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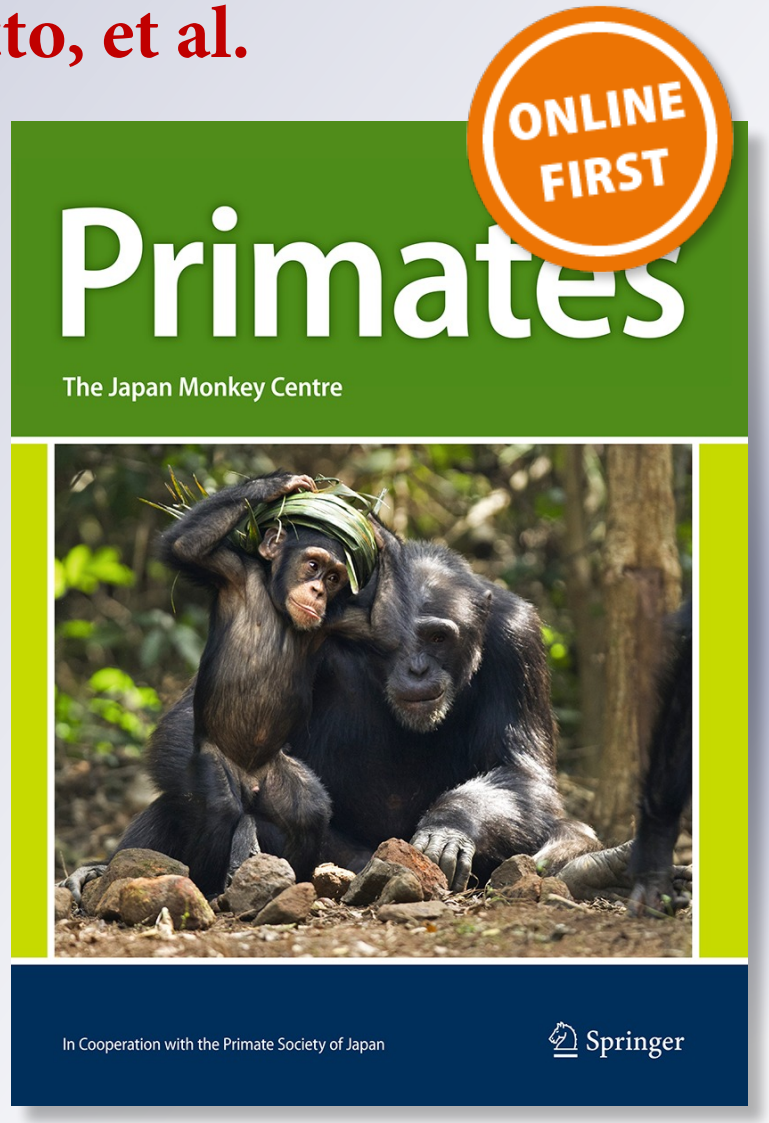

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ORIGINAL ARTICLE

Polymorphism of the 3′-UTR of the dopamine transporter gene (*DAT*) in New World monkeys

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Abstract Genetic polymorphism in the 3′-untranslated region (3′-UTR) of the dopamine transporter (*DAT*) gene has been reported in both human and nonhuman primates, and the variable number of tandem repeats (VNTR) polymorphism has been related to several neurological and psychiatric disorders. As New World primates have been employed as models in biomedical research in these fields, in the present study we assessed genetic variation in the *DAT* gene in 25 robust capuchin monkeys (*Sapajus* spp.) and 39 common marmosets (*Callithrix jacchus*). Using enzymatic amplification followed by sequencing of amplified fragments, a VNTR polymorphism in the 3′-UTR region of the *DAT* gene was identified in both robust capuchins and common marmosets. The polymorphic tandem repeat of 40-bp basic units is similar to the human VNTR consensus sequence, with size variants composed of 9, 10, and 11 units in marmosets and 8, 9, 13, and 17 basic units in capuchins. We found behavioral evidence that carrying the 10-repeat *DAT* allele promotes flexible choice and maximization of foraging in marmosets tested in an operant choice paradigm. Moreover, in an intertemporal choice task, capuchins with longer repeat variants show less self-controlled choices than capuchins with at least one short repeat variant. Future research should focus on the relationship between these *DAT* polymorphisms, dopamine reuptake via the dopamine transporter, and behavioral and cognitive variation across New World monkey individuals.

Keywords Dopamine transporter gene · *Callithrix jacchus* · *Sapajus* spp. · Common marmoset · Robust capuchin monkeys · New World primates · Platyrrhini · Gene polymorphism

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Introduction

Molecular genetics and pharmacological studies suggest the involvement of the dopamine transporter gene (*DAT*) in a wide spectrum of neurological and psychiatric disorders, including Parkinson's disease, depression, schizophrenia, drug abuse, affective disorders, Tourette's syndrome, and attention deficit hyperactivity disorder (ADHD) (Bannon et al. 2001; McHugh and Buckley 2015), as well as in the etiology of pathological gambling (Ibánez et al. 2003). The human dopamine transporter (hDAT) is expressed most strongly in the striatum, where it plays an important role in the regulation of dopaminergic levels through the reuptake of dopamine (DA) from the synaptic cleft into the presynaptic terminal. Concurrent with the cloning and chromosomal mapping of human *DAT* to the short arm of chromosome 5, a variable number of tandem repeats

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polymorphism (VNTR) was identified in the 3′-untranslated region (UTR) (Vandenbergh et al. 1992). The 40-bp VNTR element is repeated between 3 and 13 times, and in most human populations occurs with the greatest frequency in the 9- and 10-repeat forms (Doucette-Stamm et al. 1995; Kang et al. 1999; Mitchell et al. 2000). The 3′-UTR sequence may influence mRNA stability or translation efficiency (Fuke et al. 2005). Given the prominent role of DA neurotransmission in normal and abnormal behaviors, the *DAT*-VNTR became the object of numerous genetic linkage and association studies, in vitro reporter gene experiments, in vivo single-photon computed emission tomography (SPECT) molecular imaging studies, and pharmacogenetic examinations of the well-documented interindividual variation in the response to treatment with DAT inhibitors (see McHugh and Buckley 2015 for a review).

