
Detection of ACTN3 Gene in Hypertension Patients Using Specific Primers

M. Manohar,* Omran M. Abdrahman,* M. Nazurudeen,**

Abstract

Hypertension is a growing global problem, which mostly affects the adults. There two main problems which are mostly affected the world populations, one is diabetics mellitus and another one is Hypertension. It is a risk factor for death from stoke myocardial infection congestive heart failure, ischemic heart disease, and progressive renal damage. They are responsible for morbidity and mortality rate will be high. The early studies to detect the X-ray technique and Scanning techniques were used in the diagnosis of heart disease. To use it several times it will be side effects in some other problems and have poor rate of detention and treatment. The recent studies SNP detection will be used to solve the problems and responsible for good treatment. In recently RFLP analysis to easy detect the diseases in Mc Ardle's disease from women's, Human muscular dystrophy, many types of cancer, coronary heart disease glycogensisa type V disease to diagnosis and treatment of the diseases and improvement of the patients health.

Introduction:

Hypertension is defined by arbitrary limits, but it represents the upper end of this distribution of blood pressure determined by a contribution of genetic and environmental factors (Zhang *et al.*, 2002). Hypertension affects about 25% of most adult populations and is a risk factor for death from stroke myocardial infection, and congestive heart failure (Lida, *et al.*, 2003). The largest group of hypertensive patients have essential hypertension (EH). Human essential hypertension (EH) is a complex and multifactorial trait that is influenced by environmental and genetic determinants. Among environmental factors influencing this disease we note high dietary intake of sodium and alcohol, obesity and stress.

Hypertension is a growing global problem and is responsible for considerable morbidity and mortality. Despite this, recent studies have still shown a poor rate of detection, treatment and more importantly to control the target blood pressure levels (Wirth 2000). At present it is widely accepted that approximately 30-50% of cases of hypertension can arise from genetic susceptibility. Human hypertension is a complex, polygenic disease in which one or more gene control the level of blood pressure.

SNP: (Single Nucleotide polymorphism)

Single nucleotide polymorphisms (SNPs) are DNA sequence variations that occur when a single nucleotide in the genome sequence is

altered. The use of SNP is expected to lead to a greater elucidation of the genetic basis for diseases and to realize the potential for clinical diagnostics and pharmacogenetics (Delrieu and Bowman, 2006). There is thought to be one SNP for every 500-1000 bases and 3-5million SNPs in the entire human genome.

SNP analysis at multiple loci can also be useful in the study of polygenic disease by targeting specific families of SNPs or by genome-wide linkage disequilibrium mapping. The use of SNPs in genetic disease detection is facilitated by the recent discovery of more than 4000000 SNPs in the human genome that have the potential to be a rich source of genetic markers to establish genetic linkage and as indicators of disease.

SNP identification and functional assessment is becoming an increasing more important tool in molecular diagnostics and Biology. (Alderborn *et al.*, 2000; Griffin and Smith, 2000; Livak, 1999). SNPs are the most common type of human genetic variation. These single base sequence variations occur at a frequency of approximately one SNP every 1000bp throughout the human genome (Chang *et al.*, 2001). The high frequency of SNPs suggest that SNP genotyping will play a key role in future research and aimed to identify genetic variants involved in disease.

*) Department of Microbiology, Faculty of Medical Science, Sebha University, Sebha, Libya.

**) P. G. Microbiology, K.R. College of Arts and Science, K. R. Nagar 628 503.

ACTN 3 GENE:

At present Real-Time PCR is the most used technology for detection of SNPs (Kwok, 2002). This assay requires only a small amount of human DNA in clinical samples which is more than sufficient. The α -actinins belongs to family of actin - binding and cross linking proteins related to dystrophin, utrophin and spectrin (Blanchard *et al.*, 1989).

In skeletal muscle, α -actinin-2 is expressed in all fibers, whereas α -actinin-3 is found at the Z lines of a subset of type 2 fibers (all type 2b and 50% type 2a). Using synthetic peptides was designed from unique amino-terminal sequences, and developed isoform-specific antisera with which to characterize protein expression.

Using synthetic peptides designed from unique amino-terminal sequences, and developed isoform-specific antisera with which to characterize protein expression. Expression of the two isoforms diverged through mammalian evolution, with α -actinin-2 being expressed in skeletal muscle fibers and cardiomyocytes, while the expression of α -actinin-3 is almost exclusively restricted to glycolytic, type II muscle fibers. Everyone has two copies of ACTN 3 gene with one copy inherited from each parent. The ACTN 3 gene instructs our body to produce a protein called α -actinin-3.

