Meta Gene 14 (2017) 105–113

Contents lists available at ScienceDirect

Meta Gene

journal homepage: www.elsevier.com/locate/mgene

Short poly-glutamine repeat in the androgen receptor in New World monkeys

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ARTICLE INFO

Keywords:
Androgen receptor
Microsatellite repeat
Polymorphism
New World monkey

ABSTRACT

The androgen receptor mediates various physiological and developmental functions and is highly conserved in mammals. Although great intraspecific length polymorphisms in poly glutamine (poly-Q) and poly glycine (poly-G) regions of the androgen receptor in humans, apes and several Old World monkeys have been reported, little is known about the characteristics of these regions in New World monkeys. In this study, we surveyed 17 species of New World monkeys and found length polymorphisms in these regions in three species (common squirrel monkeys, tufted capuchin monkeys and owl monkeys). We found that the poly-Q region in New World monkeys is relatively shorter than that in catarrhines (humans, apes and Old World monkeys). In addition, we observed that codon usage for poly-G region in New World monkeys is unique among primates. These results suggest that the length of polymorphic regions in androgen receptor genes have evolved uniquely in New World monkeys.

1. Introduction

The androgen receptor (AR) is a ligand-activated nuclear hormone receptor that plays a critical role in the development of the male phenotype (Gelmann, 2002; Li and Al-Azzawi, 2009). A dimer of the receptors works as a transcription factor in the nucleus when it is combined with internal androgen hormones, testosterone and its metabolite, 5α-dihydrotestosterone. AR is almost ubiquitously expressed in tissues and mediates various physiological and developmental functions in addition to virilization. Androgens in the brain are involved in circuit formation during development and maintaining proper function in adulthood. Thus, expression of AR is considered to affect not only reproductive but also non-reproductive behavior patterns dealing with aggression and emotion (Li and Al-Azzawi, 2009).

The androgen receptor gene (*AR*) is located on the X-chromosome and consists of eight exons in humans (Brown et al., 1989; Kuiper et al., 1989). The similar gene structure in mice, monotremes and marsupials indicates *AR* is highly conserved in mammals (Choong et al., 1998; Faber et al., 1991; He et al., 1990; Migeon et al., 1981; Spencer et al., 1991). The N-terminal domain of AR is coded in exon 1 and relates to transcription regulation by *AR*. Other exons code DNA binding domain and ligand binding domains (Fig. 1) (Li and Al-Azzawi, 2009; Quigley et al., 1995).

In exon 1, there are trinucleotide microsatellite repeat regions, (CAG)n and (GGN)n. These repeat regions yield poly glutamine (poly-Q) and poly glycine (poly-G) repeats in the N-terminal domain. The poly-Q region is further segmented into QI and QII regions (Fig. 1). Significant variation in the length of poly-QI is known in humans. Normal repeat lengths of QI range approximately from 10 to 37 and the most frequent repeat length is 23 (Hsing et al., 2000; Huhtaniemi et al., 2009). Shorter repeats of poly-QI increase the risk of prostate cancer and extremely long repeats (> 40) are related to spinal and bulbar muscular atrophy and a variable degree of androgen insensitivity although causation is controversial among studies (Rajender et al., 2007). The length polymorphism of poly-G region is also found in humans (ranges approximately from 14 to 27 repeats), while no great variation was reported for poly-QII.

The length polymorphisms in these regions are also found in non-

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http://dx.doi.org/10.1016/j.mgene.2017.08.006
Received 22 May 2017; Received in revised form 21 July 2017; Accepted 25 August 2017
Available online 01 September 2017
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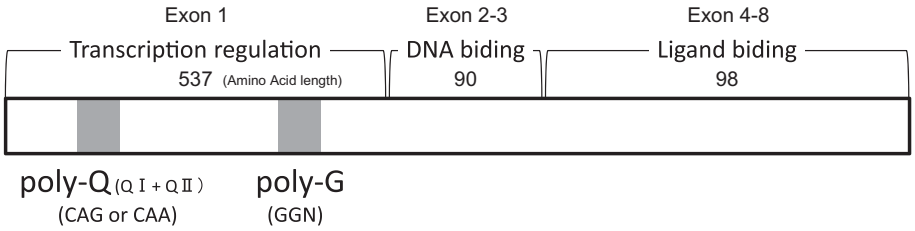

Fig. 1. Schematic illustration of the structure of AR. An example amino acid length of human AR is shown. (Modified from Gelmann, 2002)

human primates. Interestingly, if a species is phylogenetically closer to humans, it possesses longer repeats and higher polymorphism comparable to humans (Choong et al., 1998; Hong et al., 2006; Mubiru et al., 2012). For example, our closest relative, the chimpanzee, has length polymorphism ranging from 15 to 27 at poly-QI but no polymorphism was found in rhesus macaques (Hong et al., 2006; Mubiru et al., 2012). The tendency of allele combination between longer poly-QI and shorter poly-G repeats in chimpanzees (Hong et al., 2006) and insusceptibility to idiopathic infertility in haplotypes with longer poly-QI and shorter poly-G combination in humans (Ferlin et al., 2004) indicates the underlying constraints on the combination of these regions. The association between these polymorphisms and personality traits such as aggressiveness has also been an intriguing topic of personality research on non-human animals as well as humans (Jonsson et al., 2001; Konno et al., 2011; Rubinow and Schmidt, 1996).

