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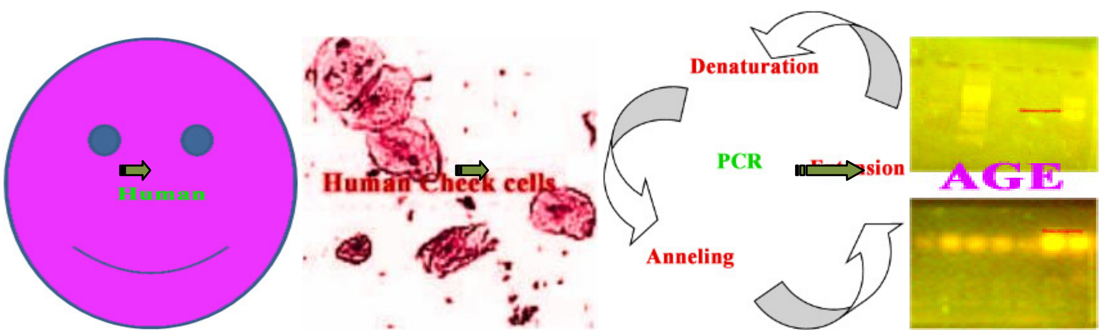
DNA PROFILING USING POLYMORPHIC AUTOSOMAL DNA LOCI PV92 IN A DRAVIDIAN POPULATION AND EVALUATION OF HARDY WEINBERG LAW

ABSTRACT

The study was made on the DNA profiling using polymorphic autosomal DNA loci, *Alu* PV 92 in a Dravidian population and the application of Hardy Weinberg Law was evaluated. The population that comprised of different castes. The populations present inter caste marriage and mixing of gene did not occur over several hundred years. In this present study, polymorphic autosomal DNA loci *Alu* PV 92 to evaluate the genotype of known Dravidian population. PCR was used to amplify a nucleotide sequence from chromosome 16 to look for a short DNA *Alu* element. Theoretically in some geographically isolated population all individuals may be homozygous (+/+) or the individuals may be homozygous (-/-) while in the melting pot population the three genotypes may exist in equilibrium. In our observation, it was observed that the presence of Homozygocity and Heterozygocity. The absence of evolutionary process like mutation genetic drift and selection gene frequencies remain constant from generation to generation as they are set to be in Hardy Weinberg law equilibrium.



Keywords: Dravidian population, DNA profile, *Alu*, Hardy Weinberg, Autosomal DNA.

1. INTRODUCTION

DNA finger printing techniques permit detection of different dispersed chromosomal minisatelite loci under condition of moderate hybridization stringency, Individuals-specific DNA fingerprint generated with the aid of polypore probes are utilized in forensic medicine as well as in numerous areas in genetics [1 & 2]. DNA fingerprinting techniques have also been used in population studies on many different kinds of organisms. In most of these studies mutinous probes were used (33.6, 33.15) [2]. M13minisatilites are found in genomes of all known living organisms and M13 minisatelites are randomly distributed on chromosomes, whereas the Jeffrey's probes are clustered near telomeres [3]. The chemical structure of everyone's DNA is the same. The only difference between people is the order of the base pairs in each person's DNA. Using these sequences every person could be identified solely by the sequence of their base pairs, the task would be very times – consuming. Instead, scientists are able to use a shorter method, because of repeating patterns in DNA [4-6]. These patterns do not, however, give an individual "fingerprint", but they are able to determine whether two DNA samples are from the same person [7-11], related people or non-related people. Hence in the present study DNA of people belonged to different castes living as a Dravidian community were analyzed to find out whether they belonged to the same ethnic group.

2. MATERIALS AND METHODS

2.1. Isolation of DNA from human cheek cells:

A total of 100 persons belonged to five different castes Nadar, Thever, Mudaliar, Bramin and Vellalar were selected. The sample consists

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of 53 females and 47 males. Oral swabs from the inner region of cheek were collected with cotton tipped applicator. Twirl the applicator while vigorously swabbing inside both the both cheeks between gum line and under the tongue. The cells attached with the swabs were washed and collected. From this collected cheek cell's DNA was isolated.

2.2. Amplification of the PV92 locus:

The isolated DNA was amplified using PCR reaction contains Taq DNA Polymerase, the four deoxytriphosphate, Mg^{2+}, buffer solution and PV92 primer.

2.3. Electrophoresis:

Amplified PV92 DNA fragment was separated by Agarose Gel Electrophoresis.

