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Cytogenetic mapping and STR polymorphism of two candidate genes (*DRD2* and *HTR1D*) for behaviour traits in four canids

(short communication)

Abstract

The dopamine D2 receptor (*DRD2*) and serotonin receptors 1D (*HTR1D*) are candidate genes for behavioural traits. In the present study, we show chromosomal location and polymorphism of these genes in four species from the family *Canidae*: dog (CFA), red fox (VVU), arctic fox (ALA) and the Chinese raccoon dog (NPP). Using fluorescence *in situ* hybridisation (FISH) the *DRD2* gene was localized in the following chromosomes: CFA5q12-13, VVU12q21, ALA10q14 and NPP3q14 and the *HTR1D* gene was mapped to: CFA2q25, VVU2q22, ALA8q25 and NPP10q25. A microsatellite marker $(TG)_n$ in intron 3 of the *DRD2* gene and $(CA)_n$ motif located in a 3'-flanking region of the *HTR1D* gene were polymorphic in all studied species. The obtained results can be helpful in further studies on effects of polymorphisms of these genes on behaviour traits in canids.

Key Words: behaviour, *Canidae*, *DRD2*, *HTR1D*, gene mapping, microsatellite polymorphism

Zusammenfassung

Titel der Arbeit: **Zwei Kandidaten-Gene (*DRD2* und *HTR1D*) für Verhaltenseigenschaften: Cytogenetische Lokalisation und STR Polymorphismen in vier *Canidae*** (Kurzmitteilung)

Der Dopamin D2 Rezeptor (*DRD2*) und die Serotonin Rezeptoren 1D (*HTR1D*) sind Kandidaten-Gene für Verhaltenseigenschaften. In der vorliegenden Studie werden die chromosomale Lokalisation und Polymorphismen dieser Gene in vier Arten der Familie *Canidae*: Hund (CFA), Rotfuchs (VVU), Polarfuchs (ALA) und Chinesischer Marderhund (NPP), gezeigt. Mit Hilfe der Fluoreszenz *in situ* Hybridisierung (FISH) konnte die Lokalisation des *DRD2*-Gens auf den folgenden Chromosomen identifiziert werden: CFA5q12-13, VVU12q21, ALA10q14 und NPP3q14. Das *HTR1D*-Gen wurde auf CFA2q25, VVU2q22, ALA8q25 und NPP10q25 lokalisiert. Ein Mikrosatellitenmarker $(TG)_n$ im Intron 3 des *DRD2*-Gens und ein $(CA)_n$ Motif, welches in der 3`-Region des *HTR1D*-Gens lokalisiert ist, sind in allen Arten polymorph. Die erzielten Ergebnisse können in weiteren Studien, die sich über die Effekte von Polymorphismen dieser Gene auf das Verhalten in *Canidae* befassen, hilfreich sein.

Schlüsselwörter: Verhalten, *Canidae*, *DRD2*, *HTR1D*, Kartierung von Genen, Mikrosatelliten-Polymorphismen

Introduction

The family *Canidae* is consisted of 36 species, including dog (*Canis familiaris)* and three fur-bearing animals: red fox (*Vulpes vulpes)*, arctic fox (*Alopex lagopus*) and Chinese raccoon dog (*Nyctereutes procyonoides procyonoides)*. Due to extensive variability of behaviour traits observed in different dog breeds, the dog and silver fox are considered as a useful models to study genetic background of it (OSTRANDER and WAYNE, 2005; SPADY and OSTRANDER, 2007).

Among candidate genes for behaviour traits there are dopamine D2 receptor (*DRD2*) and serotonin receptors 1D (*HTR1D*) genes. The DRD2 is one of five dopamine receptors and the encoded protein inhibits adenylyl cyclase activity and mitogenesis. It has been shown that mutation of this gene can be associated with human schizophrenia (GLATT et al., 2003). The serotonin is a neurotransmitter and genes encoding its receptors were mentioned to be associated with human mental disorders (BERGEN et al., 2003).
In this report we show chromosomal FISH-localization and STR polymorphism of *DRD2* and *HTR1D* genes in four species of the family *Canidae*: dog (CFA), red fox (VVU), arctic fox (ALA) and Chinese raccoon dog (NPP).