Little is known, however, about the characteristics of these regions in other primates. Only one species of New World monkeys (platyrrhine), the marmoset, and several species of prosimians have been surveyed so far and no polymorphism has been found in these taxa (Mubiru et al., 2012; Poux et al., 2005). Hormonal studies have reported that the androgen concentration in several species of New World monkeys is higher than that in Old World monkeys (Snipes et al., 1969), and their androgen concentration is associated with social status (Bales et al., 2006; Schoof and Jack, 2013). These findings indicate that androgen plays an important role in behavior of New World monkeys and highlights the importance of understanding the structure and variation of AR in this taxon. Therefore, we aimed to characterize the *AR* gene in New World monkeys and to understand the entire picture of AR variation in primates by focusing on length polymorphism of AR poly-Q and poly-G regions and codon usage bias in 17 species of New World monkeys.

2. Materials and methods

2.1. DNA samples

DNA samples were obtained from 120 individuals that belong to one of 17 species of New World monkeys. Number of individuals in each species surveyed is summarized in Table 1. Most samples were obtained from animals kept at various sites in Japan (Japan Monkey Center; Primate Research Institute, Kyoto University; Graduate School of Letters, Kyoto University; Kyoto City Zoo and Omuta City Zoo). DNA was extracted from tissues when animals were dead, or from blood or hairs when animals were alive by using QIAamp DNA mini Kit (Qiagen, California, USA). For 22 capuchin monkeys, DNA extracted from blood samples of monkeys kept at National Institute of Child Health and Human Development (NICHD) were used. For individuals whose sex was not known at sampling, we determined their sex by PCR amplification of sex-determining region Y (*SRY*). The 22 tufted capuchins in NICHD and nine tufted capuchins, four common squirrel monkeys and six Bolivian squirrel monkeys at Kyoto University were kept in several groups at each site and some individuals were related to each other. No clear genealogy and kinship were available for other individuals. We have complied with the ethical standards in the treatment of animals with the guidelines laid down by the Primate Society of Japan. The experiment was approved by the Animal Experiments Committee of the Graduate School of Letters (16–37) and the Wildlife Research Center (WRC-2016-002A), Kyoto University, and the Animal Care and Use Committee of NICHD (ASP#12-064) in accordance with the European Directive 2010/63 on the Protection of Animals in Scientific Experimentation.

2.2. Determination of repeat length and sequencing

Poly-Q and poly-G regions were PCR amplified by using following primer sets. Poly-Q: ARhf 5′-TCCAGAATCTGTTCCAGAGCGTGC-3′ and ARhr 5′-GCTGTGAGGGTTGCTGTTCCTCAT-3′. Poly-G: ARGFH 5′-CAGTGCCGCTATGGGGACCTGGCGA-3′ and ARGR 5-GGACTGGGAT AGGGCACTCTGCTCACC-3′.

To determine the length of repeated region, forward primers were fluorescent labeled (FAM for ARhf and HEX for ARGFH). A PCR was carried out with approximately 20 ng genomic DNA, forward and reverse primers 0.5uM each, 0.4 mM each for dNTP mix mixture, LA Taq polymerase (Takara bio, Shiga, Japan) 0.5 unit, 1 × Takara PCR buffer and ddH2O at 10ul scale. PCR condition was as follows: after initial incubation at 95 °C for 2 min, 35 cycles of 95 °C for 30 s, 55 °C for 30 s, 74 °C for 2 min, followed by a final extension at 74 °C for 10 min. After the PCR amplification, PCR samples were diluted a hundred times with ddH2O and subjected to electrophoresis by Applied Biosystems 3130xl Genetic Analyzer with Gene Mapper software (Applied Biosystems, Foster City, California, USA). After determination of the repeat length, we chose individuals who were hemi- or homozygous for these repeat sites and the sequence was determined for each species by using Applied Biosystems 3130xl Genetic Analyzer. For the species that had polymorphism at these repeat regions, the sequence was determined for each allele from hemi- or homozygous individuals if available. We aligned sequences of a representative allele (most frequent allele if polymorphic) from each species including previously reported primates and house mouse using CLUSTAL W (Thompson et al., 1994) implemented in MEGA version 5 (Tamura et al., 2011). We counted the number of glutamine and glycine coded in the region corresponding to the poly-QI, poly-QII and poly-G regions of other primates for which sequences were previously reported. We included all glutamine or glycine sites in repeat numbers even when other amino acids were inserted in the corresponding regions.

2.3. Calculation of codon bias and visualization of similarity among species

As an indicator of codon bias, we calculated the ratio of each codon for poly-Q and poly-G regions in representative alleles if the representative sequence was available. To visualize the relationship of these regions among species from various taxa, we applied multi-dimensional scaling (MDS) analysis using the nine variables (six codon usage ratios, length of poly-QI, poly-QII and poly-G in the

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