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Isolation and Characterization of the Canine Serotonin Receptor 1A Gene (*htr1A*)

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Abstract

Although the serotonergic system and *htr1A* have been studied extensively, little is known about the canine serotonin receptor 1A. We are interested in this receptor in the dog because it is likely to be involved in behavioral disorders such as anxiety. Therefore, we isolated a canine bacterial artificial chromosome (BAC) clone containing *htr1A*, and, with the help of this clone, the complete canine coding sequence of this gene was determined. Radiation hybrid (RH) mapping showed that *htr1A* is part of a conserved linkage group also including the survival of motor neuron 1 (*smn1*) gene. *Htr1A* is estimated to be located about 7.3 Mb from *smn1* on *cfa02*. In addition, we report a possible breed-specific variant of the gene in four golden retrievers.

Having survived more than 750 million years of evolution, serotonin (5-hydroxytryptamine, 5-HT) is expected to be of great importance in many living organisms (Peroutka 1995). 5-HT functions as a hormone, a mitogen, and a neurotransmitter. Its significance is not only evident from its evolutionary age but also demonstrated by the fact that it is used by one of the most extensive signaling systems found in the brain: from the raphe nuclei of the brain stem, serotonergic neurons project widely throughout the central nervous system, and 14 receptors have already been identified that mediate its biological effects (Hoyer et al. 1994). There has always been much interest in the serotonergic system because it plays a role in central nervous system processes regulating fear, anxiety, aggression, control of sleep, and modulation of feeding behaviors (Gingrich and Hen 2001).

One of the 14 serotonin receptors known to date is serotonin receptor 1A. Its activity is mediated by G-proteins that inhibit adenylate cyclase activity, and it can be found as an autoreceptor on serotonergic cell bodies and dendrites in the raphe nuclei, as well as on postsynaptic targets of serotonin release in a number of limbic structures (see Barnes and Sharp 1999 for a review). Stimulation of 5-HT_{1A} autoreceptors inhibits cell firing and serotonin release, which enables the receptor to modulate the activity of the serotonergic system. (Corley et al. 1992; Jolas et al. 1995; Sprouse and Aghajanian 1987). Although results are conflicting (Olivier et al. 2001; Pattij 2002), the 5-HT_{1A} receptor has often been associated with anxiety, depression, aggression, and stress response. In humans as well as animals, (partial) 5-HT_{1A} receptor agonists have been reported to have anxiolytic effects (Cervo et al. 2000; DeVry 1995; File et al. 1996; Oshima 2001), and several strains of *htr1A* knockout mice show heightened anxiety, stress response, and an antidepressant-like phenotype. (Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998).

Kobilka et al. (1987) first cloned and mapped the human gene encoding serotonin receptor 1A (*htr1A*). Currently, the complete coding sequence of *htr1A* is known for several organisms, including human (*Homo sapiens*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), *Caenorhabditis elegans*, and *Drosophila melanogaster*. The *htr1A* gene is intronless, has seven predicted transmembrane-spanning domains, and contains sites for glycosylation and phosphorylation. It is localized on the human chromosome 5q11.2-q13.

Table 1. Primer pairs used in this study

Primer	Sequence (5′–3′)	Position[a]	Length (bp)	T_A (°C)
1	GAC TAC GTG AAC AAG AGG AC	427–446[b]		
2	AAG GTG CCC ATG ATG ATG C	1043–1061[b]	635	60
3	GGC AAC AAC ACC ACC TCG TC	25–44		
4	CTT GTT CAC GTA GTC GAT GG	422–441	417	56
5	GAC TAC GTG AAC AAG AGG AC	427–446		
6	TGC ACT TCA ATC ACC TCC AG	868–887	461	56
7	CCT GGA GGT GAT TGA AGT GC	867–886		
8	ACT TGC ACC TGA CGA TCT TC	1236–1255	389	54
9	GCA GGC ATG GAG GGG CTC AG	–6–14		
4	CTT GTT CAC GTA GTC GAT GG	422–441	447	56
10	GGC GCT ACA ACC TCA ATT TTC	503–523[c]		
11	TTG GAT GTA AAA CAG AAA ACA TCA T	743–767[c]	265	55
12	GTG GAA AGT TGG TGA CAA ATG	258–278[d]		
13	CTC CTG AGT ATC CTG TTC TAC	439–459[d]	202	55
14	GGG ACG CTC GGC AAC GCT ACT GG	58–80		
15	GGC TCG CCG TTC ACG CTC TTC CTG	750–773	716	67
16	CCT TTG GCG CTT TCT ACA TCC	599–619		
17	ACC GGG CGG GCC TTC TCG TC	1286–1305	707	60

T_A = annealing temperature.