3. RESULTS

3.1. DNA profiling and evaluation of Hardy Weinberg Law in males:

For determining of the genotype distribution of *Alu* gene, male population was taken for the study (N=47) (Table 1). Homozygocity (+/+) was observed in band 731bp or Homozygous (-/-) was observed in band 416bp. Heterozygous observed two bands in 731bp and 416bp. Heterozygous (+/-) was observed to be 11 and homozygous (+/+) was observed to be 24 and homozygous (-/-) was observed to be 12 (Table 2).

3.1.1. Homozygocity and Heterozygocity in males:

Since the humans are diploid the total number of alleles taken for the study is 94 (47 X 2) from our observation.

The allele frequency for Alu^+ is 2X24 (homozygous)

+11(heterozygous)/94 =59/94= 0.63.

The allele frequency for Alu^- is 2X12 (homozygous)

+11(heterozygous)/94 =35/94= 0.37.

3.1.2. Evaluation of Hardy Weinberg law:

By calculating p and q values the distribution of the genotypes are described by equation $p^2 + 2pq + q^2 = 1$.

Substituting p and q values in above equation:

P = 0.63 and q = 0.37: $(0.63)^2 + 2\,(0.63 X\, 0.37) + (0.37)^2 = 1$

The above equation observed to satisfies the Hardy Weinberg law.

3.1.3. Chi square test:

The above values were analyzed by Chi square test, (Table 3)

Formula= $\sum \frac{(O-E)^2}{E}$ E

Chi square (X^2) = 0.068, the chi square values was less than that of the table (Table 3) value (0.455 df = 1) at 95.5% level confidence. Here our observation accepts the null hypothesis and the population taken for the study is in the study taken is in a equilibrium and accepts Hardy Weinberg law.

3.2. DNA profiling and evaluation of Hardy Weinberg Law in females:

The genotype distribution of *Alu* gene in female population taken for the study (N=47) (Table 4), showed Homozygous +/+ observed single band in 731bp or Homozygous -/- observed single band in 416bp. Heterozygous (two bands) was seen in 731bp and 416bp. Heterozygous (+/-) was found in 29 sample and homozygous (+/+) was observed to be 14 and homozygous (-/-) was observed 10 persons (Table 4).

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3.2.1. Homozygocity and Heterozygocity in females:

Since the humans are diploid the total number of alleles taken for the study is 106 (53 X 2) from our observation. (Table 5)

The allele frequency for *Alu*$^+$ is 2X14 (homozygous)

+29(heterozygous)/106 =57/106= 0.54.

The allele frequency for *Alu* $^-$ is 2X10 (homozygous)

+29(heterozygous)/106 =49/106= 0.46.

3.2.2. Evaluation of Hardy Weinberg law:

By calculating p and q values the distribution of the genotypes are described by equation $p^2 + 2pq + q^2 = 1$.

Substituting p and q values in above equation:

P = 0.54 and q = 0.46: $(0.54)^2 + 2\,(0.54X\,0.46) + (0.46)^2 = 1$

The above equation observed to satisfies the Hardy Weinberg law.

3.2.3. Chi square test:

The above values were analyzed by Chi square test, (Table 6)

Chi square (X^2) = 0.064, the chi square values was less than that of the table (Table 3) value (0.455 df = 1) at 95.5% level confidence. Here our observation accepts the null hypothesis and the population taken for the study is in a equilibrium and accepts Hardy Weinberg law.

3.3. DNA profiling and evaluation of Hardy Weinberg Law in both males and females:

Determining of the genotype distribution of *Alu* gene in female population taken for the study N=100 (Table 1 & 4), and there by counting genotypes ie Homozygous +/+ observed one band in 731bp or Homozygous -/- observed one band in 416bp. Heterozygous observed two bands in 731bp and 416bp. Heterozygous (+/-) was observed to be 40 and homozygous (+/+) was observed to be 38 and homozygous (-/-) was observed to be 22 (Table 1 & 4).

3.3.1. Homozygocity and Heterozygocity in both males and females:

As the humans are diploid the total number of alleles taken for the study is 200 (100 X 2) from our observation. (Table 7)

The allele frequency for *Alu*$^+$ is 2X38 (homozygous)

+40(heterozygous)/200 =116/200= 0.58.

The allele frequency for *Alu* $^-$ is 2X22 (homozygous)

+40(heterozygous)/200 =84/200= 0.42.

3.3.2. Evaluation of Hardy Weinberg law:

By calculating p and q values the distribution of the genotypes are described by equation $p^2 + 2pq + q^2 = 1$.

