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ORIGINAL ARTICLE

Reduced CAG repeats length in androgen receptor gene is associated with violent criminal behavior

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Abstract Androgens mediate their functions through androgen receptors (AR). The two triplet repeats in the *AR* gene (CAG and GGN) are highly polymorphic among various populations and have been extensively studied in diverse clinical conditions and antisocial personality disorders. Several studies have reported either higher levels of testosterone among rapists or the correlation of shorter CAG repeats with criminal activities. However, to date, no study has analyzed *AR* gene in rapists worldwide, and no study has been conducted on criminals from Indian subcontinent. Therefore, we have analyzed the AR-CAG repeat length in 645 men, of which 241 were convicted for rape, 107 for murder, 26 for both murder and rape, and 271 were control males. The aim was to explore if there was any correlation between CAG repeat length and criminal behavior. The study revealed significantly shorter CAG repeats in the rapists (mean 18.44 repeats) and murderers (mean 17.59 repeats) compared to the control men (mean 21.19 repeats). The criminals who committed murder after rape had a far shorter mean repeat length (mean 17.31 repeats) in comparison to the controls or those convicted of rape or murder alone. In short, our study suggests that the reduced CAG repeats in the *AR* gene are associated with criminal behavior. This, along with other studies, would help in understanding the biological factors associated with the antisocial or criminal activities.

Keywords Androgen receptor · CAG repeat · Rapists · Murdered · Antisocial activities · Criminal activities

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Introduction

Antisocial or criminal behavior is a serious social problem, which not only have heavy costs but also greatly affect the physical, mental, and social status of numerous individuals [1]. From twin and adoption studies, it was demonstrated that genetic components as well as environmental factors may affect the development of antisocial behavior [2]; however, the genetic mechanism underlying the development of antisocial behavior is still unknown. Androgens, chiefly testosterone and 5-α dihydrotestosterone, are C-19 steroids, which control the development and maintenance of male characteristics and exert their effects primarily through the stimulation of androgen receptors (ARs) [3]. The androgen-related signaling molecules may be altered as part of the neurobiological substrate of antisocial or violent criminal behavior for several reasons. First, higher testosterone levels have been found to be associated with male aggression in several studies [4–7]. Second, significant psychiatric symptoms, including aggression and violence, have been associated with androgen-related drug abuse [8].

The rate of substance abuse and dependence is higher among men than women [9]. Dabbs and Dabbs reported that soldiers with high testosterone levels were more likely to abuse drugs and alcohol [5]. Furthermore, higher levels of plasma testosterone have been correlated with violent rape activities [10, 11]. Finally, through surveys from the general population, it has been demonstrated that antisocial personality disorder (ASPD) is more common in males (4.2%) than females (1.9%) [12]. The latter reason also indicates that it may be androgens in the males which are responsible for the gender bias for the criminal nature.

AR is a member of the nuclear hormone receptor superfamily of transcription factors. The *AR* gene has been mapped to the long arm (Xq11–12) of the X-chromosome [13]. Exon 1 of the gene consists of two polymorphic triplet repeat (CAG and GGN) motifs, encoding variable lengths of polyglutamine and polyglycine stretches, respectively, in the N-terminal region (transactivation domain) of the AR protein [13, 14]. CAG, a simple repeat, varies in length from 8 to 35 repeats, while GGN, a complex repeat represented by $(GGT)_3GGG(GGT)_2(GGC)_n$, varies in length from 10 to 30 repeats [14]. The CAG repeat length and the AR transactivation potential are inversely correlated [15, 16]. *AR* alleles with more than 40 CAG repeats showed reduced transcription activity *in vitro* in comparison to the molecules with 25, 20, or no repeats [15, 16]. Therefore, it seems that the length of CAG repeat should inversely affect AR activity and, hence, the susceptibility to disorders related to the androgens action. AR has strong effects on the function of the central as well as peripheral nervous system and plays a crucial role in maintaining masculine reproductive behavior [17]. Jonsson et al. [18] reported that shorter CAG trinucleotide repeats related to personality scales characterized by dominance, high verbal aggression, high monotony avoidance, and low lack of assertiveness in normal populations. Taking into consideration the possibility of alteration in androgen-related signaling molecules in susceptibility to antisocial or violent criminal behavior, we undertook the present study on Indian men convicted for rape or murder or both to find out if AR-CAG repeat length variation was associated with criminal behavior.