[a] Unless otherwise stated, all positions are based on the canine *htr1A* sequence published in this paper.

[b] The position of this primer is based on the human instead of canine *htr1A* sequence (accession no. X57829).

[c] The positions of primers 10 and 11 are based on the human *crtl1* sequence (accession no. NM_001884).

[d] The positions of primers 12 and 13 are based on the canine *smn1* sequence (accession no. U50746)

This study on canine *htr1A* was performed in the context of a research project involving canine fear and aggression. Extreme levels of fear and aggression in dogs can result in biting incidents, with serious implications for the victim (obviously) and for the dog, because some owners consider euthanasia for their pets in such cases (Galac and Knol 1997). In order to reveal the etiology of these behavioral problems, we are studying parts of the canine serotonergic system. Although an enormous number of studies have been performed on the serotonergic system in general and the 5-HT_{1A} receptor in particular, little is known about the canine serotonin receptor 1A. In this article we describe the isolation of a canine bacterial artificial chromosome (BAC) clone containing *htr1A*, the complete coding sequence of this gene, and analysis of its position on the physical map of the dog. In addition, we report a possible breed-specific variant of the gene in four golden retrievers.

Materials and Methods

DNA Isolation and Dogs

Human and golden retriever genomic DNA was isolated from whole blood lymphocytes by the salt extraction method of Miller et al. (1988). Great Dane genomic DNA was extracted from spleen.

The four golden retrievers used in this study were privately owned, unrelated dogs. The retrievers visited our clinic in the context of a research project involving canine fear and aggression, but their owners did not consider the dogs to be either aggressive or anxious.

BAC Library Screening

The human coding sequence of *htr1A* (GenBank accession no. X57829) was used to design primers 1 and 2 (Table 1). These primers amplified a 635 bp fragment in a PCR using 200 ng human genomic DNA, 0.5 μM primers, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 2.5 units *Taq* DNA polymerase in 1× Gibco-BRL buffer in a 100 μl reaction volume. This 635 bp probe (*htr1A*-635) was labeled with [α-^{32}P] dATP with a megaprime DNA labeling kit (Amersham, Piscataway, NJ).

The canine genomic BAC library RPCI-81, derived from a Doberman pinscher (Li et al. 1999), was screened with probe *htr1A*-635 as previously described (van de Sluis et al. 1999). This resulted in only one positive colony: BAC 160O12. For further applications, the alkaline lysis method as described on the BacPac Web site (http://www.chori.org/bacpac/) was used for BAC DNA isolation.

Southern Blot

To confirm the identity of the BAC clone, genomic golden retriever DNA, genomic Great Dane DNA and BAC clone 160O12 DNA were digested with *Eco*RI. The resulting fragments were separated on a 0.7% agarose gel, transferred to Hydrobond N^+ filter (Amersham) and hybridized at 65°C with the *htr1A*-635 probe.

Sequence Analysis of the Canine *htr1A*

The major part of the canine *htr1A* sequence was determined by sequencing subcloned BAC 160O12 fragments. BAC 160O12 DNA was digested with *Sau*3AI; the resulting

