaffected animal); lane 8: molecular weight marker (φX174 RF DNA/*Hae*III fragments).

and 266 C/G (Fig. 5), which should lead to the amino acid change 28-Gly(GGC) to Arg(AGG), we could exclude GC-E as a causative gene of the retinal dystrophy, as affected dogs carried different alleles in homozygous form (Fig. 5). This latter amino acid change is in the variable putative signal sequence of guanylate cyclase and represents a common polymorphism as the same change has also been found in the control group.

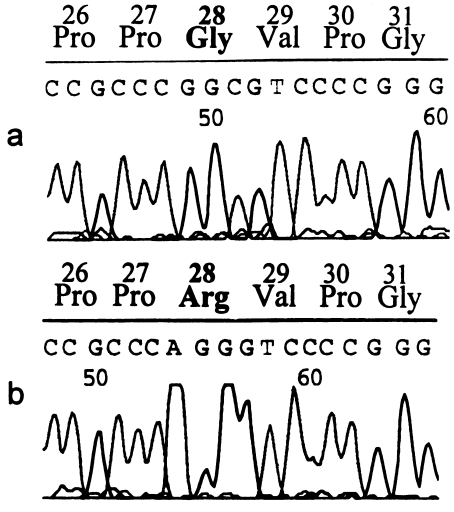

Fig. 5. Detection of the sequence polymorphisms 264G/A and 266C/G in canine GC-E gene exon 2 by direct sequencing. (a) Affected dog homozygous for 264G and 266C (28Gly); (b) Affected dog homozygous for 264A and 266G (28Arg).

4. Discussion

In the present communication, we report the characterization of the gene and cDNA encoding canine retinal guanylate cyclase (GC-E). The cDNA is 4687-bp long and the longest open reading frame predicts a 1109 amino acid protein with a calculated molecular weight of 119.92 kDa (prior to protein modification). Although both the rat and bovine primary GC-E transcripts are much larger, about 8.5 [2] and 7.5 kb [28], respectively, the size of the proteins is quite similar (115–120 kDa) in all three species. The protein is slightly acidic with an isoelectric point of 6.82. The coding sequence is very GC rich (67%). The percent of similarity of the canine retGC-E, at the amino acid level, is the highest with the bovine homologue (88.0%) and the lowest with the mouse (85.7%). The most obvious differences among the various retinal GC-E homologues are in the 5′-end of the transcript, which contains 50 amino acids representing the putative N-terminal hydrophobic signal peptide [7]. The cyclase catalytic domains are the most conserved regions, followed by the kinase-like domains.

The GC-E gene structure seems to be highly conserved among vertebrates. All functionally important domains are encoded by a separate set of exons, a feature which is in line with the hypothesis that most membrane guanylate cyclase genes are derived from a common ancestral gene [22]. Exon sizes range from 81 to 1158 nucleotides. Each of the splice junctions is in good agreement with the GT/AG rule [30] and also the adjacent sequences follow the consensus pattern (Table 1). In introns 3, 7, 8, 19, and 20 of the canine GC-E gene, we found different repetitive elements characteristic to all vertebrates. The SINE elements detected share 70–93.9% identity with the original canine specific SINE element found by Minnick et al. [31]. All together, the different repetitive elements recognized account for about 17% of the gene.

The expression pattern of the canine GC-E gene is of special interest. The presence of two abundant

Table 1
Position and nucleotide sequence of exon/intron boundaries of the canine GC-E gene. Exon sequence is shown in uppercase letters; intron sequence is in lowercase letters

Exon	Exon length (bp)	Splice donor site	Intron length (bp)	Splice acceptor site
1	110	TCCGgtgagtggca	279	ggggttgcggAGAG
2	803	AGAGgtaggttccc (1)[a]	94	cggtccgcagCGGT (2)[b]
3	305	GCAGgtagagggac (3)	2690	cctggcgcagGTGT (1)
4	352	GGAGgtgagggcaa (1)	380	gcttctgcagGAGT (2)
5	85	TGAGgtgagtatgg (2)	234	cctctaccagGCAT (3)
6	103	AAAGgtgggacagc (3)	391	taccctccagGTGG (1)
7	102	TGAAgtgagtttta (3)	1994	tctactccagGGAG (1)
8	81	CAAGgtgagggtgg (3)	1172	tccctcccagCTCC (1)
9	207	CAAGgtgcggggta (3)	87	gccgccccagGGAA (1)
10	157	GAGGgtaagattcc (1)	443	cggcccccagACCA (2)
11	150	GAGGgtacgcagcc (3)	595	atgccttcagAAGT (1)
12	149	CCAGgtcaggggct (3)	484	ctccaactagTTTA (1)
13	164	CTCCgtgcgtcccc (3)	91	ccatctctagTTCT (1)
14	193	CAAGgtgggctgtg (3)	216	cttcccgcagGTGG (1)
15	175	TCGGgtacctagag (1)	248	gtctccgcagGCCC (2)
16	99	CTGCgtgagtggag (1)	117	gtcattccagCTTA (2)
17	95	GAAGgtgaggctat (3)	120	gttcccccagGGCA (1)
18	86	CGGGgtgaggtgcc (2)	128	tctcccgcagCGCC (3)
19	110	GCAGgtgctgcccc	559	tctctctcagGGTA
20	1158			

[a,b]Numbers indicate, respectively, the position of the last and first exonic nucleotide within the respective codon.

mRNA species in the retina may indicate the use of alternative poly(A) addition sites, alternative promotor usage with different transcription start sites, or alternatively-spliced guanylate cyclase variants. While our Northern blot data show abundant expression of GC-E in the retina and are therefore in good agreement with those obtained by other authors [2,28], RT-PCR analysis revealed also low expression in cerebrum (occipital lobe). Heterotopic expression of retina-specific guanylate cyclase has been shown for rat GC-E in pineal gland but not in brain [2]. Yet, further careful studies are needed to confirm this novel finding and exclude a possible artifact.

Among the various polymorphisms found in the cGC-E gene, the substitution 380-Ala(GCC) to Asp(GAC) is the most interesting as all species characterized so far (human, cow, mouse, and rat) carry invariantly alanine at this position in the extracellular domain of guanylate cyclase. The polymorphisms 264G/A and 266 C/G found in exon 2 were helpful to exclude this gene as causative for the retinal degeneration found in Swedish Briard and Briard–Beagle dogs. Due to the high degree of inbreeding, affected animals are expected to be homozygous not only for the disease causing mutation but also for all other nonpathogenic sequence variations of the same gene. Therefore, a heterozygous pattern for any sequence change or homozygosity in affected animals for two different alleles of the same sequence variant provides a genetic proof to exclude the gene in question as the one implicated in the retinal disorder in this strain. The involvement of six further retina-specific genes in the eye disease of this strain of dogs has already been excluded previously, e.g., those encoding rhodopsin, the β-subunit of rod photoreceptor cGMP phosphodiesterase, peripherin/RDS, arrestin, rod outer membrane protein (ROM1), and the α-subunit of rod cGMP-gated cation channel [27,32,33]. Nevertheless cCG-E and all the other genes listed before are good candidates for the numerous other canine retinal degenerations found in different breeds.

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