Materials and Methods:

Blood sample collection:

Blood samples were obtained from people of different age groups (30-50) in and around

Virudhunagar (District) Tamil Nadu, India. . Samples were collected from known sources and from Diagnostic Laboratory and Nursing homes. 5ml of blood was collected and was refrigerated by adding EDTA for further use.

Isolation of DNA:

Human Genomic DNA was isolated from the Lymphocytes by using modified Miller's protocol.

Agarose gel electrophoresis of DNA samples:

The 1% agarose gel was seen to be appropriate for electrophoresis of human genomic DNA (Chikh Bengra ., 2002).

Polymerase Chain Reaction (PCR) of exon 15 region:

PCR was carried out to amplify the exon 15 of the human ACTN 3 gene, in which the polymorphism (C>T transition) is present. The primers were designed based on the DNA sequence of the human ACTN 3 gene.

A) Primers:

The primers were obtained in the lyophilized form from IDT, Coralville, USA.

The primers were obtained in the lyophilized form from IDT, Coralville, USA. The primers were diluted with TE to get a working solution of 250 ng / μ l respectively. It was stored at -20° C.

Table.4.1

Sl.No	Primer Name	Primer Sequence	Primer length	GC%	Tm	Product size
1.	ABF	5' CTG TTG CCT GTG GTA AGT GGG 3'	21 mer	57.14	66°C	291 bp
2.	ABR	5' TGG TCA CAG TAT GCA GGA GGG 3'	21 mer	57.14	66°C	291 bp

Primers ABF – 40 times diluted i.e. 1 μ l stock + 39 μ l TE

Primers ABR – 45 times diluted i.e. 1 μ l stock + 44 μ l TE

B) Taq polymerase:

Taq Polymerase was obtained at a concentration of 3 U/ μ l and was stored at -20° C.

The typical amplification cycle is represented below.

1. Initial Denaturation : 94°C 3min
2. Denaturation : 94°C 30sec
3. Annealing : 66°C 30sec
4. Extension : 72°C 60sec
5. Repeat steps 2-4, 34 times
6. Final Extension : 72°C 2min
7. Holding temperature : 4°C

The annealing temperature was standardized to 66°C

Agarose Gel Electrophoresis:

After each PCR reaction the products were checked by electrophoresis on a 2% agarose gel. 600mg of agarose was melted in 30 ml of 1X TBE buffer. 5 µl of the reaction products were loaded with 2 µl of loading dye to check for amplification.

RFLP Analysis:

Genotyping was performed by RFLP (Restriction Fragment Length Polymorphism) analysis. PCR amplicons were digested with Dde I enzyme. In 577 R allele, Dde I enzyme recognize 5'CTNAG 3' site and produce two fragments (205bp and 86 bp). In 577X Dde I will recognize two sites with the above sequence and products three fragments (108bp, 97bp and 86bp). In heterozygous mutant type Dde I produced four fragments (205bp, 108bp, 97bp and 86bp).

Methodology:

The digested amplicons were analyzed by electrophoresis on a 2% agarose gel. 600 mg of agarose was melted in 30 ml of 1X TBE buffer. 8 µl of the digested sample were mixed with 2 µl of loading dye for the analysis of digestion. The digested DNA sample in the gel was viewed under UV trans-illuminator and photographed.

Results**Blood sample collection:**

The sixty blood samples were collected from the hypertension patients of different age groups between 30 –52, out of 60 patients 29 patients had ACTN3 gene. Among 29 patients 16 male patients and 13 female patient's

Table 5.1

S.No	Age	Male	Female	ACTN3 GENE	
				Male	Female
1	30 – 35	10	5	5	3
2	36 – 40	6	9	3	4
3	41 – 45	11	4	3	4
4	46 – 52	10	5	5	2
Total		37	23	16	13

Number of Blood Samples 60

Male 37

Female 23

ACTN3Gene collected in Blood Samples in Male 16

ACTN3Gene collected in Blood Samples in Female 13

Total No. of ACTN3 Gene collected in Blood Samples 29

Percentage of ACTN3 Gene in Male Patients. $16/37 \times 100 = 23.17$

Percentage of ACTN3 Gene in Female Patients. $13/23 \times 100 = 17.69$

Total No. of Blood Samples 60

Total No. of ACTN3 Gene in Patients Blood 29

Percentage of ACTN3 Gene in Patients Blood $29/60 \times 100 = 20.7$

presence in ACTN3 gene. (Table – 5.1, 5.2, 5.3, 5.4 Figures 5.2.1, 5.3.1, 5.4.1)

Isolation of DNA:

The DNA was isolated using the salting out method. After the identification of DNA it undergoes for the purity process. After the purity of DNA, it confirm the DNA bands with the help of 1% agarose gel electrophoresis (Plate 1).

