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ORIGINAL ARTICLE

Association of AVPR1A Polymorphism with Criminal Intent

ANAM IQBAL[1], USMAN SHAHID BUTT[2], NASREEN AKHTAR[3], RIASAT ALI[4], TOOBA ALI[5], SADIA ZAHEER[6]
[1]*Senior Demonstrator, Forensic Medicine, Al-Aleem Medical College Lahore*
[2]*Assistant Professor, Forensic Medicine, Govt. Khawaja Muhammad Safdar Medical College, Sialkot*
[3]*Associate Professor, Forensic Medicine, Al-Aleem Medical College Lahore*
[4]*Associate Professor, Forensic Medicine, King Edward Medical University, Lahore*
[5]*Senior Demonstrator Biochemistry, Al-Aleem Medical College Lahore*
[6]*Assistant professor, Medical Education, Sialkot Medical College, Sialkot*
Correspondence to Dr. Usman Shahid Butt, Email: buttrulez@gmail.com, Cell: 0321-6113203

ABSTRACT

Background: The human behavior is influenced by genetic as well as environment components. Likewise, the aggressive behavior having an intent of criminality is also governed by both environmental and genetic makeup.
Aim: The genetic element has been explored by analyzing the microsatellite RS1 and RS3 of AVPRIA gene which showed strong variations in short tandem repeats (STRs) of convicted offenders when they were compared with normal population.
Methods: Blood samples of 100 convicted offenders were taken and DNA was extracted using PCI protocol. The PCR was then carried out using primers and the products were send for gene sequencing. The results were compared with that of general population having no history of crime or psychological abnormality.
Results: The microsatellite RS1 and RS3 of AVPRIA gene showed strong variations in short tandem repeats (STRs) of convicted offenders when they were compared with normal population.
Keywords: AVPR1A, criminal intent, PCR

INTRODUCTION

The researches carried out on human behavior shows that it is influenced by both genetic and environmental factors. Similarly, the aggressive behavior having an intent of criminality is also governed by both environmental and genetic makeup. In this study we have analyzed and explored genetic factor. Genes play a pivotal role in aggression, and different researches on both human and animals show that different variants of a same gene make some subjects to show deviant violent behavior and emerging criminal intent. Aggression is considered a phenomenon in which many genes interact with each other and yields an aggressive phenotype (Rueve and Welton, 2008). It is a complex behavior, regulated by multiple factors, including environmental, cognitive, neurobiological and genetic (Teodorović and Uzelac, 2015). Genes explain approximately half of the variance in human aggressiveness (Denson et al., 2014).

AVPRIA (arginine vasopressin receptor 1A) is type of gene that plays an important role in exhibiting various type of social and antisocial behavior including aggression. It is also labelled as ruthlessness gene according to Nature.com. It plays a major role in aggression which is evident from the fact that antagonists of AVPR1A in hamsters reduced their offensive aggressive behavior (Ferris et al., 2006) and its administration in rats reduced their anxiety and depression (Ebner et al., 1999). There are more than ten million prisoners all around the world and nearly one million prisoners (Shirazi et al., 2016), in each decade, are added to the world's prison population (Fazel and Seewald, 2012). One out of seven people being incarcerated worldwide suffers from severe mental ailments (Thomas et al., 2016). Prisoners mostly suffer from poor physical and mental health during their detention (Macciò et al., 2015).

According to specific researches it became evident that the polymorphic microsatellites present in gene surroundings has been amalgamated with the aggressive behavior present in borderline personality disorders and socially deviant individuals including autistic spectrum disorders. It has also been investigated that antisocial and prosocial behavior including altruistic behavior is associated with RS1 and RS3 microsatellites (Israel et al., 2008).

The 5'-flanking region of the gene contains three polymorphic microsatellite repeats (Thibonnier et al., 2000). Of these RS3, a complex repeat of (CT)4-TT-(CT)8 - (GT) n 3625bp (base pairs) upstream of the transcription start site, with 16 different alleles in the population, and RS1, a (GATA)n repeat with 9 alleles located 553bp from the start site.

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MATERIAL AND METHODS

Blood samples of two groups (offenders and general population) were collected after permission from IRB. DNA extraction was done using standard phenol chloroform isoamyl alcohol (PCI) protocol (Green and Sambrook, 2012). To check the quality as well as quantity of DNA, agarose gel electrophoresis was used. DNA samples were run on agarose gel containing ethidium bromide 0.8% agarose gel was used to check the quality of DNA via gel-electrophoresis. Conventional PCR was used for amplification of DNA using the following primers. The following primers were used (Meyer-Lindenberg et al., 2009).

