

Androgen receptor gene polymorphisms are associated with aggression in Japanese Akita Inu

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We tested for an association between variable number of tandem repeats in the canine *androgen receptor* (*AR*) gene and personality differences in Japanese Akita Inu dogs. The polymorphic trinucleotide (CAG) repeat region coding for glutamine in exon 1 of the *AR* gene was genotyped using genomic DNA obtained from 171 dogs. Three alleles (23, 24 and 26 repeats) were detected, and the allele frequency differed with the coat colour. We assessed the personality profiles of 100 fawn-coloured dogs (54 males and 46 females) based on a questionnaire answered by each dog's owner. The questionnaire consisted of five sub-scales (sociability, playfulness, neuroticism, aggressiveness, distractibility), and the psychometric properties were acceptable based upon internal consistency of the subscales. We found that male dogs with a short allele conferring increased *AR* function had higher aggressiveness scores than male dogs with longer alleles. By contrast, no evidence was found for a relationship between *AR* gene variants and personality in females. To our knowledge, our findings provide the first evidence of polymorphism in the *AR* gene being associated with canine aggression.

Keywords: aggression; androgen receptor gene; dogs; gene polymorphisms; personality

1. INTRODUCTION

Androgens such as testosterone play a key role not only in sexual differentiation of males but also in modulating human behaviour [1,2]. The human *androgen receptor* (*AR*) gene, located on the X chromosome and composed of eight exons, contains a highly polymorphic trinucleotide (CAG) repeat in exon 1, and the level of transactivation is affected by the length of the polyglutamine stretch [3]. *In vitro* studies have shown that a relatively short CAG repeat sequence enhances the transcriptional activity of the *AR* by promoting interactions between the receptor and coactivators [4]. The CAG repeat polymorphisms have been shown to affect reproductive capability and

various disease risks in men [5–7]. Moreover, recent studies on human males have reported that shorter CAG repeats are associated with violent criminal behaviour and amygdala reactivity [8,9] and longer CAG repeats with cognitive ageing and male-to-female trans-sexualism [10,11]. With respect to personality, it has been suggested that there are relationships between relatively short repeat polymorphisms and extraversion, verbal aggressiveness and depression [12–14].

The aim of the present study is to explore the potential link between *AR* gene polymorphism and personality in domestic dogs (*Canis familiaris*). There are a large number of dog breeds showing behavioural variation, and consequently dogs are recognized as a model species for understanding the genetic basis of behaviour. Most of the attention has been focused on the *dopamine receptor D4* gene (*DRD4*) which is related to novelty seeking in humans [15]. Previous studies demonstrated that polymorphism in the dog *DRD4* exon 3 were associated with aggressiveness and activity/impulsivity [16,17], as well as those in intron 2 with social impulsivity [18]. The canine *AR* gene contains two CAG repeats referred to as the *Q1* and *Q2* regions of exon 1 [19]. The form and extent of influence of the *AR* gene on personality measures in dogs, however, is still unknown. It has been reported that *AR* allele frequencies differ among dog breeds [19,20]. Therefore, we focused on a specific breed, Japanese Akita Inu, which descended from bear-hunting dogs in Japan. This breed is thought to be temporarily bred for fighting and has strong guarding instincts. The genetic constitution of East Asian native breeds, including Japanese Akita Inu, has been shown to be similar to those of wolves [21]. These primitive-type dogs maintain a high genetic diversity owing to a very ancestral origin and short history of artificial selection, which enabled us to investigate the genetic control of canine behavioural variability. In our study, we tested for an association between *AR* gene variants in Japanese Akita Inu and personality scores obtained from a questionnaire answered by the dogs' owners.

2. MATERIAL AND METHODS

We collected genomic DNA from buccal cells of 171 Japanese Akita Inu (83 males and 88 females; 102 fawn, 53 brindle and 16 white-coloured dogs) which were registered by the Akita Inu Introduction Foundation in Japan. Litter siblings were excluded from analysis to remove closely related dogs. All dogs were kept in their owner's house as pets. The methods for genotyping the canine *AR* gene have been previously reported ([19,20]; see the electronic supplementary material). Since very few polymorphisms were observed in the *Q1* region in Japanese Akita Inu, only the CAG repeats in the *Q2* region of the *AR* gene were genotyped [20]. As shown in table 1, we detected three different alleles (23, 24 and 26 repeats) in our population, and the observed allele frequencies did not differ significantly from Hardy–Weinberg equilibrium ($\chi^2 = 0.02$, $p = 0.999$). The distribution of allele frequencies according to coat colours showed that *AR* allele frequencies were different when comparing fawn and brindle dogs ($\chi^2 = 23.52$, $p < 0.001$).

To eliminate the confounding effect of coat colour, our personality assessment was limited to 100 dogs with fawn-coloured coats (54 males and 46 females). All subjects were intact and sexually mature dogs with a mean age of 3.75 years old, ranging from 13 months to 7 years. Allele 23, which has the smallest number of tandem repeats, was the most common *AR* allele observed in Japanese Akita Inu. Hence, we categorized allele 23 as short-type (*S*), and the other relatively scarce alleles 24 and 26 as long-types (*L*). Based on this grouping, five genotype categories were set as follows: *S/S* (23/23, $n = 30$) and *L/L* (24/24 and 26/26, $n = 24$) for males; and *S/S* (23/23, $n = 13$), *S/L* (23/24 and 23/26, $n = 19$)

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Table 1. Allele frequency of *AR* in Japanese Akita Inu (number of male dogs is shown in parentheses). (Heterozygosity (H) was calculated according to the following equation: $H = 2n(1 - \sum q_i^2)/(2n - 1)$.)