Materials and methods

Subjects

Blood samples were collected from 374 men who had been imprisoned at the Central Jail, Hyderabad, India for various antisocial activities. The samples were collected with due permission from the higher police authorities and informed written consent of the subjects. Of the total 374 men, 236 were convicted for single rape, five for multiple rapes, 26 for murder after rape and 107 for murder. All these individuals were proven to be responsible for the crime by the forensic analysis at Andhra Pradesh State Forensic Science Laboratory, Hyderabad, India by matching short tandem repeat (STR) profiles of the criminals' and the forensic sample (semen from victim's clothes, hair or blood) recovered from the crime spot. The age of the subjects ranged from 16 to 58 years at the time of crime. All the subjects belonged to three major populations of Andhra Pradesh with similar linguistic affiliations. It was difficult to select an ideal control group for this study; therefore, we selected a total of 271 men from the same three populations in the same ratio as cases to match the ethnicity. The control group comprised of the individuals with known history of no violent activities or other evident psychopathies, as evidenced by the information from other family members and social records of no criminal activity from these individuals. The control group was composed of individuals with age ranging from 16 to 57 years.

DNA isolation

DNA was extracted from peripheral blood lymphocytes by the method described in our earlier study [19]. The procedure used for isolating the DNA from the semen was also adopted from our earlier study [20]. DNA isolation from hair was done according to a previously described method [21].

Genetic analysis

To ascertain the identity of the criminals, 16 STR loci (AmpF*l*STR, Identifiler Plus, Applied Biosystems, Foster City, CA, USA) were genotyped for both the DNA samples (i.e., blood and forensic sample), in a multiplex PCR using the protocol of the manufacturer. For genotyping, 1 μl of the amplified product was mixed with 0.3 μl of LIZ500™ (Applied Biosystems, Foster City, CA, USA) and 8.7 μl of Hi-Di formamide (Applied Biosystems, Foster City, CA, USA) in a 96-well plate. The mixture was denatured by heating at 95°C for 5 min followed by cooling on ice for 5 min and size fractionation on 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The results were further analyzed using GeneMapper software (Applied Biosysytems, Foster City, CA, USA) to ascertain the allele sizes. The CAG repeat region of *AR* gene was amplified using the following primers flanking the repeat region; forward: 5′ FAM-CAGAATCTGTTCCAGAGCGTGC-3′, reverse: 5′-AAGGTTGCTGTTCCTCATCCAG-3′. Polymerase chain reactions (PCRs) were set up in 10 μl volume consisting of 1.0 μl PCR buffer (10X), 1.0 μl $MgCl_2$ (25 mM), 1.0 μl