This polymorphic variation may be a relatively recent trait, as a VNTR homologue has been observed in humans (*Homo sapiens*), chimpanzees (*Pan troglodytes*), and crab-eating macaques (*Macaca fascicularis*), but not in other mammals such as the rat (*Rattus norvegicus*) and the mouse (*Mus musculus*) (Wu and Gu 1999; Miller et al. 2001). In chimpanzees, the *DAT*-VNTR was found to be polymorphic with one-repeat (82.7 %) and two-repeat (17.3 %) alleles (Inoue-Murayama et al. 2002). Such short repeated alleles have not been described in humans. Among the other nonhuman primates examined, only a two-repeat allele was detected in gorillas (*Gorilla gorilla*) and orangutans (*Pongo pygmaeus*), a five-repeat allele was noted in African green monkeys (*Chlorocebus aethiops*), while the 12-repeat allele is the most common (99.5 %) in crab-eating monkeys (Inoue-Murayama et al. 2002).

New World monkeys (Platyrrhini) are important and highly utilized animal models within several areas of biomedicine where the dopaminergic system plays an important role. To our knowledge, there are no data on VNTR polymorphism of the *DAT* gene for New World primates. Common marmosets (*Callithrix jacchus*) are used in neurobiological (Mitchell et al. 2014; Stassart et al. 2015), toxicological (Orsi et al. 2011; Seehase et al. 2012), and immunological (t'Hart et al. 2015) studies, as well as in the field of neurodegenerative disorders (Uchida et al. 2015). Furthermore, the common marmoset is increasingly popular for experiments in neuroscience, including studies into the mechanisms of decision-making disorders (Adriani et al. 2013; Clarke et al. 2015) and pathological gambling (Tokuno and Tanaka 2011; Zoratto et al. 2014).

Capuchin monkeys have been successfully employed as models of Huntington's disease (Roitberg et al. 2002), Parkinson's disease (Emborg and Colombo 1994), and tardive dyskinesia induced by chronic therapy with dopamine receptor-blocking agents (Blanchet et al. 2012) but, as suggested (Phillips et al. 2010), they may also be used as models of nonmotor disorders such as ADHD, in which there is an involvement of frontal lobes and basal ganglia (Castellanos et al. 1996; Casey et al. 2007; Qiu et al. 2009; Silk et al. 2009). Also, a promising model of human pathological gambling might be developed with robust capuchins, since they show a preference for a risky option over a safe option (De Petrillo et al. 2015a). Furthermore, capuchin self-control abilities have been extensively explored in several behavioral studies using different experimental paradigms (for a review, see De Petrillo et al. 2015b), with results often similar to those reported for great apes, our closest living relatives (Addessi et al. 2011).

The work reported in the present paper aimed to evaluate whether a VNTR polymorphism is present in the 3′-UTR region of the *DAT* gene in two New World primates, the common marmoset and the robust capuchin monkey. This information will also be valuable in order to assess whether this polymorphism originated before the New World primate lineage split from the Old World primate lineage about 35 million years ago (Schrago and Russo 2003) or it is a more recent acquisition. In addition, the study included a reanalysis of marmoset data on individual capacity to maximize foraging and/or to flexibly change own choice strategy (Adriani et al. 2013) and capuchin data on individual performance in a self-control paradigm (Addessi et al. 2011) in relation to the *DAT* genotype of the individuals tested in the above studies.

As recent research into capuchin monkey morphology and phylogenetics has revealed that there may be up to eight distinct species within the robust capuchin genus, *Sapajus* (Lynch Alfaro et al. 2012a, b, 2014), a further goal of this study was to identify capuchin monkeys of the Institute of Cognitive Sciences and Technologies (ISTC) colony at the species level and compare the VNTR *DAT* polymorphism frequencies across species.

Methods

Animals

We analyzed DNA samples from 39 common marmosets (18 females and 21 males) and 25 robust capuchin monkeys (12 females and 13 males). Twenty-four marmosets (11 females and 13 males) were housed at the Istituto Superiore di Sanità (Section of Behavioral Neuroscience, Department of Cell Biology and Neuroscience) in Rome, Italy, and originated from four different founding pairs. Data on the kinship among these founders are not available. Fifteen marmosets (7 females and 8 males) were housed at Aptuit (Preclinical Technologies Department), Verona, Italy. This colony originally derived from two

distinct groups from the University of Strasbourg and Harlan UK. No data on kinship are available.

All capuchins were housed at the Primate Centre of the Institute of Cognitive Sciences and Technologies (CNR, Rome, Italy). One male subject originated from the Helping Hands breeding colony (Dr. Moshe Bouchmitz D.V.M., Israel), two subjects (one male and one female) originated from a female from Peru that was confiscated at Rome Airport, and two subjects (one male and one female) were the offspring of two different females from South American Primates Inc., Miami, FL, USA (wild-caught in Guyana and probably unrelated). Seven subjects (4 females and 3 males) were the offspring of a female (Punk) housed at Rome Zoo since 1982 and of unknown origin. The remaining 13 subjects were also all second- or third-generation descendants of Punk.