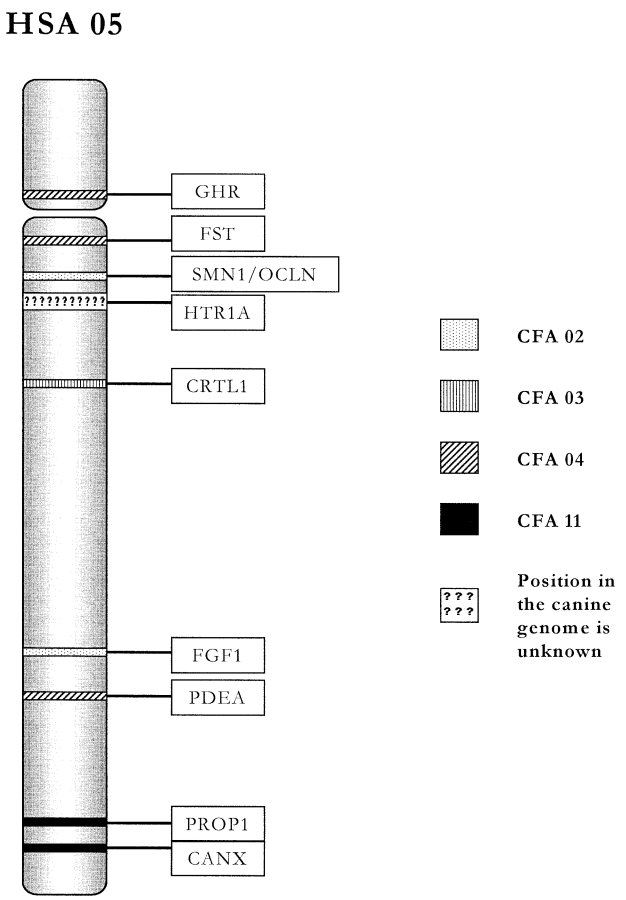

Figure 1. Schematic representation of the human chromosome 5. The bands mark several genes that were mapped in both the human and the canine species. The position of the bands along the chromosome is based on genomic contigs of *hsa05* on the Human Map Viewer Web site (http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/map_search, accessed in October 2002), and the pattern of a particular band represents the position of the gene in the canine genome (http://www-recomgen.univ-rennes1.fr/cgi-dog/display-hsa.prog?hsa=5, accessed in October 2002). The position of *htr1A* on the canine genome was not yet known when we started this study. *ghr* = growth hormone receptor; *fst* = follistatin; *smn1* = survival of motor neuron 1, telomeric; *ocln* = occludin; *crtl1* = cartilage linking protein 1; *fgf1* = fibroblast growth factor 1 (acidic); *pdea* = phophodiesterase 6A, cGMP-specific, rod, alpha; *prop1* = prophet of Pit1, paired-like homeodomain transcription factor; *canx* = calnexin.

Figure 2. Southern blotting results. Genomic golden retriever DNA (lane A), genomic Great Dane DNA (data not shown, but similar), and BAC 160O12 DNA (lane B) were digested with *Eco*RI, separated on a 0.7% agarose gel, transferred to Hydrobond N^+ filter, and hybridized at 65°C with the *htr1A*-635 probe.

fragments were ligated into *Bam* HI digested pZeRO^{tm}–1 vector with T4 DNA ligase, transformed into TOP10F' bacterial cells, and plated on low-salt LB plates with 50 μg/ml zeocin. Zeocin-resistent colonies were transferred to Hydrobond N^+ filters that were hybridized with the *htr1A*-635 probe. Positive colonies were picked and grown overnight. DNA was isolated from these cultures by miniprepping with a Promega DNA purification system. The subcloned DNA was sequenced with T7 (5′-TAA TAC GAC TCA CTA TAG GG-3′) and SP6 (5′-ATT TAG GTG ACA CTA TAG-3′) primers using an ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) with BigDye Termination Mix.

The first 50 bp of the gene were sequenced by means of direct BAC DNA sequencing. Twenty pmol of a reverse primer (5′-GAG GTG ATC ACT TGG TAG CTG-3′) was used in a 30 μl tercycle reaction with 4 mM $MgCl_2$, 12 μl Big Dye Terminator Ready Reaction Mix, and 750 ng BAC DNA. The tercycle consisted of 5 min at 95°C, followed by 34 cycles of 30 s at 95°C, 10 s at 56°C, and 4 min at 60°C. Tercycle products were purified with multiscreen 96-well filtration plates (Millipore) and sequenced in 10 μl distilled water with an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA)