Forward and reverse primer for RS1 and RS3 microsatellite (Table1.1)

Micro-satellite	5'-Labeled Primer	Reverse Primer
RS1	VIC-AGGGACTGGTTCT	GTTTCTTACCTCTCAAGTT
RS3	6FAM-TCCTGTAGAGAT	GTTTCTTTTTGGAAGAGAC

Table 2: PCR tubes were used to carry out PCR with total reaction volume of total 10 ul

Constituents	Volume
Green Mix	3.8
Water	3.7
Forward Primer	1.5
Reverse Primer	1.5
DNA	1.5

A negative containing master mix only and a positive containing master mix and successfully isolated DNA were set up for each experiment. After amplification the product of Polymerase Chain Reaction were checked on 2% agarose gel. Polyacrylamide gel electrophoresis (PAGE) is usually carried out to separate DNA fragments that are less than 100bp in size. Gel was examined under UV light and photographs were documented. After doing all the processes mentioned above, PCR product of both RS1 and RS3 were send for gene sequencing. The two microsatellites were sequenced, and length polymorphism was observed.
Data analysis: The statistical analysis of gene sequencing was carried out by using independent sample t test.

RESULTS

Fig. 1: Poly Acrylamide Gel Electrophoresis for RS3

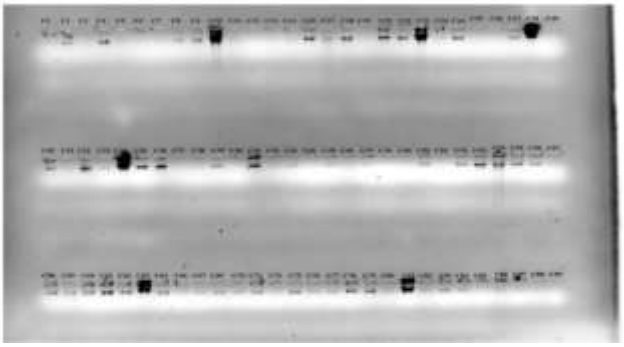


Fig. 2:Ethidium bromide stained 0.8% gal image of extracted samples of convicted criminals

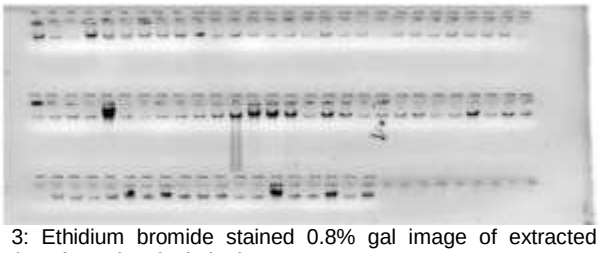


Fig. 3: Ethidium bromide stained 0.8% gal image of extracted samples of convicted criminals

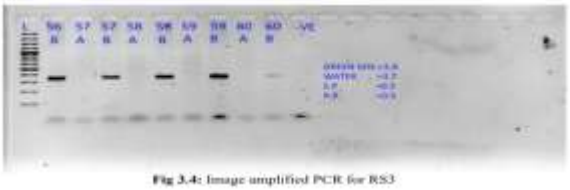


Fig 3.4: Image amplified PCR for RS3

Fig. 4: Amplified PCR of RS1 and RS3

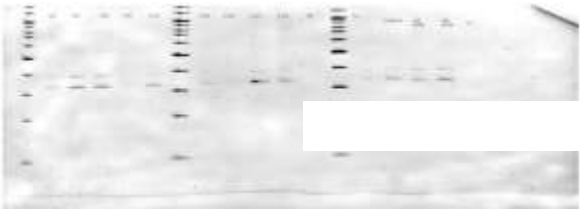
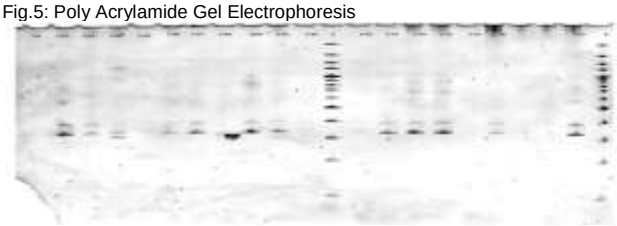


Fig.5: Poly Acrylamide Gel Electrophoresis



GATA Repeats

Table 3.4: Mean and Standard Deviation of GATA repeats of offenders and General population

	Group	N	Mean	Std. Deviation	Std. Error Mean
Repeats	Offenders	50	10.8400	1.11319	.15743
	GATA normal value	50	14.0000	.00000	.00000

Table 3.5: Independent sample t test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
Repeats	Equal variances assumed	82.065	.000	-20.073	98	.000	-3.16000	.15743	-3.47241	-2.84759
	Equal variances not assumed			-20.073	49.000	.000	-3.16000	.15743	-3.47636	-2.84364

P Value <0.05 shows that there is a significant difference exist between the Normal RS1 STRS and Convicted RS1 STRs

RS3 Microsatellite

Table 3.6: Mean and Standard Deviation of CT, TT, TC and TG repeats of RS3

	Group	N	Mean	Std. Deviation	Std. Error Mean
CT	RS3(Offenders)	52	4.0000	.00000[a]	.00000
	Normal values	52	4.0000	.00000[a]	.00000
TT	RS3(Offenders)	52	1.0000	.00000[a]	.00000
	Normal values	52	1.0000	.00000[a]	.00000
TC	RS3(Offenders)	52	9.7885	1.52543	.21154
	Normal values	52	8.0000	.00000	.00000
TG	RS3(Offenders)	47	22.5957	2.37432	.34633
	Normal values	52	24.0000	.00000	.00000

CT and TT cannot be computed because the standard deviations of both groups are 0.