Materials and Methods

Fragments of two genes (*DRD2* and *HTR1D*) were amplified by PCR using a T-gradient thermocycler (Biometra, Goettingen, Germany). Primers for the *DRD2* gene used in this study were previously described by MYEONG et al. (2000). The genome sequence of this gene is also deposited in GenBank (AF_293962). The primers for *HTR1D* were as follows: F:5'ACTACGTATGTCTGGCAAAACTTC3' and R:5'TCTACACTCTCCCTTAAACACTGG3' (GenBank NW_876292). Amplifications were carried out in 20 µl containing 100 ng of DNA, 15 pmol of each primer, 200 µM of dNTP, 1×PCR buffer with 1.5mM $MgCl_2$ and 0.6 units *Taq* polymerase (Novazym, Poznan, Poland). The following PCR reaction conditions were applied: the initial denaturation at 94ºC for 5 minutes, 35 cycles of denaturation at 94°C for 40 seconds, annealing of primers at 63°C for *DRD2* gene and 52°C for *HTR1D* gene for 40 seconds, elongation at 72ºC for 45 seconds, final elongation at 72ºC for 10 minutes. One primer of each pair was labeled with Cy5 and DNA fragments were separated and detected on 8% denaturing polyacrylamide gel using automatic sequencer ALFexpressII (Amersham Bioscience, Buckinghamsire, UK). To confirm the presence of repetitive motifs in analysed fragmets of both genes, the samples of PCR products from one animal were sequenced at Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland.
Chromosome preparations were obtained from short-term lymphocyte cultures and stained by Q-banding technique prior to FISH. Chromosome nomenclatures for the dog (SWITONSKI et al., 1996), red fox (MÄKINEN et al., 1985a), arctic fox (MÄKINEN et al., 1985b) and the Chinese raccoon dog (PIENKOWSKA et al., 2002) were applied.
A canine genomic bacterial artificial chromosome (BAC) library (SCHELLING et al., 2002) was screened for *DRD2* and *HTR1D* genes by PCR. DNA of the two BAC clones, containing sequences of the selected genes (S091P05C10 – for *DRD2*, S002P01E05 for *HTR1D*) was isolated and labeled with biotin 16-d-UTP by random priming. Fluorescence *in situ* hybridization was carried out as described earlier (SZCZERBAL et al., 2003). Slides were analyzed under a fluorescence microscope (Nikon E 600 Eclipse) equipped with a cooled digital CCD camera, driven by computer aided software Lucia.

Results

The selected BAC probes gave positive FISH signals in all studied species (Fig.). The *DRD2* gene was localized in the following chromosomes: CFA5q12-13, VVU12q21,

ALA10q14 and NPP3q14 and the *HTR1D* gene was assigned to: CFA2q11, VVU2q22, ALA8q25 and NPP10q25.

