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Androgen receptor CAG repeat polymorphism in males of six non-human primate species

James N. Mubiru[1], **Nicole Cavazos**[2], **Peggah Hemmat**[2], **Magdalena Garcia-Forey**[1], **Robert E. Shade**[1], and **Jeffrey Rogers**[1,3]

[1]Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, USA

[2]Department of Biology, St. Mary's University, San Antonio, TX, USA

[3]Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, USA

Abstract

Introduction—Androgen receptor $[CAG]_n$ microsatellite has been linked to human diseases.

Methods—Six nonhuman primates were genotyped for the $[CAG]_n$ microsatellite.

Results—Marmosets and macaques are monomorphic while mangabeys, baboons and chimpanzees are polymorphic.

Conclusions—Nonhuman primates that are polymorphic for the microsatellite are candidate animal models for CAG-related diseases.

Introduction

The androgen receptor (AR) gene is more than 90 kb long and is located on chromosome Xq11-12 in humans [6, 15]. The gene contains eight exons and encodes a protein with three functional domains: the *N*-terminal domain, a DNA-binding domain, and an androgen-binding domain [6, 13]. The androgen receptor helps direct the development of male sexual characteristics and regulate other important functions in both males and females. The AR gene contains two trinucleotide repeat segments in exon 1: $[CAG]_n$ codes for the polyglutamine tract (polyQ) while $[GGN]_n$ codes for the polyglycine tract (polyG) in the AR protein. In humans, the number of CAG repeats in the AR gene ranges from fewer than 10 to about 36 [4, 10,12]. Longer polyglutamine segments in humans lead to spinal bulbar muscular atrophy (SBMA) also called Kennedy's disease [14]. Short polyglutamine tracts have been linked to increased risk [11] and early onset [9] of prostate cancer. A negative correlation has also been reported between CAG repeats and serum levels of prostate specific antigen (PSA) in subfertile men [16]. The androgen receptor has also been associated with coronary heart disease (CHD) [1, 19].

The aim of this study was to genotype AR CAG polymorphisms in nonhuman primate species used in biomedical research and then use this information to identify the best animal model for studying the relationship between AR CAG repeats, and prostatic diseases and CHD risk factors.

Correspondence: James N. Mubiru, Southwest National Primate Research Center, Texas Biomedical Research Institute, San Antonio, TX, USA. jmubiru@TxBiomed.org.

Materials and Methods

Animal tissues

DNA samples from 230 baboons (*Papio hamadryas*), 23 cynomolgus macaques (*Macaca fascicularis*), 54 rhesus macaques (*Macaca mulatta*), 56 sooty mangabeys (*Cercocebus atys)*, 48 marmosets (*Callithrix jacchus)*, and 48 chimpanzees (*Pan troglodytes)* were used. Only male animals were used.

Baboon, chimpanzee, marmoset, rhesus and cynomogus monkey samples were obtained from the Southwest National Primate Research Center (SNPRC), Texas Biomedical Research Institute, San Antonio. Sooty mangabey DNA samples were from Yerkes National Primate Research Center, Atlanta. Founders of the baboon captive population were wild-caught olive baboons (*P. hamadryas anubis*) and yellow baboons (*P. h. cynocephalus*) from East Africa (360 females and 40 males). The cynomolgus monkeys and chimpanzees are the progeny of many founder animals that were obtained from various sources. The marmosets are descendants of more than 40 animals obtained from several sources in Europe and the USA. The genetic background of the rhesus monkey and sooty mangabey colonies is not very diverse as they are descended from few founder animals.

$[CAG]_n$ microsatellite genotyping

DNA for the microsatellite assays was extracted from frozen tissues using the DNeasy kit (Qiagen). Polymerase chain reactions were run using Platinum *Taq* DNA polymerase (Invitrogen). The primer sequences used for CAG microsatellite amplification are located in AR exon 1(see below).

AR Forward: 5′-ACCGAGGAGCTTTCCAGA AT-3′

AR Reverse: 5′-GAAGGCTGCTGTTCCTCATC-3′

PCR products were separated on a 2% agarose gel, and bands were cut out from the gel and then purified using the Qiaquick Gel Extraction Kit (Qiagen). The Advanced Nucleic Acid Core Facility at the University of Texas Health Science Center at San Antonio performed the nucleotide sequencing.

Results

CAG repeat polymorphisms

Three species (cynomolgus macaques and rhesus macaques, and marmosets) were found to be monomorphic. Only one genotype of 3 CAG repeats was found in marmosets and only one genotype of 8 CAG repeats was found in the macaques. Baboons, sooty mangabeys and chimpanzees, on the other hand, show a high level of polymorphism. In baboons there were 10 alleles ranging from 4 to 13 repeats, while in sooty mangabeys there were 3 genotypes ranging from 8 to 10. In chimpanzees alleles ranged from 14 to 26 repeats. The allele frequencies for each species studied are shown in Table 1. Using data from the present study combined with published data on CAG repeats from other primates, we have constructed a phylogenetic tree that show an increase in CAG microsatellite length as we move from species that are distantly related to humans towards those that are closely related to humans (Figure 1). A similar tree was published previously [3, 17].