All individuals were socially housed and environmental enrichment was provided, in compliance with the commitments on accommodation and care for laboratory animals as described by the relevant current Italian law (D.Lgs.26/2014). The present study adhered to the American Society of Primatologists and the ASAB/ABS guidelines for the treatment of animals in behavioral research and teaching (ASAB/ABS, 2014).

Polymerase chain reaction (PCR) and DNA sequence VNTR at the 3′-UTR region of DAT

Genomic DNA was extracted from 0.5–1 ml of an EDTA peripheral blood sample following the standard protocol. The 3′-UTR repeated sequence of the *DAT* gene was amplified by the polymerase chain reaction (PCR). The primer sequences employed were 5′-GCCTGTGGGTCCT TGTGGTGTA-3′ (DAT F-Primate, forward) (Inoue-Murayama et al. 2002) and 5′-CTTCCTGGAGGTCACGGCT CAAGG-3′ (DAT R, reverse) (Vandenbergh et al. 1992) for capuchins, and 5′-TGTGGGTGCTTGTGGTGTAG-3′ (DAT F-Mar, forward) and 5′-AGGCCAGGCAGAGTGT GG-3′ (DAT R-Mar, reverse) for marmosets.

PCR amplification was carried out in a final volume of 50 µl containing 50 ng genomic DNA, 1.5 mM $MgCl_2$, 200 µM dNTP, 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.25 µM of each primer, and 2 U of Promega Taq DNA polymerase. Amplification conditions were: 94 °C for 45 s, 60 °C for 30 s, 72 °C for 30 s repeated for 35 cycles. PCR products were analyzed by electrophoresis on 6 % acrylamide gel stained with ethidium bromide. The genotype was estimated from the size of the PCR product and confirmed by nucleotide sequence. The PCR products were purified by gel electrophoresis and then sequenced in both strands using the dye termination method and an ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA) for sequence and repeat number assessment, as well as for genotyping. The primers used for DNA sequencing were the same as those used for PCR amplification.

The sequences were deposited in the GenBank with the following accession numbers:

KU696967 *Callithrix jacchus* dopamine transporter 3′UTR, 9-repeat allele.
KU696968 *Callithrix jacchus* dopamine transporter 3′UTR, 10-repeat allele.
KU696969 *Callithrix jacchus* dopamine transporter 3′UTR, 11-repeat allele.
KT279589 *Sapajus* spp. dopamine transporter 3′UTR, 8-repeat allele.
KT279590 *Sapajus* spp. dopamine transporter 3′UTR, 9-repeat allele.
KU696965 *Sapajus* spp. dopamine transporter 3′UTR, 13-repeat allele.
KU696966 *Sapajus* spp. dopamine transporter 3′UTR, 17-repeat allele.

The marmoset, capuchin, and human sequences were aligned using the DNA Block Aligner (DBA) program (http://www.ebi.ac.uk/Tools/psa/wise2dba). This program aligns two sequences from noncoding regions under the assumption that they share a number of collinear conserved blocks separated by potentially large and varied lengths of DNA in the two sequences. The conserved blocks may have one or two gaps in them, and each block falls under one of four different identity-substitution probabilities, roughly equivalent to sequence conservation at 65, 75, 85, or 95 % identity. Linear gaps have been modeled in the blocks at a fixed probability of 0.05, and the blocks are expected to comprise around 1 % of the DNA sequence (Jareborg et al. 1999).

D-loop and cytochrome *b* (cyt *b*) mitochondrial genes

Although nuclear markers for capuchin species identification are not yet available, we used the mitochondrial markers cytochrome *b* (cyt *b*) and D-loop to identify the capuchin matrilines by haplotype. D-loop and cyt *b* were amplified by PCR, sequenced, and analyzed to distinguish among the *Sapajus* spp. Primers for D-loop were: CR_HR F 5′-CTRCCRTCAACACCCAAAG-3′ and CR_AR R 5′-CATCCAGTGACGCGGTTAAGA-3′, and amplification conditions were: 94 °C for 30 s, 55 °C for 30, 72 °C for 60 s repeated for 35 cycles (Boubli et al. 2012). PCR conditions for cyt *b* amplification were: 94 °C for 30 s, 58 °C for 60 s, and 72 °C for 60 s. The forward primer was CB1F 5′-ATCTGCCCACACCATCCAAC-3′ and the reverse primer was CB2R 5′-CCTCAGAATGATATTTGG CCTC-3′ (Lynch Alfaro et al. 2012a). Sequences recovered

for each of the five robust capuchin matrilines represented in our captive group were deposited to GenBank [accession numbers: *S. cay* KX592673 (cyt *b*), KX592678 (D-loop); *S. macrocephalus* KX592674 (cyt *b*), KX592679 (D-loop); *S. apella* KX592676 (cyt *b*), KX592677 (D-loop), and *S. nigritus* KX592675 (cyt *b*), KX592680 (D-loop)].

To determine the most likely capuchin species for each matriline, we performed a phylogenetic analysis in MrBayes 3.2 with the concatenated cyt *b* and D-loop sequences from the ISTC capuchins and previously published capuchin sequences of known origin and taxonomic affiliation (see Lynch Alfaro et al. 2012b).

Phylogenetic analysis was performed as previously described (Lynch Alfaro et al. 2012; Boubli et al. 2012). We partitioned cyt *b* into all three positions for the codons in the analysis. Samples from ISTC matrilines recovered in the Bayesian tree in clades with specimens from known species with a posterior probability of 1 were considered to be from that species.