PCR analysis:

After confirm the specific DNA, it undergo for the amplification process. From, that the ACTN3 gene alone amplify with the help of specific primers. The PCR amplification cycle undergo up to 35 cycles.

Followed, the amplification process, then the ACTN3 gene was amplified or not, it will be confirmed with the help of 2% agarose gel electrophoresis. The control sample shows no band formation, but the positive samples show the banding formation. It indicates that the presence of SNP marker. (Plate 2)

RFLP analysis:

The ACTN3 gene carries the restriction site, Dde I enzyme. The restriction enzymes added into the PCR product, and incubate for 2 hours, after that run in the agarose gel electrophoresis. To visualize the restriction fragments in the ACTN3 gene, but in the control sample cleaves into two bands and the mutant type produce four fragments (Plate 3).

Table – 5.2

Sl. No.	Total No. of Patients in blood samples	Total No. of ACTN3 gene patients in blood samples
1.	60	29

Figure – 5.2.1

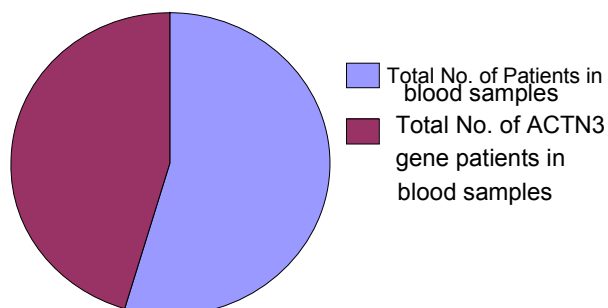


Table – 5.3

Sl. No.	Total No. of blood samples in male patients	ACTN3 gene in male patients
1.	37	16

Figure – 5.3.1

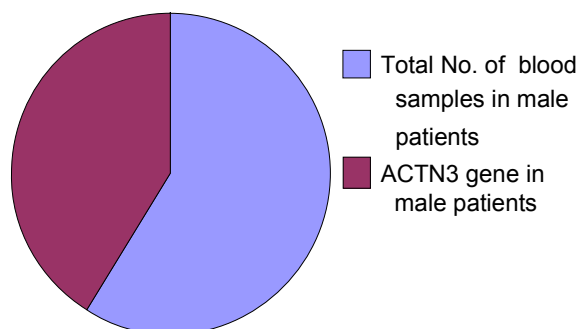
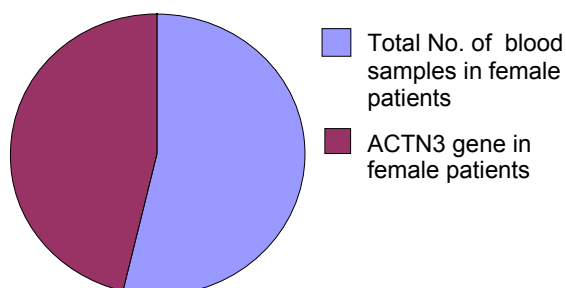


Table – 5.4

Sl. No.	Total No. of blood samples in female patients	ACTN3 gene in female patients
1.	23	13

Figure – 5.4.1



Discussion:

Hypertension disorder is a major risk factor of cardiovascular disease such as coronary heart disease and stroke. They often occur concomitantly, it is the same individuals and have an evident familial aggregation. This suggests that common genetic and environmental effects on these factors are present and genes conferring susceptibility to the development of hypertension might be closely linked with the genes leads to hypertension. Several genes linked for hypertension activity LPL (lipoprotein lipase), LDL (low-density lipoprotein) ACTN 3 and others (Laur *et al.*, 2001).