Discussion

Of the six species we sequenced, the marmosets represent New World monkeys, the macaques, baboons and sooty mangabeys represent Old World monkeys and the chimpanzees are anthropoid apes. Previous studies have reported a linear increase in $[CAG]_n$

repeat length proportional to the time of species divergence from humans [3]. Our study builds upon those previous studies by both examining more samples of each species and including previously unstudied species to confirm the previous findings. The results reported here confirm that the increase in $[CAG]_n$ repeat length from (3 repeats) in marmosets to macaques (8 repeats), baboons (4–13 repeats) to chimpanzees (14–27 repeats) parallels their evolutionary relationships. One drawback of our study could be the lack of genetic diversity in the primate colonies studied, although this effect is limited as regards baboons, cynomolgus monkeys, chimpanzees and marmosets because they are the progeny of many founder animals. The absence of polymorphisms in the rhesus colony could be due to the fact that this colony is genetically close.

It is clear from this study that the normal physiological range for androgen receptor $[CAG]_n$ repeats in nonhuman primates is very variable. The $[CAG]_n$ microsatellite is in the coding region of the gene, but there is little understanding of its role. It seems that a considerable length of these $[CAG]_n$ repeats can be tolerated. This might suggest that the $[CAG]_n$ repeats may not always play a critical function but raises the question of why they are retained in all the species studied so far. One possibility is that the variation in $[CAG]_n$ repeats length represents a mechanism for fine-tuning the activity of this gene [18]. This could be plausible considering that the $[CAG]_n$ microsatellite is located in the *N*-terminal domain, which has been reported to be a part of the gene that influences transcription efficiency [2].

In humans the $[CAG]_n$ microsatellite is highly polymorphic. Longer repeats (≥40) have been reported to lead to Kennedy's disease whereas shorter CAG repeats have been reported to result in higher activation of the receptor thus increasing the risk for several diseases [4, 7, 8].

In summary we have shown that the AR gene is polymorphic in baboons, sooty mangabeys and chimpanzees but monomorphic in macaques and marmosets. Nonhuman primates that are polymorphic for the CAG repeat could potentially be good models for investigation of the control of the AR gene activity, as well as human disease conditions that are associated with variation in this microsatellite.

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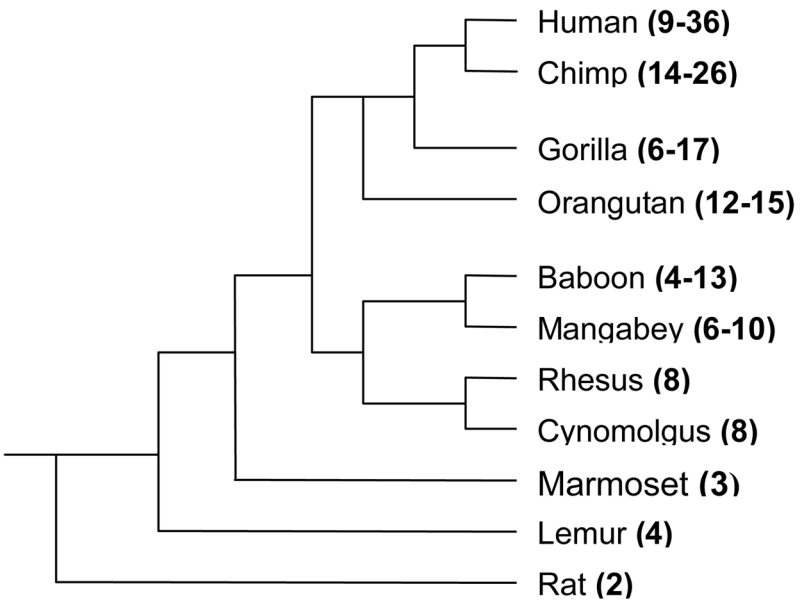

Figure 1.
Species differences in the number of CAG microsatellite repeats in the androgen receptor. The number of CAG repeats are in brackets. Rat and lemur CAG repeat numbers were based on data from Choong et al 1998. The human data was based on Huhtaniemi et al 2009, gorilla data was based on Djian et al 1996, the orangutan data was based on on GenBank sequence #AB207232 and the sequence available on University of California Santa Cruz genome browser gateway (chrX:65097837-65098089). A version of this figure was previously published in Perelman et al 2011.

Table 1

Androgen receptor CAG genotypes in 6 male nonhuman primate species

Baboon (n=230)	
CAG repeats	Frequency (%)
4	0.87
5	3.04
6	6.52
7	4.78
8	19.57
9	41.30
10	16.52
11	6.52
12	0.43
13	0.43
Chimpanzee (n=48)	
CAG repeats	Frequency (%)
14	4.35
17	15.21
18	13.04
19	10.87
20	4.35
21	19.56
22	21.74
23	4.35
24	4.35
25	2.17
26	2.17
Mangabey (n=56)	
CAG repeats	Frequency (%)
8	69.64
9	3.57
10	26.79
Cynomolgus monkey (n=23)	
CAG repeats	Frequency (%)
8	100
Rhesus monkey	(n=54)
CAG repeats	Frequency (%)
8	100
Marmoset (n=48)	
CAG repeats	Frequency (%)