Behavioral data associated with VNTR genotype in marmosets and capuchins

In a previous study, marmosets were exposed to an operant choice paradigm and classified for the individual capacity to maximize foraging and/or to flexibly change their own choice strategy (Adriani et al. 2013). They had a series of choices between a Small and Soon (SS) and a Large and Late (LL) food reward. Initially, in the absence of delay, there was a preference for the large reward. The delay associated with this reward was progressively increased (from 0 to 60 s), so that choosing LL would have been suboptimal. Subjects were classified as either "flexible" or "nonflexible" based on a decrease (or not) in the LL preference as the delay increased. Each subject was also classified as a "maximizer" (or "nonmaximizer") based on its (in)capacity to maximize the food payoff as the delay increased (see Adriani et al. 2013 for more details). Here, we analyzed the performance of the marmosets tested in the above study in relation to their *DAT* genotype. Specifically, the eight subjects carrying at least one *DAT* 10 allele (genotypes 9/10 and 10/10) were compared with the seven subjects carrying no such allele (i.e., with genotype 9/9).

Similarly, in a previous study, capuchins were tested in an intertemporal choice paradigm with an adjusting-delay procedure, in which they were presented with a series of choices between a Small and Soon (SS) and a Large and Late (LL) food reward (Addessi et al. 2011). Whereas in the first session both rewards were available immediately, in subsequent sessions the delay to LL was adjusted according to the subject's preference for either reward in the previous session. Each subject was tested up until its indifference point, i.e., the delay at which both food rewards were chosen to a similar extent. The capuchins' delay tolerance showed a wide interindividual variation, and their performance is evaluated here on the basis of their *DAT* genotypes. Specifically, the performance of eight subjects carrying at least one *DAT* 8 allele (genotypes 8/8 and 8/13) was compared with that of seven subjects carrying no such allele (genotypes 9/13, 13/13, and 17/17).

Results

We succeeded in amplifying the region of the *DAT* gene corresponding to the VNTR for the first time in two New World monkey genera. In both marmosets and robust capuchin monkeys, no PCR amplification was detected using the oligonucleotide sequences that specifically amplify the VNTR in human DNA (Vandenbergh et al. 1992), or by using the primer pair described for the other nonhuman primates (Inoue-Murayama et al. 2002). In capuchin monkeys, successful PCR amplification resulted from the use of the forward primer that is reported to amplify the region corresponding to the VNTR in Old World nonhuman primates (Inoue-Murayama et al. 2002) and the reverse primer for the human sequence. The capuchin VNTR region showed fragments 408, 447, 608, and 768 bp in size. The sequences of the amplified fragments were determined and deposited in GenBank. We found that the region corresponding to the VNTR is polymorphic with respect to the number of 40-bp basic units. In the PCR genotyping, 8-repeat (26 %), 9-repeat (20 %), 13-repeat (46 %), and 17-repeat (8 %) alleles were recognized. Confirmation of repeat number inheritance was obtained by analyzing related capuchins. By genotyping the mitochondrial markers D-loop and cyt *b* and performing a phylogenetic analysis, we identified matrilineal haplotypes corresponding to four different *Sapajus* species in the ISTC colony (Fig. 1). Specifically, we identified 20 *S. cay* (Punk matriline), two *S. macrocephalus* (Peru matriline), two *S. apella* (Guyana matriline), and one *S. nigritus* (Helping Hands matriline). Polymorphism of *DAT* was detected in all species: in particular, in *S. cay* we found alleles with 8 (27.5 %), 9 (17.5 %), and 13 repeats (55 %); in the two *S. apella* individuals, alleles with 9 (75 %) and 13 (25 %) repeats were present; in *S. nigritus*, only the allele with 8 repeats was found, while in the two members of *S. macrocephalus* we detected the longest allele, containing 17 repeats present in homozygosity (Table 1). Note that we designated "species" based on matrilineal haplotype only, and there was a high index of hybridization within the colony.

The oligonucleotides that allowed the amplification of the region corresponding to 3′-UTR in the *DAT* gene in