Three primer pairs (3 and 4, 5 and 6, 7 and 8; see Table 1) were designed based on the preliminary canine coding

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CCCCCCCGCCGGGCGCGCAGGC

ATG GAG GGG CTC AGC CCC CGA CAG GGC AAC AAC ACC ACC TCG TCC GAG GGG CCC  54
 M   E   G   L   S   P   R   Q   G   N   N   T   T   S   S   E   G   P  18
TTC GGG ACG CGC GGC AAC GCT ACT GGC ATC TCC GAC GTG ACC TTC AGC TAC CAA 108
 F   G   T   R   G   N   A   T   G   I   S   D   V   T   F   S   Y   Q  36
GTG ATC ACC TCC CTG CTG CTG GGC ACG CTC ATT TTC TGC GCG GTG CTG GGC AAT 162
 V   I   T   S   L   L   L   G   T   L   I   F   C   A   V   L   G   N  54
GCG TGC GTG GTG GCC GCC ATC GCC CTG GAG CGC TCC CTG CAG AAT GTG GCC AAC 216
 A   C   V   V   A   A   I   A   L   E   R   S   L   Q   N   V   A   N  72
TAT CTC ATC GGC TCG CTG GCC GTC ACC GAC CTC ATG GTG TCG GTG CTG GTG CTG 270
 Y   L   I   G   S   L   A   V   T   D   L   M   V   S   V   L   V   L  90
CCC ATG GCC GCG CTG TAC CAG GTG CTC AAC AAA TGG ACG CTG GGA CAG GTC ACC 324
 P   M   A   A   L   Y   Q   V   L   N   K   W   T   L   G   Q   V   T 108
TGT GAC CTA TTC ATT GCC CTC GAC GTG CTG TGC TGC ACC TCG TCC ATC CTG CAC 378
 C   D   L   F   I   A   L   D   V   L   C   C   T   S   S   I   L   H 126
CTG TGC GCC ATT GCG CTG GAC AGG TAC TGG GCC ATC ACG GAC CCC ATC GAC TAC 432
 L   C   A   I   A   L   D   R   Y   W   A   I   T   D   P   I   D   Y 144
GTG AAC AAG AGG ACG CCC CGG CGC GCC GCT GCG CTC ATC TCG CTC ACT TGG CTC 486
 V   N   K   R   T   P   R   R   A   A   A   L   I   S   L   T   W   L 162
ATC GGC TTC CTC ATC TCC ATT CCG CCC ATG CTG GGT TGG CGC ACC CCC GAA GAC 540
 I   G   F   L   I   S   I   P   P   M   L   G   W   R   T   P   E   D 180
CGC TCG GAC CCC GAC GCG TGC ACC ATC AGC AAG GAC CAC GGC TAC ACT ATC TAC 594
 R   S   D   P   D   A   C   T   I   S   K   D   H   G   Y   T   I   Y 198
TCC ACC TTT GGC GCT TTC TAC ATC CCG CTG CTG CTC ATG CTG GTC CTC TAC GGG 648
 S   T   F   G   A   F   Y   I   P   L   L   L   M   L   V   L   Y   G 216
CGC ATC TTC CGC GCC GCG CGC TTC CGC ATC CGC AAA ACA GTC AAG AAG GCG GAG 702
 R   I   F   R   A   A   R   F   R   I   R   K   T   V   K   K   A   E 234
AGG AAG GGA GCG GAC GCC CGC TCC GGG GTG TCG CCA GCC CCG CAG CCC AGG AAG 756
 R   K   G   A   D   A   R   S   G   V   S   P   A   P   Q   P   R   K 252
AGC GTG AAC GGC GAG CCG GGG GGC AGA GAA TGG AGG CAG GGT CCG GGG AGC AAG 810
 S   V   N   G   E   P   G   G   R   E   W   R   Q   G   P   G   S K(Q) 270
GCT GGG GGG CCT CTG TGC ACC AAC GGC GCG GTG AGG CGG GGC GAC GAC GGC GCC 864
 A   G   G   P   L   C   T   N   G   A   V   R   R   G   D   D   G   A 288
GCC CTG GAG GTG ATT GAA GTG CAC CGC GTG GGC AGC TCC AAA GAG CAC CTG CCG 918
 A   L   E   V   I   E   V   H   R   V   G   S   S   K   E   H   L   P 306
CTG CCC TGC GAG GCT GGC GCC ATC CCT TGC GCC CCC GCC TCC TTC GAG AAG AAG 972
 L   P   C   E   A   G   A   I   P   C   A   P   A   S   F   E   K   K 324
AAT GAG CGC AAC GCC GAG GCC AAG CGC AAG ATG GCC CTG GCC CGG GAG AGG AAA 1026
 N   E   R   N   A   E   A   K   R   K   M   A   L   A   R   E   R   K 342
ACG GTG AAG ACG CTG GGC ATC ATC ATG GGC ACG TTC ATC CTC TGC TGG CTG CCC 1080
 T   V   K   T   L   G   I   I   M   G   T   F   I   L   C   W   L   P 360
TTC TTC ATC GTG GCC CTG GTC CTG CCC TTC TGC GAG AGC AGC TGC CAC ATG CCC 1134
 F   F   I   V   A   L   V   L   P   F   C   E   S   S   C   H   M   P 378
ACC CTG CTG GGC GCC ATA ATC AAC TGG CTG GGC TAC TCC AAC TCC CTG CTC AAC 1188
 T   L   L   G   A   I   I   N   W   L   G   Y   S   N   S   L   L   N 396
CCC GTC ATC TAC GCC TAC TTC AAC AAG GAC TTC CAG AAC GCC TTT AAG AAG ATC 1242
 P   V   I   Y   A   Y   F   N   K   D   F   Q   N   A   F   K   K   I 414
GTC AGG TGC AAG TTC TGC CGC CGA CGG TGA                               1272
 V   R   C   K   F   C   R   R   R                                   423