	n	allele frequency (q_i)			heterozygosity (H)
		23	24	26	
total dogs	171 (83)	0.58	0.21	0.22	0.58
fawn	102 (56)	0.52	0.16	0.32	0.60
brindle	53 (24)	0.70	0.26	0.05	0.45
white	16 (3)	0.52	0.34	0.14	0.62

Table 2. Items of the aggressiveness scale applied in the present study.

adjective	defining statement
dominant	behaves only as he/she pleases and becomes aggressive when interrupted
defiant	becomes aggressive, even towards you or other family members when he/she is annoyed
moody	seems to be in a bad mood constantly and displays mood swings frequently
irritable	impatient and gets angry at people as well as other dogs that he/she dislikes
aggressive	threatens or initiates fights with people as well as other dogs, that he/she dislikes

Table 3. Mean scores (s.d.) of each of the personality scales as a function of genotype categories. (The p -value in *italics* indicates a significant value.)

personality scale n	male		F -value	p	female			F -value	p
	$S/-$ 30	$L/-$ 24			S/S 13	S/L 19	L/L 14		
sociability	3.20 (1.05)	3.74 (1.16)	3.09	0.09	3.71 (1.48)	3.81 (1.64)	3.83 (1.72)	0.02	0.98
playfulness	3.32 (0.73)	3.42 (1.03)	0.17	0.68	3.33 (0.90)	3.34 (0.79)	3.30 (0.78)	0.01	0.99
neuroticism	3.72 (1.07)	3.35 (1.12)	1.52	0.22	3.67 (1.13)	3.60 (1.15)	3.48 (1.01)	0.10	0.90
aggressiveness	3.16 (1.07)	2.30 (1.23)	7.50	<i><0.01</i>	2.06 (1.05)	2.01 (0.77)	1.90 (0.81)	0.12	0.88
distractibility	3.02 (0.92)	3.10 (1.00)	0.10	0.76	2.67 (0.68)	2.50 (0.77)	2.75 (0.66)	0.53	0.59

and L/L (24/24, 24/26 and 26/26, $n = 14$) for females. We asked the owners of the subject dogs (all men; mean age, 46.8 years old) to fill out a questionnaire assessing the dogs' personalities. The questionnaire consisted of 30 items designed to characterize the five personality traits of aggressiveness, distractibility, neuroticism, playfulness and sociability (items contained in the aggressiveness trait are shown in table 2). The owners indicated the extent to which a personality description was applicable to the subject dog for each item, using a six-point scale. To confirm the factor structure of the questionnaire, we performed a factor analysis. The scale scores for each dog were calculated as the average scores of items belonging to each of the factors. A one-way analysis of variance with a Bonferroni multiple test correction was used to assess statistical significance for association of the scale scores with the *AR* genotype (see the electronic supplementary material).

3. RESULTS

A factor analysis with Varimax rotation on the 30-item ratings of 100 dogs confirmed that the observed factor structure was simple and equivalent to our pre-arranged five personality dimensions (electronic supplementary material, table S1). The internal consistencies of the five personality scales assessed by Cronbach's α were found to be satisfactory, ranging from 0.71 for distractibility to 0.88 for sociability. Table 3 shows the mean scores of each of the personality traits by *AR* genotypes. We found that the *AR* genotype in males was significantly associated with only the aggressiveness scale after the Bonferroni correction ($p < 0.01$), indicating that dogs carrying the $S/-$ genotype displayed higher aggressiveness scores than dogs carrying the $L/-$ genotype ($F_{1,52} = 7.50$, $p = 0.008$). Furthermore, there was no significant difference in any personality scores between the two genotypes included in the $L/-$ category (the 24/- and the 26/- genotype; see the electronic supplementary material). When analysing females, no

significant difference was found between genotypes on any of the personality scales in females (table 3).

4. DISCUSSION

Consistent with previous reports, our genotyping demonstrated that the $Q2$ region of the *AR* gene in Japanese Akita Inu had three different alleles and maintained a high genetic diversity with Hardy-Weinberg equilibrium. To ensure the most homogeneous genetic and physiological background possible, we limited our subjects to intact and sexually mature dogs with fawn-coloured coats. We obtained the personality scores of individual dogs from a questionnaire answered by the owners. This indirect method for phenotyping has been accepted for the study of dogs if the psychometric criteria are fulfilled [22]. In this study, internal consistency of subscales was confirmed as rationales for applying the owner-assessment approach (see the electronic supplementary material for test-retest reliability). This suggests that the questionnaire used here is an appropriate tool for providing a rough assessment of personality differences among dogs.

We found that the CAG repeat polymorphism in the *AR* gene was associated with owner-assessed aggressiveness in male Japanese Akita Inu. It was reasonable that male dogs possessing a short CAG repeat, whose *AR* responsivity is assumed to be increased according to human studies, had higher aggressiveness scores than dogs with relatively long alleles. These data broaden our knowledge about the influence of the *AR* gene on men's behaviour [8,12,13] by providing a valid animal model. No evidence was found for a relationship between *AR* gene variants and personality in female

dogs. This could be attributed to a gender difference in androgenic activity. Many studies have demonstrated that males are typically more responsive than females to the behavioural and neuroendocrine actions of androgens [1]. Although the molecular function of the canine AR gene variants should be confirmed, our results suggest that dog personality traits related to aggression might be partially mediated through the androgen receptor. To our knowledge, the present study is the first demonstration of an association between AR gene polymorphism and dog aggressiveness.

The present study was approved by the ethical committee at the Wildlife Research Center, Kyoto University (no. WRC-2010-EC001).

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