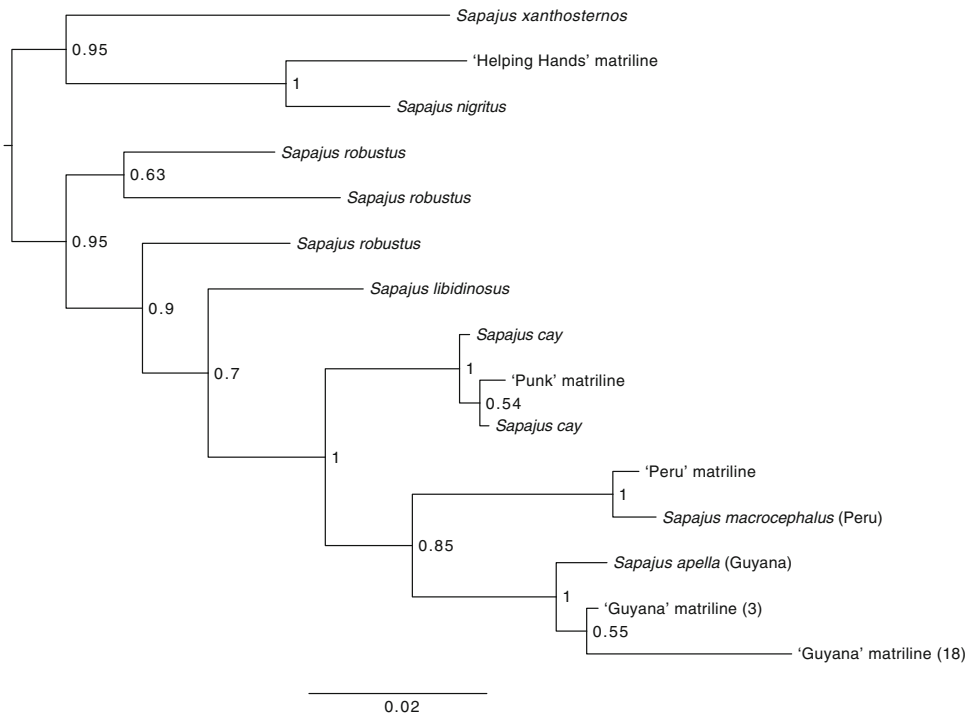

Fig. 1 Bayesian phylogeny using concatenated cyt *b* and D-loop sequences for *Sapajus* individuals representing the five matrilines within the ISTC colony, along with GenBank sequences from individuals of known species, including *Sapajus xanthosternos* (KC757410), *S. apella* [USNM 296634: JN409297 (cyt *b*)], *S. cay* [UMMZ 126131; UMMZ 124696: JN409329 (cyt *b*)], *S. macrocephalus* [AMNH 268240: JN409326 (cyt *b*)], *S. nigritus* [USNM 518524: JN409304 (cyt *b*)], *S. libidinosus* [LACM 27344: JN409299 (cyt *b*)], and *S. robustus* [USNM 518434: JQ317672 (cyt *b*), JQ317618 (D-loop); USNM 518429: JN409331 (cyt *b*), USNM 518327: JN409301 (cyt *b*)]. Unpublished sequences will be uploaded to GenBank upon publication. *Numbers at nodes* are posterior probabilities (*PP*). We assigned matrilines to species when they formed clades with representatives of those species with a PP = 1

Table 1 Polymorphisms identified in the 3′UTR region of the *DAT* gene in common marmoset and capuchin monkeys

Species	No. of samples	No. of repeats (allele frequency)
Common marmoset		
Callithrix jacchus	32	9 (45 %)
		10 (45 %)
		11 (10 %)
Capuchin monkeys		
Sapajus cay	20	8 (27.5 %)
		9 (17.5 %)
		13 (55 %)
Sapajus macrocephalus	2	17 (100 %)
Sapajus apella	2	9 (75 %)
		13 (25 %)
Sapajus nigritus	1	8 (100 %)

marmosets were derived by the alignment (using BLAST, Basic Local Alignment Search Tool) of the human and capuchin VNTR regions with the marmoset genome DNA sequence available in the GenBank (NC_013897.1). In marmosets, we used PCR to detect DNA fragments of 422 bp (9 repeats), 461 bp (10 repeats), and 501 bp (11 repeats) in size. The 11-repeat alleles were present in about 10 % of the subjects analyzed, while the 9- and 10-repeat alleles each were present in 45 % of the marmosets (Table 1). Marmoset PCR genotyping showed only one (homozygote) or two (heterozygote) alleles per individual. Sequences of marmoset DNA fragments were deposited in GenBank.

Analysis of these sequences from both capuchins and marmosets revealed the presence of repeat units 40 bp in length with nucleotide sequences similar to the VNTR present in the 3′-UTR of human DNA (Fig. 2). Among all capuchin and marmoset individuals, the similarity was 95–98 %. The sequences of the VNTR in the 3′-UTR from humans (NG_015885.1), capuchins, and marmosets were aligned using the DNA Block Aligner program in order to look for the most conserved repeat sequences present in both human and New World monkeys. The alignments showed 85–95 % similarity between human and capuchin sequences and 75 % similarity between human and marmoset sequences. Sequence comparisons with the 3′-UTR region of the *DAT* gene available for other nonhuman primates (Inoue-Murayama et al. 2002) revealed between 85 and 95 % similarity. Figure 3 shows the alignment of

I

Ss17 GCCTGTGGGTCCTTGTGGTGTAG c c g t C a c a AGAGGAGTGACCTACCCTGGGATGCATGCAGGCCCCTCAC
Cj 11 - - - -TGTGGGTGCTTGTGGTGTAG c c c t C a c AGAGGAGTGTCCTGCCCCGGGATGCATGCAGACCCCTCAC
Hs11 - - - - - - - - - - - - - - - TGTGGTGTAGGGAACGGCCTGAG -

II III

Ss17 AGGAGCGTGTCCCACCCCAGGACACATGGGCAGACCCCTCAC AGGAACGTGTCCTGCCCTGGGATGCATGCA - ACCCCTCAC
Cj 11 AGGGGCGTGTCCTGCCCTGGGATGCATGCAGACCCCTCAC AGGGGCGTGTCCTGCCCCGGGATGCATGCAGACCCCTCAC
Hs11 -

IV V

Ss17 AGGAGCGTGTCCTGCCCCGAGATGCATGCAGACCCCTCAC AGGAGCGTGTCCTg c CCCgGGACGCATGCAG acCCC gCAC
Cj 11 AGGGGCGTGTCCTGCCCCGGGATGCATGCAGATCCCTCAC AGG gGCGTGTCCTg c CCCg GGA tGCATGCAGa t CCC t CAC
Hs11 - AGGAGCGTGTCCTATCCCCGGACGCATGCAGGGCCCCCAC