CGGCGGCGCGGTCGACGAGAAGGCCCGCCCGGTCCTCTCGGGCCC
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Figure 3. Nucleotide sequence and deduced amino acid sequence of canine *htr1A*. The sequence has been deposited in the GenBank database under accession no. AY134445. A preliminary sequence was obtained by sequencing subcloned BAC 160O12 with T7 and SP6 primers. Subsequently, this sequence was checked by means of sequencing BAC DNA-based PCR products. DNA was translated with EDITSEQ (DNA Star Software). The adenine at nucleotide position 808 is printed in bold because this was cytosine in four golden retrievers studied, resulting in glutamine instead of lysine at amino acid position 270.

sequence. These primers amplified adjacent fragments in PCRs, using BAC clone 160O12 DNA as a template, and the PCR products were sequenced several times. The first and last 50 bp of the exon were checked by direct BAC DNA sequencing. In this way, every base in the preliminary sequence was checked at least once.

Homologies with human and murine sequences were calculated with SEQMAN and MEGALIGN (DNA Star Software). Sequences retrieved have the following GenBank accession numbers: X57829, AB041403, XM_003692, AF498978 (human), U39391, S67168, XM_122644, and NM_008308 (mouse). Predicted positions of the transmembrane regions in the protein were derived from the SWISSPROT Web site (http://srs6.ebi.ac.uk/srs6bin/cgi-bin/wgetz?-newId+-e+[libs%3d{SWALL_SP_REMTREMBL}-acc:P08908]).

