NOTE  Ethology

Sequence Comparison of the Dopamine Receptor D4 Exon III Repetitive Region in Several Species of the Order Carnivora

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ABSTRACT. It was previously demonstrated that the dog dopamine receptor D4 (DRD4) gene is polymorphic in terms of the repeat number and/or order of 39- and 12-bp sequences located in the third exon. To examine whether or not the repetitive region is present in other species of the order Carnivora, the homologous region of DRD4 genes were sequenced in the gray wolf, raccoon dog, Asiatic black bear, common raccoon and domestic cat. In the family Canidae, the wolf had an identical sequence to that of the dog 447b allele, and a repetitive sequence similar to the dog DRD4 was also recognized in the raccoon dog. On the other hand, no obvious repeated structure was observed in the sequences of the bear, raccoon and cat.

KEY WORDS: Carnivora, dopamine receptor D4, repetitive sequence.


Human dopamine receptor D4 (DRD4) is polymorphic in terms of the repeat numbers of the 48-bp sequence in the third exon of the gene [15]. It has been demonstrated that the DRD4 genotype is possibly related to the personality trait known as novelty seeking [1, 2]. We reported previously that DRD4 is also polymorphic in the dog (Canis familiaris) [10] and seven alleles were identified based on the number and/or order of 39- and 12-bp sequences located in the homologous region of human DRD4 [12]. In addition, a distinct difference in allele frequency was observed in several dog breeds with different behavioral traits [7, 12] and the DRD4 genotype may be related to certain behavioral characteristics (e.g. aggression to dogs) in dog breeds [11].

The sequence and repeated structure of the polymorphic region of the DRD4 gene differ considerably between primates [5, 6, 8, 9] and dogs, and a repetitive sequence does not exist in the homologous region of the rat [3], mouse [13] and carp [4] DRD4 genes. The carnivores are classified into 231 species belonging to nine genera of seven families. Therefore, analysis of various species of the order Carnivora may provide useful information for understanding the origin of the repetitive region and diversity of the DRD4 genes in dogs. In the present study, we amplified the nucleotide sequences corresponding to the repetitive region in dog DRD4 employing the following species: the gray wolf (Canis lupus, n=8) and raccoon dog (Nyctereutes procyonoides, n=3) of the family Canidae; the Asiatic black bear (Ursus thibetanus, n=13) of the family Ursidae; the common raccoon (Procyon lotor, n=7) of the family Procyonidae; and the domestic cat (Felis sylysvestris catus, n=6) of the family Felidae.

Genomic DNA was extracted from peripheral blood taken from wolves and raccoon dogs kept at the Kyoto Municipal Zoo and Tama Zoological Park, respectively. Raccoon and bear samples were derived from wild-caught specimens. Cat samples were obtained from pet animals. The DRD4 exon III region corresponding to the repetitive region in dog DRD4 was amplified by the polymerase chain reaction (PCR) employing forward D4F (5’-TTCCTCCTACCCCTGCCTGCTATG-3’) and reverse D4R (5’-GACCACCAACCGGCAGGACCCTCAT-3’) primers according to Niimi et al. [10]. The PCR products were purified and then directly sequenced by the dye termination method using a 377 DNA Sequencer (Perkin-Elmer, Applied Biosystems Division, CA).

Figure 1 shows the alignment of the deduced amino acid sequences of DRD4. The wolf had an identical nucleotide sequence to that of the dog 447b allele [10], in which 17 amino acids were deleted in the repetitive region from the sequence of the dog 498 allele. The sequences of the 5’ upstream region were conserved among the dog, wolf and raccoon dog, and a relatively short sequence homologous with the dog repetitive region was recognized in the sequence of the raccoon dog. On the other hand, amino acid substitutions were noted in the 5’ downstream regions of the sequences of the bear, common raccoon and cat. Sequences corresponding to the dog repetitive sequences were not obvious in these species. In addition, individuals within the same species shared only one genotype in their DRD4, which was estimated from the size of PCR products (data not shown).

Figure 2 illustrates the results of dot matrix analysis of the deduced amino acid sequences. Each figure contains a plot of the respective DRD4 sequence against itself. Several repeated domains were recognized in the dog 498 and wolf sequences; for example, [P(G/I)]PPPDPGSPDTG(S/P)DG(T/N)] and [P(P/N)PDPGDSP(G/D)(T/N)], which are indicated by “a” and “b”, respectively. In the wolf, one “a” domain was deleted compared to the dog 498 sequence. In the raccoon dog, a repeated domain such as PDGTPGPPP-
Fig. 1. Multiple alignment of deduced amino acid sequences of the \textit{DRD4} exon III repetitive region. PCR-derived nucleotide sequences from the wolf, raccoon dog, raccoon, bear and cat were translated and aligned with the previously reported dog sequence. Dots indicate sequence identity with the dog sequence, while dashes represent gaps introduced to optimize the alignment. The arrows above the sequence indicate the repetitive region of dog \textit{DRD4} [10]. The nucleotide sequences of the \textit{DRD4} genes can be obtained from the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB044885 (dog allele), AB069661 (gray wolf), AB069662 (raccoon dog), AB069663 (common raccoon), AB069664 (Asiatic black bear) and AB069665 (domestic cat). The wolf nucleotide sequence was identical to that of the dog allele (AB030236).

Fig. 2. Dot matrix analysis of the amino acid sequences. Repeated structures were estimated in the deduced amino acid sequences employing the Dotlet program available on the Internet (http://www.isrec.isb-sib.ch/java/dotlet/ Dotlet.html). R represents the repetitive region. The repeated domains are indicated by “a”, “b” and “c” in the dog allele, wolf and raccoon dog sequences.
PDG(S/T)PD] was included at the positions indicated by “c”. There was no obvious repeated domain in the raccoon, bear and cat sequences.

Among the species of the order Carnivora examined in the present study, only the wolf and raccoon dog in the family Canidae had repetitive sequences in DRD4. A PCR product with the same length as that of the dog 498 allele was identified in the wolf samples by electrophoresis (data not shown), suggesting that DRD4 is also polymorphic in wolves. However, its nucleotide sequence could not be determined because the amount of DNA sample was insufficient for sequencing. It has been suggested from an analysis of D loop sequences of mitochondrial DNA that dogs might have originated from or interbred with wolves [14, 16]. Additional samples from wolves are needed to make a precise estimation of the relationships among wolf and dog DRD4 alleles. However, its nucleotide sequence could not be determined because the amount of DNA sample was insufficient for sequencing. It has been suggested from an analysis of D loop sequences of mitochondrial DNA that dogs might have originated from or interbred with wolves [14, 16]. Additional samples from wolves are needed to make a precise estimation of the relationships among wolf and dog DRD4 alleles. Although a repeat structure was recognized in the raccoon dog, no difference in the size of the PCR products was observed in three samples (data not shown). Therefore, it remains obscure whether or not raccoon dog DRD4 is polymorphic.

Based on the DNA-DNA hybridization test, it has been suggested that the family Canidae might have diverged at an earlier time than other carnivore families, approximately 50 million years ago [17]. The present data imply that the repetitive region of DRD4 gene might have developed following the divergence of the family Canidae. Further analysis of various species of the family Canidae is required in order to elucidate the origin of the diversity of the DRD4 gene and the detailed significance of DRD4 polymorphism in dogs.

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