VI VII

Ss17 AGGAa CGTGT t CTg c CCCaGGACGCATGCAG a c CCCt CAC AGGAGCg TGTCCTg cCCCg GGACGCATGCAGa c CCCt CA t
Cj 11 AGG gGCa TGTCCTg c CC t gGGA t GCAaGCAG a c CCCt CAC AGG gGCg TGTCCT gcCCCa GGA t GCATGCAGa c CCCt CAC
Hs11 AGGAGCGTGTCCTATCCCCGGACGCATGCAGGGCCCCCAC AGGAGCATGTCCTATCCCTGGACGCATGCAGGGCCCCCAC

VIII IX

Ss17 AGGAGC t TGT cCTg CCCC g GgACGCATGCAGa c C t Ct CA t AGGAGC t TGTc CTgCCCC g Gg ACGCATGCAGa c CCC tCA t
Cj 11 AGGg GCGTGTc CTg CCCC g GgA t GCAaGCAG a c CCCt CAC AGGg GCGTGT cCTg CCC t g GGA tGCAa GCAGa c CCC tCAC
Hs11 AGGAGCGTGTACTACCCCAGAACGCATGCAGGGCCCCCAC AGGAGCGTGTACTACCCCAGAACGCATGCAGGGCCCCCAC

X XI

Ss17 AGGAGCGTGTc CTg CCC t g GGACGCATGCAGa c CCC t CA t aGGAGCGTGTc CT gCCCC g GGAcGCATGCAG a c CCC tCAC
Cj 11 AGGg GCGTGT cCTg CCCCg GGA t GCATGCAGa c CCC tCAC AGGg GCGTGTc CT gCCCCg GGATGCA TGCAGGGCt CCC t C
Hs11 AGGAGCGTGTACTACCCCAGGACGCATGCAGGGCCCCCAC TGGAGCGTGTACTACCCCAGGATGCATGCAGGGCCCCCAC

XII

Ss17 AGGAGCGTGTCCT g -CCCCGG- - - G –ACGCATGCAGa c CCC t CAC
Cj 11 -
Hs11 AGGAGCGTGTCCTATCCCCGGACCGGACGCATGCAGGGCCCCCAC

XIII XIV

Ss17 AGGAGtGTGT c CTg CCCCg GGACGCATGCAG a c CCC t CAC AGGAa C aTGTc CT gC tCCAGGACGa ATGCAGGa CCCCCtcAC
Cj 11 -
Hs11 AGGAGCGTGTACTACCCCAGGACGCATGCAGGGCCCCCAC AGGAGCGTGTACTACCCCAGGACGCATGCAGGGCCCCCAT

XV XVI

Ss17 AGGAGCGTGTCCTGCCCTGGGACGCATGCAGACCCCTCAC AGGAACGTGTCCCACCCCAGGACACATGGGCAGACCCCTCAC
Cj 11 -
Hs11 -

XVII

Ss17 AGGAGCACATCCTGCAGCAGGACACACAGGCAGACCCCTCAC
Cj 11 -
Hs11 -

Ss17 AGGCAGCCTGCAG - CCACACTCTGCCTGGCCTTGAGCCGTGACCTCCAGGAAG
Cj 11 gcaGCAGCC aGCAGg CCACACTCTGCCTGGCCT
Hs11 AGGCAGCCTGCAGACCACACTCTGCCTGGCCTTGAGCCGTGACCTCCAGGAAG

Fig. 2 Multiple alignment of VNTR sequences of the 3′-UTR *DAT* genes of human (*Hs*), common marmoset (*Cj*), and capuchin (*Ss*). PCR-derived nucleotide sequences from common marmoset and capuchin were aligned with the previously reported human sequence (GenBank accession number NG_015885.1). *Lowercase letters* indicate nucleotide differences from those in the human sequence. *Dashes* represent gaps introduced to optimize the alignment. The primer sequences employed are *underlined* and the *Roman numerals above the sequences* represent the serial numbers of the repeat units

the *DAT* VNTR 40-bp consensus repeat sequences of human and primates so far identified. Interestingly, upon nucleotide sequence analysis, we realized that of the 24 bp of the human *DAT* F-primer, only the first 9 bp are conserved in the *Sapajus* spp. and *Callithrix jacchus* genomes, explaining the failure of the PCR reaction under the conditions used for human DNA amplification.

For the behavioral data associated with VNTR genotype of marmosets, we considered the classification into quadrants proposed in Adriani et al. (2013): each individual was assigned to a "pie" quadrant using the index of flexibility as coordinates on the *X*-axis and the index of maximization as coordinates on the *Y*-axis. The upper right quadrant contains subjects that show the best performance, i.e., the clearest

shift from LL to SS (optimal choice) and the highest number of food rewards earned. All other quadrants contain subjects with poorer performance, because they (1) persist in performing a suboptimal LL choice (inflexibility) and/or (2) showed a reduction in the total number of trials completed over time. When the distribution of carriers vs. noncarriers of the *DAT* 10 allele in the upper right versus the other three quadrants was compared statistically, the two-tailed Fisher's exact probability test yielded a $P = 0.026$, thus indicating a significant bias in this distribution (Fig. 4). Indeed, the upper right quadrant contained five *DAT* 10-carriers and no subjects with the 9/9 genotype; conversely, all 9/9 subjects and the remaining three *DAT* 10-carriers were evenly dispersed in the other three quadrants.

For the capuchin monkeys, we compared the delay associated with LL at the indifference point between subjects carrying at least one *DAT* 8 allele and those carrying no such allele by means of a Mann–Whitney *U* test. Results showed that capuchins carrying at least one *DAT* 8 allele showed a higher delay tolerance ($U = 10.0$, $p = 0.040$, $n_1 = 7$, $n_2 = 8$) than subjects with longer repeat variations (Fig. 5).