Table 3.7: Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	T	Df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
TC	Equal variances assumed	57.688	.000	8.455	102	.000	1.78846	.21154	1.36888	2.20805
	Equal variances not assumed			8.455	51.000	.000	1.78846	.21154	1.36378	2.21314
TG	assumed		.000	-4.267	97	.000	-1.40426	.32908	-2.05738	-.75113
	Equal variances not assumed			-4.055	46.000	.000	-1.40426	.34633	-2.10138	-.70713

P Value <0.05 shows that there is a significant difference exist between the Normal RS3 STRS and Convicted RS3 STRs

DISCUSSION

The genetic factor that has an impact on aggressive behavior associated with criminal behavior was analyzes by exploring AVPR1A gene which is also known as ruthlessness gene. Two microsatellites RS1 and RS3 of this gene were studied. In order to study AVPR1A gene, first DNA was extracted from the blood

samples obtained from convicted criminals. The samples were taken from different jails of Punjab. DNA was extracted by PCI protocol. DNA extraction of 200 samples were done, out of which 100 were of general population having no history of crime and 100 were from offenders.

After extraction of DNA, its qualitative analysis was performed by running the samples on 0.8% agarose gel as shown in fig 1.1. The extracted DNA was subjected to PCR. Different primers (forward and reverse) for both microsatellites were used mentioned in Table 1.1. By optimizing conditions for PCR, again the extracted DNA was subjected to polymerase chain reaction for its amplification. PCR conditions were optimized, and master mix was prepared as shown in Table 1.2. The PCR product obtained was again checked on 2% agarose gel to check the quality of amplified DNA

After checking the PCR products on 2% agarose gel as shown in fig 1.3,1.4, they were subjected to PAGE (polyacrylamide gel electrophoresis). The purpose of this study was to find the difference in the base pairs, since it can detect up to 100bp difference. The results obtained from PAGE were not enough to find the variation between two groups as shown in fig 1.5 and 1.6. So, the amplified DNA products were sent for genetic sequencing. Because of limited resources, almost 100 samples of convicted offenders were sent for genetic sequencing. Approximately half samples were sequenced for RS3 and remaining half were sequenced for RS1. The analysis of microsatellites RS1 and RS3 of AVPRIA gene showed variation in short tandem repeats. The normal values of both the alleles were taken from previous researches conducted on AVPRIA polymorphism on autism (Wassink et al., 2004, Yirmiya et al., 2006, Kim et al., 2002).

In RS1, GATA repeats were studied. It showed mean value of 14.00 in normal population (Kim et al., 2002). When the samples were sequenced for RS1 microsatellite, mean value of 10.00 in case of offenders was observed shown in Table 1.3. Independent t-test was applied and p value of 0.00 was observed as evident from Table 1.4. Since p value signifies the error in research and its value below 0.05 is accepted. In our case p value was 0.00, so the results were statistically significant. This means variations do exist in offenders when studied against general population. In case of RS3, four repetitive STRs were studied. These include CT, TT, TC and TG. There means value along with standard error and standard deviations are shown in Table 1.5. On analyzing CT, it was found that the mean value of CT in normal population was 4.00 (taken from previous research). On scrutinizing the CT sequence in offenders, it was also found to be 4.00. since they have equal mean, so they don't show standard deviation. Hence proved no variation exists in CT repeats. Now TT sequence was studied. The mean value taken from previous researches was 1.00 in general population. When the same sequence was studies in offenders, it was surprisingly found to be same. Like CT repeats showing no variations, TT also showed no variation in offenders. Both CT and TT repeats, standard deviation is 0.0 so both were excluded from further analysis. After analyzing CT and TT, showing no variation in offenders, TC repeats were studied. The mean value was found to be 8.00 as taken from previous researches. The TC value of offenders was found to be 9.78. since standard deviation of 1.5 exists between two group, t test was applied. The p value was found to be 0.00 which was statistically significant Lastly TG repeats were studied. Their mean value of general population was found to be 24. When the sequence of offenders was analyzed, it showed mean value of 22.56. As the difference exist between two groups, standard deviation of 2.37 was observed. Independent variable t test was applied as depicted in Table 1. It showed p value to be 0.00 which was significant. In our study when we analyzed RS1, variation exist between the two groups. In the same way RS3, out of four repeats, variation was present in two repeats in samples of convicted offenders. This study reveals the association of AVPR1A polymorphism and dermatoglyphics with criminal intent.

CONCLUSION

This study may be helpful in reshaping medicolegal framework of our country by making separate legislation bodies for these genetically and socially deviant individuals. This may include lesser punishment, rehabilitation centers and providing mental care for such individuals.

Contributions of authors: AI: Write up, Data collection, USB: Write up, Data collection, **NA:** Sample collection, **RA:** Sample collection, **TA:** Data Analysis, SZ: Data Analysis

Conflict of interest: Nil

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