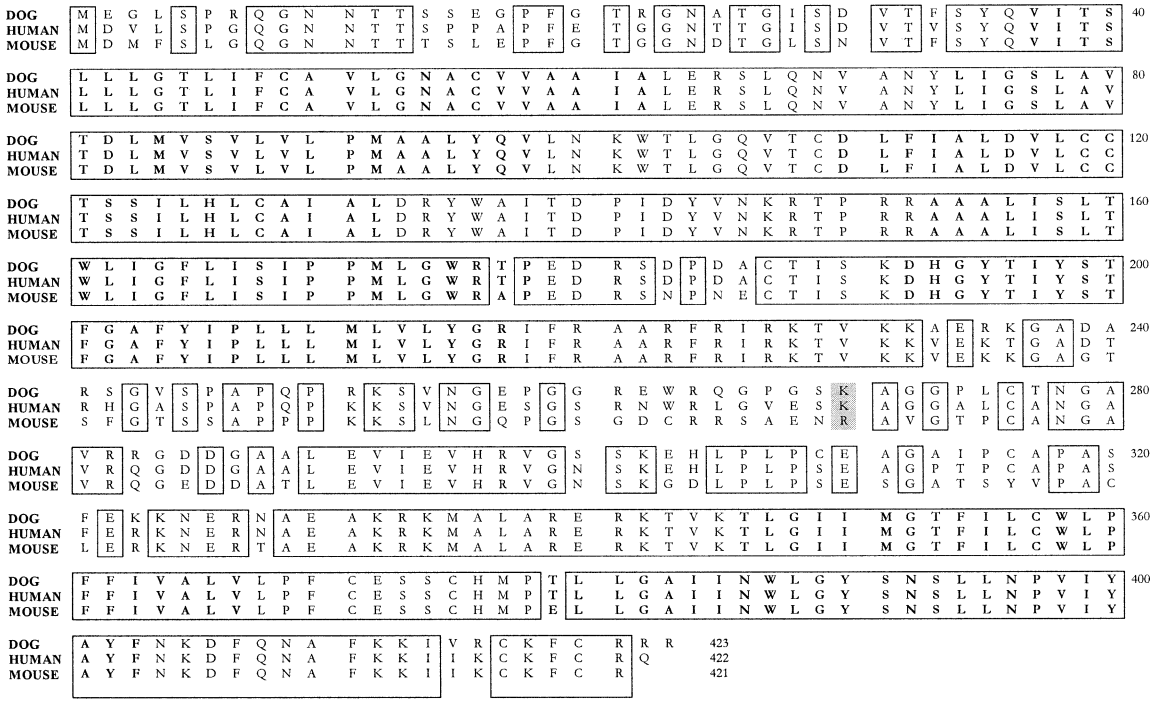

Figure 4. Comparison of the amino acid sequence of the canine serotonin receptor 1A with human (accession no. AF498978) and mouse (accession no. NM_008308) sequences. For the sake of clarity, amino acids are presented in groups of 10. Completely identical residues are boxed, and transmembrane regions, according to the SWISSPROT Web site, are printed bold. Note that the exact positions of the transmembrane segments are not known with certainty and are reported in different positions by various authors. The residues at amino acid position 270 are depicted in a gray box because there was glutamine instead of lysine at this position in four golden retrievers studied in our department.

Radiation Hybrid Mapping

The canine chromosome segments corresponding to HSA05 were studied in order to select two genes flanking *htr1A* in the human genome: cartilage linking protein 1 (*crtl1*) and survival of motor neuron 1, telomeric (*smn1*) (Figure 1). The commercially available canine whole genome radiation hybrid (RH) panel T72 (3000 RAD, Research Genetics, http://www.resgen.com/products/CRH.php3) was used to determine linkage between *htr1A* and both of these genes. Primers 9 and 4 were used to establish retention of *htr1A* in the hybrids, and primers 10 and 11 and 12 and 13 were used for *crtl1* and *smn1*, respectively (Table 1).

PCR reactions contained 25 ng DNA, 0.53 μM primers, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.6 units Platinum *Taq* Polymerase (Invitrogen), and 1× Gibco-BRL buffer in a total reaction volume of 15 μl. We used the following PCR program: 3 min 94°C initial denaturation, followed by 35 cycles of 30 s at 94°C, 30 s at T_A, and 30 s at 72°C. The final step was 4 min at 72°C. The primers were first tested on genomic canine, hamster, and 2:1 hamster/canine DNA in order to confirm their specificity for dog DNA.

A two-point LOD score was computed for both *htr1A/smn1* and *htr1A/crtl1* with RH2PT software (RHMAP, version 3.0; Boehnke et al. 1996).