Substituting p and q values in above equation:

P = 0.58 and q = 0.42: $(0.58)^2 + 2\,(0.58X\,0.42) + (0.42)^2 = 1$

The above equation observed to satisfies the Hardy Weinberg law.

3.3.3. Chi square test:

For the above values were analyzed by Chi square test, (Table 8)

Chi square (X^2) = 0.026, the chi square values was less than that of the table (Table 3) value (0.455 df = 1) at 95.5% level confidence. Here our observation accepts the null hypothesis and the population taken for the study is in the study taken is in a equilibrium and accepts Hardy Weinberg law.

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Table 1: DNA profiling and evaluation of Hardy Weinberg Law in males:

Bands in 100bp DNA ladder	731bp	416bp	*Alu* typing
Sample 1	+/+		Homozygous
Sample 2	+/+		Homozygous
Sample 3		-/-	Homozygous
Sample 4	+/+		Homozygous
Sample 5	+	-	Heterozygous
Sample 6	+	-	Heterozygous
Sample 7	+	-	Heterozygous
Sample 8		-/-	Homozygous
Sample 9	+/+		Homozygous
Sample 10	+/+		Homozygous
Sample 11	+/+		Homozygous
Sample 12	+/+		Homozygous
Sample 13	+	-	Heterozygous
Sample 14		-/-	Homozygous
Sample 15		-/-	Homozygous
Sample 16	+	-	Heterozygous
Sample 17	+/+		Homozygous
Sample 18	+/+		Homozygous
Sample 19	+/+		Homozygous
Sample 20	+/+		Homozygous
Sample 21	+	-	Heterozygous
Sample 22	+/+		Homozygous
Sample 23	+/+		Homozygous
Sample 24		-/-	Homozygous
Sample 25	+/+		Homozygous
Sample 26	+/+		Homozygous
Sample 27	+/+		Homozygous
Sample 28	+/+		Homozygous
Sample 29	+	-	Heterozygous
Sample 30	+/+		Homozygous
Sample 31	+	-	Heterozygous
Sample 32		-/-	Homozygous
Sample 33		-/-	Homozygous
Sample 34	+	-	Heterozygous
Sample 35	+/+		Homozygous
Sample 36	+/+		Homozygous
Sample 37		-/-	Homozygous
Sample 38		-/-	Homozygous
Sample 39		-/-	Homozygous
Sample 40	+	-	Heterozygous
Sample 41	+/+		Homozygous
Sample 42	+/+		Homozygous
Sample 43	+/+		Homozygous
Sample 44		-/-	Homozygous
Sample 45	+/+		Homozygous
Sample 46	+	-	Heterozygous

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Sample 47	-/-	Homozygous

Table 2: Homozygocity and Heterozygocity in males

Homozygous		Heterozygous
-/-	+/+	+/-
12	24	11
36		11

Total = 47

Table 3: Chi square analysis for male population:

Possibilities	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$	Result
Homozygous +/+	0.63	0.5	+0.13	0.017	$\frac{0.017}{0.5}$	0.034
Homozygous -/-	0.37	0.5	-0.13	0.017	$\frac{0.017}{0.5}$	0.034
					$\sum \frac{(O-E)^2}{E}$	= 0.068

Chi square $(X^2) = 0.068$

Table 4: DNA profiling and evaluation of Hardy Weinberg Law in males

Bands in 100bp DNA ladder	731bp	416bp	*Alu* typing
Sample 1	+/+		Homozygous
Sample 2	+/+		Homozygous
Sample 3	+/+		Homozygous
Sample 4	+/+		Homozygous
Sample 5	+	-	Heterozygous
Sample 6	+	-	Heterozygous
Sample 7		-/-	Homozygous
Sample 8		-/-	Homozygous
Sample 9	+	-	Heterozygous
Sample 10	+	-	Heterozygous
Sample 11	+/+		Homozygous
Sample 12	+/+		Homozygous
Sample 13	+	-	Heterozygous
Sample 14	+	-	Heterozygous
Sample 15	+	-	Heterozygous
Sample 16	+/+		Homozygous
Sample 17	+/+		Homozygous
Sample 18		-/-	Homozygous
Sample 19	+	-	Heterozygous
Sample 20		-/-	Homozygous
Sample 21	+/+		Homozygous