Hs	AGGAGCGTGT	ACTACCCCAG	GACGCATGCA	GGGCCCCCAC
Cj	AGGGGCGTGT	CCTGCCCCGG	GATGCATGCA	GACCCCTCAC
Ss	AGGAGCGTGT	CCTGCCCCGG	GACGCATGCA	GACCCCTCAC
Cy	AGGAGCGTGT	CCTAT CCCCG	GATGCATGCA	GGGCCCCCAC
Agm	AGGAGCGTGT	CCTATCCCCG	GACGCATGCA	GGACCCC - AC
Or	AGGAGCGTGT	CCTACCCCAG	GACGCATGCA	GGGCCCCC AT
Ch	AGGAGCGTGT	CCTACCCCAG	GACGCATGCA	GGGCCCCC AT
Go	AGGAGCGTGT	CCTATCCCCG	GACGCATGCA	GGGCCCCCAC

Fig. 3 Sequence alignment of the *DAT* VNTR consensus repeats of primates. *Underlines* indicate nucleotide differences from the human sequence. *Hs* human, *Cj* marmoset, *Ss* capuchin, *Cy* cynomolgus macaque, *Agm* African green monkey, *Or* orangutan, *Ch* chimpanzee, *Go* gorilla

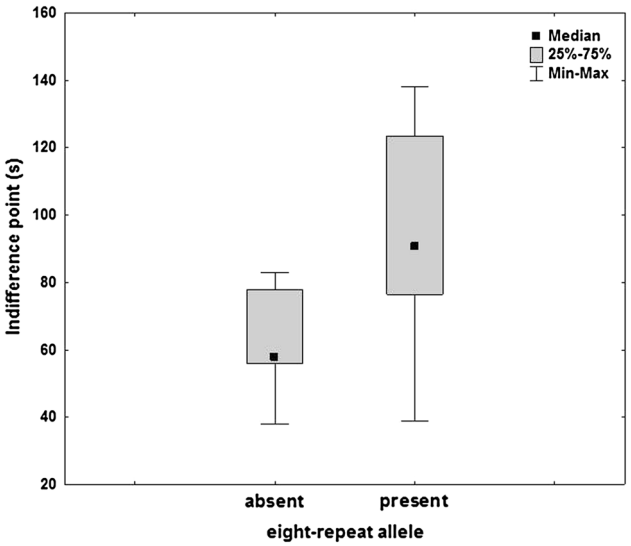

Fig. 5 Capuchin indifference points (s) in an intertemporal choice task with an adjusting-delay procedure for *DAT* 8-carriers ($N = 2$ with genotype 8/8 and $N = 6$ with genotype 8/13) and individuals without the 8 allele ($N = 3$ with genotype 9/13, $N = 3$ with genotype 13/13, and $N = 1$ with genotype 17/17). The *dot in each box* indicates the median, *the bottom and top of each box* indicate the first and third quartiles, and the *bottom and top whiskers* indicate the minimum and maximum data values

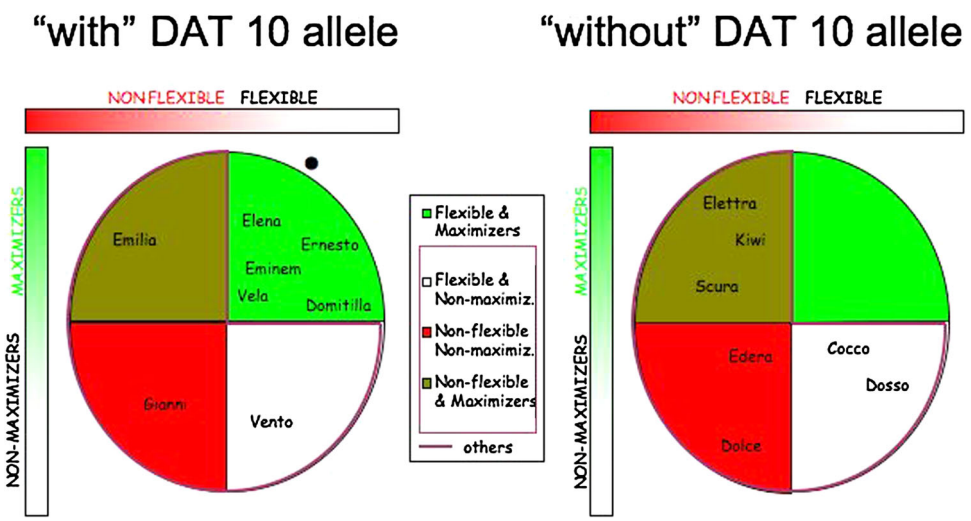

Fig. 4 Relationship of *DAT* allele variation to feeding choice in marmosets. The pie charts show the distribution of 15 experimental subjects. The pie is divided by the *horizontal line* into nonmaximizer (*lower portion*) and maximizer (*upper portion*) subjects, and by the *vertical line* into nonflexible (*left portion*) and flexible (*right portion*) subjects. Coordinates along the axes derive from calculating the slope of individual linear regressions for variables such as food earned and preference (%) for suboptimal Large-Late reward, respectively (see Adriani et al. 2013 for more detail). Five subjects in the *upper right quadrant* are both flexible and maximizers; interestingly, these five subjects all carry the *DAT* 10 allele. The other ten subjects showed less flexibility and/or less maximization capacity; these are evenly interspersed in the *upper left and lower quadrants*, and comprise three *DAT* 10-carriers and all seven subjects with a 9/9 *DAT* genotype

Discussion

Genetic variations in the noncoding regions of *DAT* in humans have been hypothesized to be involved in the etiology of neuropsychiatric disorders, neurodegenerative diseases, and susceptibility to drug addiction. In particular, the VNTR polymorphism at the 3′-UTR has been found to act as a modulator of gene transcription, associated with different levels of DAT expression. New World monkeys are an important model for biomedical research in these fields (Emborg and Colombo 1994; Roitberg et al. 2002; Blanchet et al. 2012), but a deeper understanding of their genome is required in order to select the most translationally relevant models of human diseases. Understanding *DAT* allele variation and comparing it to physiological and behavioral phenotypes in marmosets and capuchins may also provide a window into behavioral and cognitive variation in these species.