Partial Sequence Analysis of *htr1A* in Four Golden Retrievers

Analysis of *htr1A* sequences in genomic DNA of four golden retrievers was performed by means of PCR product sequencing, using primers 14 and 15 and 16 and 17. Reliable sequencing results were obtained for base pairs 115–1250 in each of these dogs. Note that there is a mismatch in primer 14: the eighth base is a T, where the corresponding position in the exon shows a G, but this apparently did not affect the specificity of the primer.

Results

BAC Library Screening and Southern Blot

BAC library screening resulted in one positive BAC clone: 160O12. Both this clone and genomic dog DNA showed a single 9.4 kb band in a Southern blot analysis using a 635 bp human probe (Figure 2). Because the *htr1A* exon contains no *Eco*RI sites, it was not considered necessary to repeat the Southern blotting procedure with a full-length probe. These results imply that we have isolated a genomic clone of canine *htr1A* and that the *htr1A* gene is single copy in the canine genome.

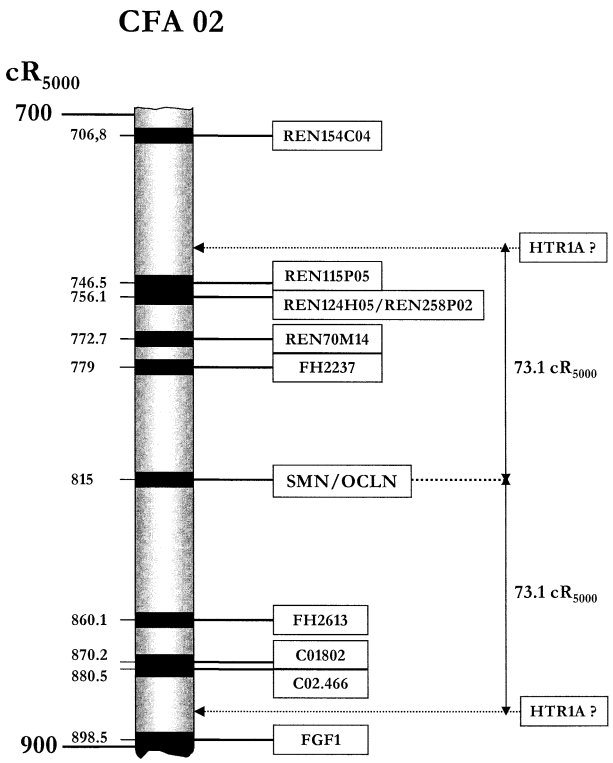

Figure 5. Schematic representation of a part of canine chromosome 2, running from 700 cR_{5000} to 900 cR_{5000}, based on radiation hybrid data from http://www-recomgen.univ-rennes1.fr/cgi-dog/display-hsa.prog?hsa=5 (accessed in October 2002). The two possible positions of *htr1A* were calculated on the basis of radiation hybrid mapping of *htr1A* and *smn1*, with the help of a commercially available radiation hybrid panel. Markers REN154C04, REN115P05, REN124H05, REN258P02, REN70M14, FH2237, FH2613, C01802, and C02.466 are all microsatellite markers, whereas *smn1* (survival of motor neuron 1, telomeric), *ocln* (occludin), and *fgf1* [fibroblast growth factor 1 (acidic)] are all gene-based markers.

Sequence Analysis

With the help of the 160O12 BAC clone, we have managed to reveal the entire coding sequence of *htr1A* in the domestic dog, including 22 bp at the 5′-flanking side and 45 bp at the 3′-flanking side. The gene consists of only one exon, which is 1272 bp long (Figure 3). In dogs, the gene is longer than in humans (1269 bp) and mice (1266 bp). Homology of the exonic nucleotide sequence with human and murine sequences is high (89% and 85% sequence identity, respectively). The corresponding protein consists of 423 amino acids. Amino acid homology reached 92% with human and 85% with mouse.

Several regions in the protein were even more homologous between the species (Figure 4). The seven hydrophobic (transmembrane) regions consist of amino acid residues 37–62, 74–98, 110–132, 153–178, 192–217, 346–367 and 379–403 in humans, mice, and dogs (where the initiator ATG codon is designated number 1). Amino acid composition of these seven regions is 100% identical between dog and human. The murine amino acid composition is different at two positions (residues 177 and 379). At amino acid positions 147–151 and 227–232 two sites for phosphorylation by protein kinase C can be found in the human, canine, and murine protein. These regions are 100% conserved. Furthermore, three asparagine residues (10, 11, and 24) that were indicated as potential sites for glycosylation by Kobilka et al. (1987) can be found at identical positions in human, mouse, and dog.