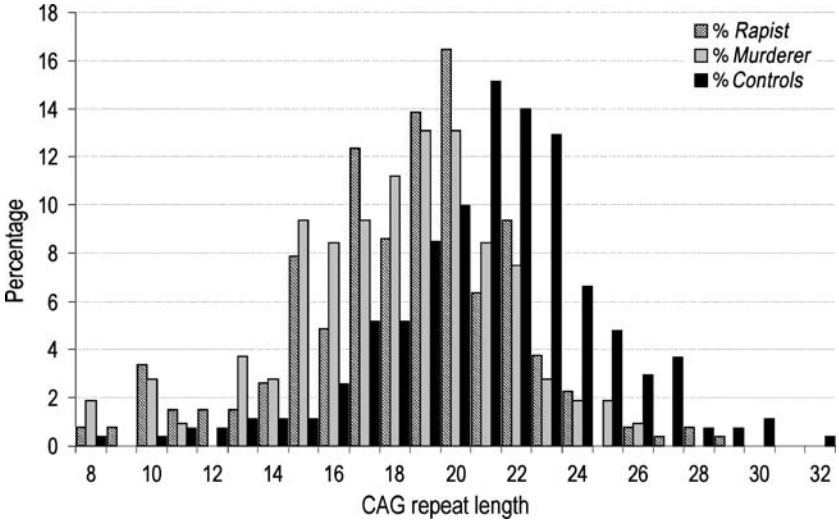

Fig. 1 Distribution of various AR-CAG alleles between cases and controls

dNTPs (10 mM), 5 pM of each primer, 0.5 units Ampli Taq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 10 ng genomic DNA. PCRs were performed under the following conditions: initial denaturation at 94°C for 12 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and polymerization at 72°C for 1 min with a final extension at 72°C for 20 min. For genotyping, 3.0 μl of the PCR product was mixed with 0.3 μl of LIZ500™ and 6.7 μl of Hi-Di formamide and analyzed on 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA). The raw data were further analyzed using GeneMapper software to ascertain the size of AR alleles. PCR and GeneScan analysis were repeated for all the samples to confirm the number of repeats.

Statistical analysis

All the statistical analyses were done using Statistical Package for the Social Sciences software (version 13, Inc., Chicago, IL, USA). Descriptive statistics were computed to determine the mean values of the CAG repeats. The differences in the mean repeats lengths between cases and controls and the three groups individually were computed by "univariate analysis of variance." "Effect size" and "power" statistics were run to estimate the significance of the correlation(s) observed. Test of homogeneity of variances was run to check for the statistical significance of the differences in the variances between groups. We also compared the distribution of the CAG repeats in three gross categories, i.e., repeat length below average (<21 repeats), average (21 repeats), and above average (>21 repeats), by chi-square test between the two categories of the cases and the control samples. To predict data stratification, we classified the cases and controls into three groups according to the parent populations and calculated mean repeat length individually.

Results

Analyses of the subjects with 16 autosomal STR markers (Identifiler plus™) confirmed their criminal activities (rape or murder). We observed significant differences in the distribution of various CAG alleles between cases and controls (Fig. 1). The mean CAG repeat length and the range of repeats were different among different groups (Table 1). Analysis of variance also showed that the mean repeat lengths were significantly different among the three groups (Table 2). The effect size statistics "Partial Eta Squared" showed the significance of the differences observed with a good statistical power (Table 2). Test of homogeneity showed no significant difference in the variances between three groups. Mean repeat lengths both in the rapists and the individuals detained for murder were significantly shorter than the control populations (Table 3). Analyses of the repeat lengths upon dividing the samples in three populations showed almost same mean repeat length (18.07, 18.17, 18.38 repeats) in all the categories. Similarly,

Table 1 CAG repeat distribution and mean length in various categories of criminals versus controls

	Number	Mean	SD	SE	CI for mean (95%)	
					Lower bound	Upper bound
Murder	133	17.59	3.521	0.309	16.98	18.19
Rape	241	18.44	3.583	0.229	17.98	18.89
Controls	271	21.19	3.565	0.217	20.76	21.61

SD Standard deviation, *SE* standard error, *CI* confidence interval

Table 2 Univariate analysis of variance of the CAG repeats distribution among the three groups

	Sum of squares	*df*	Mean square	*F*	Sig.	Partial Eta Squared	Noncent. parameter	Observed power[a]
Between groups	1525.94	2	762.97	60.11	0.000	0.158	120.22	1.000
Within groups	8149.04	642	12.69					

The calculations were done assuming CAG repeats as dependent variable and various groups of individuals as factors
[a] Computed using alpha=0.05

the mean repeat lengths in the three categories of controls were same (21.10, 21.25, 21.29 repeats), ruling out a stratification in these populations. The mean number of repeats in the individuals convicted for "murder after rape" was even smaller (mean 17.31 repeats, SD 3.89) than the individuals convicted for rape or murder alone. In the gross distribution of the three categories of CAG repeats, we observed a higher frequency of less than average (<21) repeats among rapists and murderer, and a higher frequency of average (21) and more than average (>21) repeats among the controls (Fig. 2). Various effect size measures for the chi-square test showed the significance of the differences observed in this test (Tables 4, 5, 6).