Previous studies showed that the 3′-UTR of the *DAT* gene was shared across several ape and Old World monkey species. This sequence was found to be polymorphic in the chimpanzee, with a single repeat being the most frequent (82.7 %), while no polymorphism has been detected in the 3′-UTR region of the *DAT* gene in the other species analyzed to date. While the functional implications of most of these alleles remain unclear, significant differences in reporter gene expression were found between the 1- and 2-repeat alleles in chimpanzee compared to the human alleles (Inoue-Murayama et al. 2002). These differences suggest that the VNTR sequence in the 3′-UTR may be an element involved in the expression of the *DAT* gene in chimpanzees too.

In the present study, we identified a polymorphic VNTR in the region corresponding to the 3′-UTR in New World monkey species. The DNA sequences we obtained showed the presence of repeat elements with a consensus sequence that was similar to those present in the 3′-UTR of the *DAT* genes in humans and the other nonhuman primate species analyzed (Inoue-Murayama et al. 2002) (Fig. 3). The results of our study indicate that the basic structure comprising the human 40-bp sequence at the 3′-UTR of the *DAT1* gene is shared across New World monkeys and is polymorphic with respect to the number of repeats.

It is generally agreed that New World monkeys have been separated from the Old World primates for at least 35 million years (Schrago and Russo 2003). Consequently, the 40-bp repeat structure found in humans, capuchins, and marmosets appears to be highly conserved across all anthropoids (Catarrhini and Platyrrhini). Moreover, the region corresponding to 3′-UTR shows four different length alleles in capuchins and three in marmosets, in contrast to the Old World nonhuman primate species studied so far (Inoue-Murayama et al. 2002), where only one or two VNTR alleles were found. At least in this preliminary sample, the polymorphic *DAT* alleles were present at a different frequency across the *Sapajus* species tested, suggesting a potential involvement of the different-length alleles in a different modulation of *DAT* gene expression as observed in humans. Other studies in capuchins are needed in order to confirm this observation, analyzing the region corresponding to the VNTR in a greater number of nonhybrid individuals belonging to different *Sapajus* species.

Cross-species comparison studies provide valuable insights into genome evolution, speciation, and selection mechanisms, and can identify common variants, thereby highlighting sites that are potentially of functional or evolutionary importance. The *DAT* gene is an important candidate for a primary role in promoting survival and fitness in the wild, as it is a well-known modulator of the mesolimbic, mesocortical, and nigrostriatal pathways, essential for reinforcement, motivation, and goal-oriented behavior. Pilot data suggest that 9- vs. 10-repeat VNTR polymorphism may well affect the behavior of marmosets in an operant choice paradigm. When marmosets were tested for their ability to maximize food payoff in a two-choice task, they had to be flexible enough to abandon a previous preference for a large reward when it was delayed and became suboptimal (Adriani et al. 2013). Our results show that only *DAT* 10-carrier subjects displayed the best performances, were highly flexible, and therefore maximized food gain. In contrast, *DAT* 9/9 carriers performed poorly, either because they were suboptimally attracted by the larger unitary reward or because they failed to maintain adequate motivation to perform all the choice tests presented (see Adriani et al. 2013 for a discussion).

Capuchin individuals with different *DAT* genotypes showed some behavioral differences when tested in a self-control task. The task consisted of an intertemporal choice task with an adjusting-delay procedure in which capuchins were presented with choices between a smaller immediate reward and larger delayed reward, and the delay to the larger reward was progressively increased or decreased depending on the subject's preference up until the two options were chosen to a similar extent (Addessi et al. 2011). The comparison between subjects with at least one 8-repeat allele and those not carrying that allele suggests that individuals with longer repeat variants perform less self-controlled choices than those with at least one shorter repeat variant. Thus, we have a preliminary indication that different repeat alleles of *DAT* may have distinct impacts on ecologically relevant functions such as food-related choices, foraging maximization, and self-control in the two New World primate genera tested.

Other examples of VNTR polymorphic alleles have been described in New and Old World monkeys (Inoue-Murayama et al. 2008; Pascale et al. 2012), indicating the presence of ancestral alleles unique to anthropoid primates and suggesting that a one-time insertion event occurred before the divergence between Old and New World monkeys. Subsequently, length polymorphisms arose independently along the human and several other lineages, and natural selection seems to have acted to maintain high frequencies of particular alleles in some environments for these taxa (Mountain et al. 1992).

Further studies are needed to determine the effects of the different VNTRs upon the physiological function of the *DAT* gene in New World monkeys. Analyses of larger sample populations derived from unrelated individuals will be required to clarify the evolutionary history of this highly polymorphic region.

Our present data showing the presence of tandem repeats in New World monkeys and behavioral variation associated with those differences are a first step towards clarifying the role and origin of tandem repeats of the *DAT* gene.

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare.

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