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Sample 22	+/+		Homozygous
Sample 23		-/-	Homozygous
Sample 24	+	-	Heterozygous
Sample 25	+	-	Heterozygous
Sample 26	+	-	Heterozygous
Sample 27		-/-	Homozygous
Sample 28	+/+		Homozygous
Sample 29		-/-	Homozygous
Sample 30		-/-	Homozygous
Sample 31	+	-	Heterozygous
Sample 32	+	-	Heterozygous
Sample 33	+/+		Homozygous
Sample 34		-/-	Homozygous
Sample 35	+	-	Heterozygous
Sample 36	+	-	Heterozygous
Sample 37	+	-	Heterozygous
Sample 38		-/-	Homozygous
Sample 39	+	-	Heterozygous
Sample 40	+/+		Homozygous
Sample 41	+	-	Heterozygous
Sample 42	+	-	Heterozygous
Sample 43	+	-	Heterozygous
Sample 44	+	-	Heterozygous
Sample 45	+	-	Heterozygous
Sample 46	+	-	Heterozygous
Sample 47	+/+		Homozygous
Sample 48	+	-	Heterozygous
Sample 49	+	-	Heterozygous
Sample 50	+	-	Heterozygous
Sample 51	+	-	Heterozygous
Sample 52	+	-	Heterozygous
Sample 53	+	-	Heterozygous

Table 5: Homozygocity and Heterozygocity in females

Homozygous		Heterozygous
-/-	+/+	+/-
10	14	29
24		29

Total = 53

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Table 6: Chi square analysis for female population

Possibilities	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$	Result
Homozygous +/+	0.54	0.5	+0.04	0.016	$\frac{0.016}{0.5}$	0.032
Homozygous -/-	0.46	0.5	-0.04	0.016	$\frac{0.016}{0.5}$	0.032
					$\sum \frac{(O-E)^2}{E}$	= 0.064

Chi square (X^2) = 0.064.

Table 7: Homozygocity and Heterozygocity in both females and males

Homozygous		Heterozygous
-/-	+/+	+/-
22	38	40
60		40

Total = 100

Table 8: Chi square analysis for female population

Possibilities	Observed frequency (O)	Expected frequency (E)	(O-E)	$(O-E)^2$	$\frac{(O-E)^2}{E}$	Result
Homozygous +/+	0.58	0.5	+0.08	0.0064	$\frac{0.0064}{0.5}$	0.013
Homozygous -/-	0.42	0.5	-0.08	0.0064	$\frac{0.0064}{0.5}$	0.013
					$\sum \frac{(O-E)^2}{E}$	= 0.026

Chi square (X^2) = 0.026.

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4. DISCUSSION

The study was made on the DNA profiling using polymorphic autosomal DNA loci, *Alu* PV 92 in a Dravidian population and evaluation Hardy Weinberg Law. The human genome contains a variety of length and sequence specific polymorphic loci that can be used, to distinguish individuals (12). In this present study, polymorphic autosomal DNA loci *Alu* PV 92 were used to evaluate the genotype of known Dravidian population and to assess the Hardy Weinberg Law. According to Rudin and Inmen (2002) and National Research council report I and II, allelic frequency for polymorphic loci can be determined and used to calculate genotypic frequency. Similar pattern of study was carried out in the present investigation. PCR used to amplify a nucleotide sequence from chromosome 16 to look for a short DNA *Alu* element (13 & 14).

The value of *Alu* insert is having a non – coding region of the TPA gene which is not related to a particular disease nor does it code for any protein sequence. It is just non – coding DNA that can be used as a tool to study human genotype frequencies. Because *Alu* – repeats have become integrated into the general population at random, the *Alu* insert in the TPA gene is very useful in the study of the gene frequencies localized in human population (15-17). Theoretically in some geographically isolated population all individuals may be homozygous (+/+) or the individuals may be homozygous (-/-) while in the melting pot population the three genotypes may exist in equilibrium. In our observation Homozygocity and Heterozygocity were found.

This can be compared with that of Hardy Weinberg Law in that the absence of evolutionary process like mutation genetic drift and selection, so in the present study it was found that all the five castes studied are isolated group without any inter caste wedding. Gene frequencies remain constant from generation to generation and it's set to be in Hardy Weinberg law equilibrium.

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R.Mariselvam, E.Muthukumar, S.Suresh, A.J.A. Ranjit Singh* and K.R.Narayanan

Department of Zoology, Paramakalyani College, Alwarkurichi, Tamilnadu, India.

Corresponding Author,

A.J.A Ranjit Singh

Principal, Sri Paramakalyani College,

Alwarkurichi,

Tamilnadu, India.