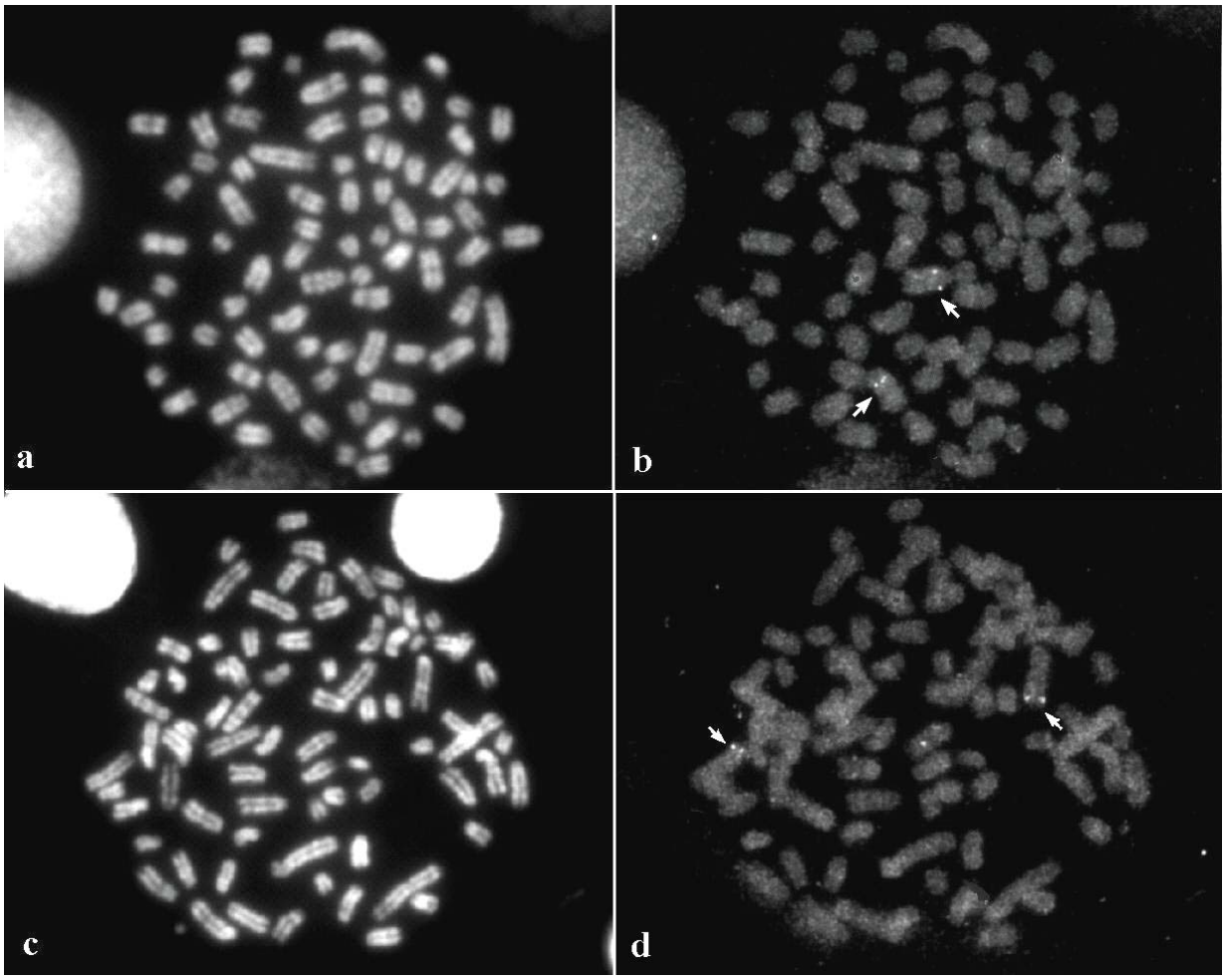

Fig.: The dog, Q-banded metaphase spreads (a, c) and the same spreads after FISH with the BAC probes harbouring *DRD2* (b) and *HTR1D* genes (d) Hybridization signals are indicated by arrows (Der Hund, Q-Banden von Metaphasechromosomen (a, c) und FISH auf denselben Metaphasechromosem mit BAC Sonden der *DRD2* (b) und *HTR1D*-Gene (d). Hybridisierungssignale sind durch Pfeile gekennzeichnet)

Obtained results are in agreement with data from comparative chromosome painting studies performed for canid species (GRAPHODATSKY et al., 2001; NIE et al., 2003). Moreover, in case of the *DRD2* gene our results confirm previous data obtained by radiation mapping for the dog (JEOUNG et al., 2000).

Within both genes polymorphism of the STR (microsatellite) markers was also studied. In the *DRD2* gene a polymorphic sequence $(TG)_n$ in the intron 3, described earlier in the dog (MYEONG et al., 2000), was analysed. In case of the *HTR1D* gene *in silico* analysis revealed a potentially polymorphic STR - $(CA)_n$ - in the 3'flanking region.

Polymorphism was studied in DNA samples originated from 36 dogs, representing 18 breeds, and 70 fur animals kept on a farm (24 Chinese raccoon dogs, 24 red foxes and 22 arctic foxes). Sequencing of PCR products confirmed the presence of $(TG)_{11}$ repeated motif in intron 3 of the *DRD2* gene and $(CA)_{23}$ motif in 3'flanking region of the *HTR1D* gene. Number of alleles, size range, heterozygosity (HET) and polymorphism information content (PIC) values are shown in the Table. The analyzed

STR markers appeared to be highly polymorphic and thus will be useful in the association studies.

Table
Chromosome location and polymorphism of *DRD2* and *HTR1D* genes in dog, red fox, arctic fox and Chinese raccoon dog (Chomosomenlokalisation und Polymorphismen der DRD2 und HTR1D-Gene bei Hund, Rotfuchs, Polarfuchs und Chinesischem Marderhund)

	Gene	Dog	Red fox	Arctic fox	Chinese raccoon dog
DRD2	**Chromosome location**	5q12-13	12q21	10q14	3q14
	Number of allels	5	5	4	2
	Size range[a)]	11-17	12-16	10-15	10-11
	HET	0.676	0.699	0.678	0.191
	PIC	0.612	0.630	0.599	0.169
HTR1D	**Chromosome location**	2q25	2q22	8q25	10q25
	Number of allels	12	11	8	5
	Size range[b)]	18-29	6-21	10-19	16-22
	HET	0.872	0.869	0.730	0.480
	PIC	0.845	0.835	0.679	0.440

a) - number of repeated motif (TG) in intron 3; b) - number of repeated motif (CA) in 3'flanking region

Discussion

To understand genetic background of behaviour traits several other candidate genes were recently analyzed. The dopamine receptor D4 (*DRD4*) gene polymorphism was studied in 23 dog breeds, including 1535 unrelated individuals (ITO et al., 2004). The authors divided the studied dogs into two groups, taking into consideration behaviour traits, and found that some alleles were more frequent in the group with a higher average score of "aggressiveness" and the others in a group with the lower score. Also other genes (*HTR1A*, *HTR2A*, *HTR1B*, and *HTR2C*), encoding canine serotonin receptors, were considered (KLUKOWSKA-ROTZLER et al., 2005; MASUDA et al., 2004; VAN DEN BERG et al., 2005). In the *HTR1A* gene microsatellite markers (KLUKOWSKA-ROTZLER et al., 2005) and two nonsynonymous SNPs were found (VAN DEN BERG et al., 2005). Interbreed allele and genotype frequency differences in case of four SNPs, found in *HTR1B* gene, were identified (MASUDA et al., 2004).

To our knowledge this study brings new data on chromosome gene localisation and microsatellite polymorphism of two genes (*DRD2* and *HTR1D*) which were not yet been studied in the fur-bearing species. This may enable further studies on verification of the major role of these candidate genes.

Recently, a linkage mapping of 320 microsatellite markers in the silver fox, performed on thirty-four pedigrees, originating from breeding tame and aggressive strains, were published (KUKEKOVA et al., 2007). Genome scanning of such reference families, will probably result in identification of chromosomal regions harbouring genes for behaviour.

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