Discussion

The antisocial activities (aggression, psychoticism, and tendency to rape or murder), once thought to be personality specific and influenced by environment rather than by genes, are gaining more attention among geneticists. Our present study on AR-CAG repeat length in individuals convicted for rape or murder revealed a significant difference in the mean length and distribution of the AR alleles between criminals and the control men. The comparison of the repeat distribution showed higher percentage of shorter repeats with a shorter mean repeat length among rapists and murderers in comparison to the controls (Fig. 1, Table 1). Taking clues from the *in vitro* studies demonstrating inverse correlation between CAG repeat length and AR activity [15, 16], we hypothesize that the higher AR activity as a result of shorter length of CAG repeats observed in these criminals might be one of the factors associated with criminal behavior.

Although no earlier study has analyzed AR-CAG repeats in rapists, several studies have provided evidence that increased signaling through AR correlate with an increased tendency for the antisocial or criminal activities such as the tendency to develop ASPD, psychiatric behavior, loss of self-control, and a tendency to commit offenses such as rape [10, 11, 21]. Aromaki et al. [11], in their study on rapists and child molesters, reported no difference in the average level of testosterone but observed that the antisocial personality disorder index was directly correlated with the testosterone level, and sexual activity (masturbation and intercourse) was directly correlated with the testosterone level in the rapists and child molesters but not in the control subjects. Giotakos et al. reported higher levels of testosterone and dihydrotestosterone in the rapists [22]. Given that CAG repeats inversely correlate with AR activity, shorter CAG repeats may produce effects equivalent to high testosterone levels.

AR-CAG repeat has been previously studied in association with antisocial activities other than rape. Cheng et al., by their study on violent male criminals, reported shorter repeats (<17) among more violent criminals than controls

Table 3 Comparison of the mean among various groups using univariate analysis of variance

(I) Group	(J) Group	Mean Difference (I−J)	SE	LSD (equivalent to no adjustments)			Bonferroni adjustment for multiple comparisons		
				Sig.	CI for Difference (95%)		Sig.	CI for Difference (95%)	
					Lower bound	Upper bound		Lower bound	Upper bound
Murder	Rape	−0.853	0.385	0.081	−1.777	0.070	0.160	−1.609	0.098
	Control	−3.602*	0.377	0.000	−4.507	−2.696	0.000	−4.342	−2.861
Rape	Murder	0.853	0.385	0.081	−0.070	1.777	0.160	−0.098	1.609
	Control	−2.748*	0.315	0.000	−3.506	−1.991	0.000	−3.368	−2.129
Control	Murder	3.602*	0.377	0.000	2.696	4.507	0.000	2.861	4.342
	Rape	2.748*	0.315	0.000	1.991	3.506	0.000	2.129	3.368

SE Standard error, *LSD* least significant difference, *CI* confidence interval
*The mean difference is significant at the 0.05 level

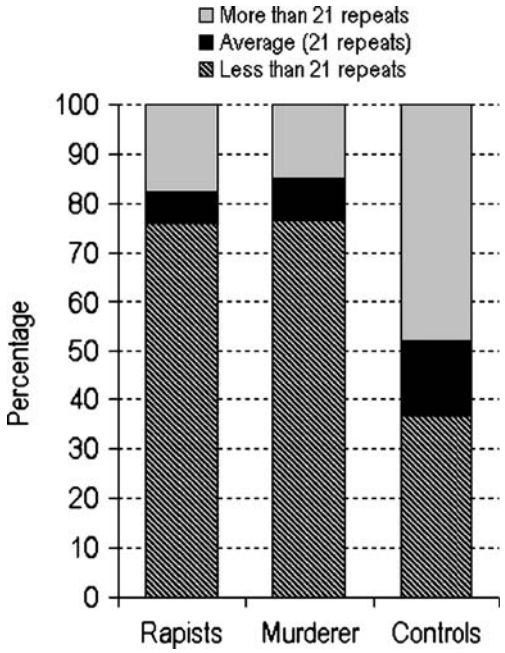

Fig. 2 Comparison of the distribution of the CAG repeats below average (<21), average (21) and above average (>21) among criminals and controls, taking 21 repeats as the average repeat size