Radiation Hybrid Mapping

htr1A was retained in 26 hybrids (28.5%). The calculated distance between *htr1A* and *smn1* was 34.8 cR_{3000} (LOD score = 9.7). Because 1 cR is estimated to be 210 kb for this RH panel (van de Sluis et al. 2000) and because *smn1* has been mapped to *cfa02*, *htr1A* is expected to be localized about 7.3 Mb from *smn1* on canine chromosome 2. These results imply that *htr1A* will be positioned either between microsatellite markers REN154C04 and REN115P05, or between microsatellite marker C02.466 and gene-based marker FGF1 on the 2001 integrated dog map, assuming that 1 cR_{5000} corresponds to 100 kb (Breen et al. 2001; see Figure 5). Not surprisingly then, no linkage (LOD score = 0) was found between *htr1A* and *crtl1*, which has been mapped to canine chromosome 3.

Partial Sequence Analysis of *htr1A* in Four Golden Retrievers

Sequence analysis of base pairs 115–1250 in four golden retrievers revealed that all of these dogs had cytosine instead of adenine at position 808 in their *htr1A* sequence. Amino acid 270 will therefore be glutamine instead of lysine in these dogs (Figures 3 and 4). This finding might point to breed-specific differences in the structure of serotonin receptor 1A. No other differences were found between the BAC 160O12 sequence and the genomic golden retriever sequence.

Discussion

We have isolated a canine BAC clone containing the gene encoding serotonin receptor 1A, and we have determined the entire coding sequence of this gene. The 1272 bp intronless gene is very similar to human and murine *htr1A*, especially in transmembrane regions and at the sites for phosphorylation and glycosylation. Canine *htr1A* is more homologous to human *htr1A* than to murine *htr1A*; 89% of the canine nucleotides are identical to the human sequence, whereas human and mouse share only 85% of their nucleotides. At the amino acid level, dog and human are 92% identical, whereas mouse and human are 88% the same.

In the short stretch of 5′-flanking region that we have sequenced, several promoter elements can be recognized. Both human and mouse 5′-flanking regions of *htr1A* have

been shown to contain complex TATA-less promoters (Parks and Shenk 1996). It will be very interesting to find out more about the *htr1A* promoter region in the dog by sequencing farther in the 5′ direction.

Radiation hybrid mapping showed that *htr1A* resides on *cfa02*, at a distance of 34.8 cR_{3000} from *smn1*. These results are in accordance with the human situation, where *htr1A* and *smn1* are separated by about 6 Mb on *hsa05* (Human Map Viewer, http://www.ncbi.nlm.nih.gov/cgi-bin/Entrez/maps.cgi?org=hum&chr=5). Additional detailed mapping will reveal whether *htr1A* is positioned proximally or distally from *smn1*.

An A→C substitution was found in four golden retrievers, resulting in a different amino acid at position 270. As can be seen in Figure 4, this amino acid is not situated in a very conserved region of the protein. The region forms one of the extracellular loops of the receptor, and, because the ligand binding site of the 5-HT_{1A} receptor is localized in the membrane domains (Shih et al. 1991), it is not expected that this substitution of residue 270 results in altered ligand-binding properties of the receptor. It will be very interesting to determine the rest of the *htr1A* sequence in these golden retrievers and to compare these sequences with those in other breeds. Moreover, a comparison between anxious or aggressive members of a certain dog breed and "normal" dogs of the same breed will be useful.

In conclusion, we have cloned, characterized, and mapped one of the genes of the serotonergic system in the dog. These data are valuable for candidate gene studies of behavioral disorders in dogs, and they provide a new Type I marker on the canine genome map. Future studies will be directed at determination of the *htr1A* sequence and its promoter region sequence in several dog breeds and in dogs with behavioral disorders.

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