[23]. Turakulov et al. reported an association between the psychoticism scale and shorter CAG repeats [24]. Our study supports the results of Chang et al. regarding the violent criminals, and we report the association of shorter CAG repeats with the rape activity for the first time. The antisocial activities, including rape, contribute to the ASPD. Therefore, all these studies along with the present one emphasize the association of the shorter CAG repeats with the criminal or antisocial behavior. Some earlier studies have mentioned drug abuse to be common among sexual offenders, which may affect their mental status because certain drugs are potent to alter testosterone levels [8]. However, in the present study, we could not take drug abuse into account because of unavailability of the information regarding drug abuse among the offenders.

Further, we observed that the mean number of CAG repeats was far shorter (17.31 repeats, SD 3.89) in the individuals convicted for rape or multiple rapes followed by murder (Table 1). This may indicate that smaller CAG repeats may associate with criminal behavior characterized by repeated crimes and severe offences; however, we admit the limitation due to smaller sample size (N=26) in the later category of criminals. It would be highly informative if the overall results of our study could be supported by a study on social and sexual habits of general population, measuring testosterone level and genotyping AR-CAG alleles in them. Furthermore, most of the studies on criminals or rapists to date have studied either testosterone levels or the CAG repeat length in isolation. Given that both the increased testosterone level and the shorter CAG repeats associate with antisocial or criminal activities, it would be interesting to study both these aspects simultaneously on violent criminals or antisocial activists.

In conclusion, the shorter CAG repeat length in *AR* gene associated with the antisocial activities, i.e., murder and rape. Nevertheless, the antisocial behavior and tendency to rape or murder is highly influenced by environmental factors. But the association of increased androgens levels or the shorter CAG repeat length with antisocial activities may indicate additional factors associated with the criminal activities. The genetic study on the criminals may help in understanding if there is some biological factor, which may affect psychological state of an individual, and also in

Table 4 Cross tabulation of the three categories of CAG repeats (<21, 21, >21 repeats) between the three groups of cases and controls

Group	CAG repeat length			
	<21	21	>21	Total
Murder	106	10	17	133
Rape	181	16	44	241
Controls	100	41	130	271
Total	387	67	191	645

Table 5 Chi square statistics of the three categories of CAG repeats (<21, 21, >21 repeats) between the three groups of cases and controls (Table 4)

Chi-square statistics	Value	*df*	Asymp. Sig. (2-sided)
Pearson's chi-square	106.103	4	0.000
Likelihood ratio	108.585	4	0.000
Linear-by-linear association	85.914	1	0.000
N of valid cases	645		

Table 6 Symmetric measures of chi square for the three categories of CAG repeats (<21, 21, >21 repeats) between the three groups of cases and controls (Table 4)

		Symmetric measures of chi-square			
		Value	Asymp. SE[a]	Approx. T[b]	Approx. Sig.
Nominal by nominal	Phi	0.406			0.000
	Cramer's V	0.287			0.000
Interval by interval	Pearson's R	0.365	0.034	9.949	0.000[c]
Ordinal by ordinal	Spearman correlation	0.383	0.035	10.520	0.000[c]
N of valid cases		645			

SE Standard error
[a] Not assuming the null hypothesis
[b] Using the asymptotic standard error assuming the null hypothesis
[c] Based on normal approximation

proper management and reducing the social burden due to these offenses, if the biological basis of such offenses is established.

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