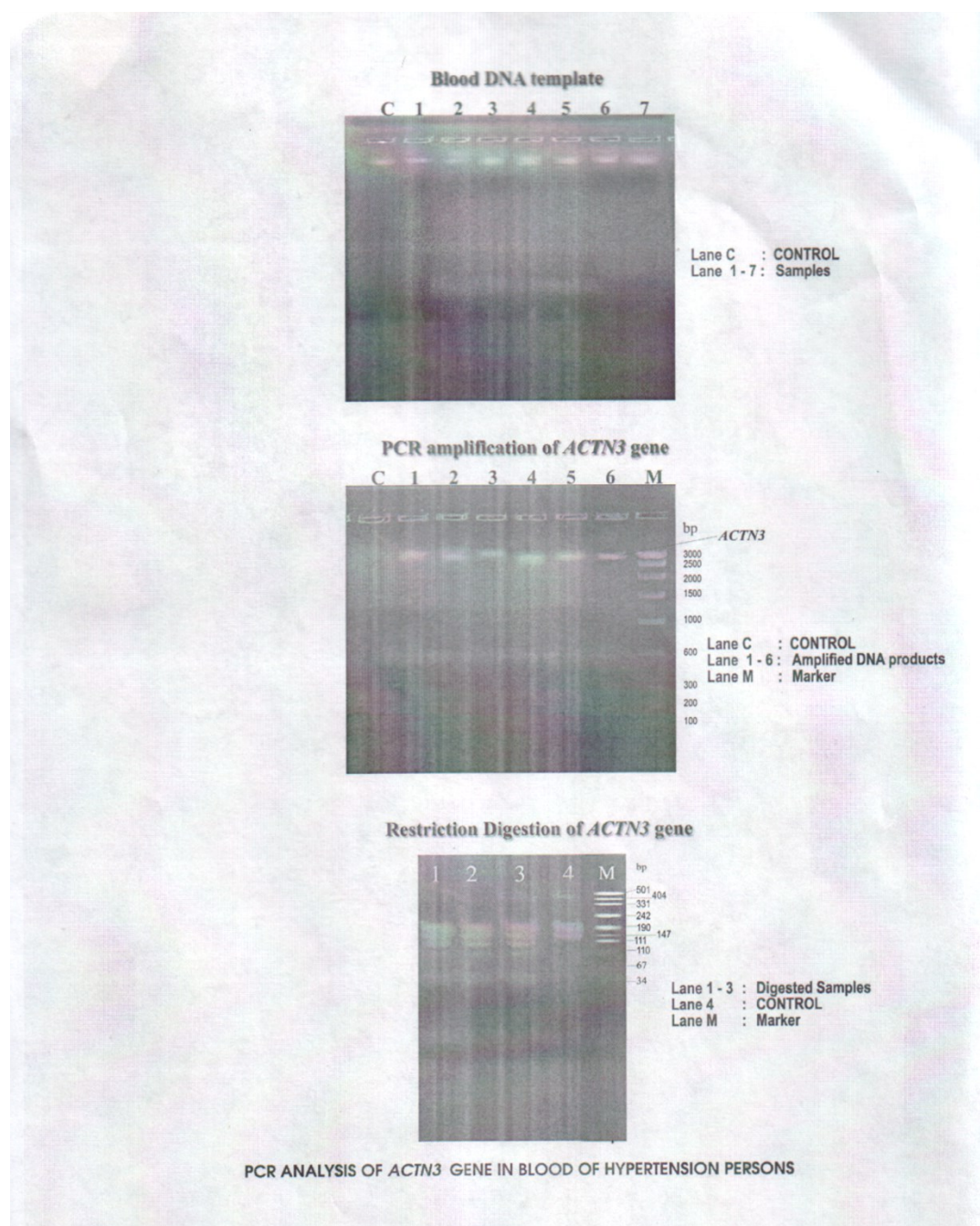
In this study, we have evaluated ACTN3 gene for the presence of hypertension in patients having different age group between 30 – 52. ACTN3 gene is one of the very common genes present in hypertension patients. This is used as the marker gene in several studies; hence we used this ACTN3 gene in human population studies. In our study, sixty different blood samples were taken from hypertension patients. Out of 60 patients 29 patients were ACTN3 gene. From 29 patients 16 male patients and 30 female patients several marker genes available for hypertension detection like LPL gene, LDL gene and ACTN3 gene. This supported by results in Eschenbacher and Mannia. 1990, and Kawabe *et al.*, (2007) reported that the influence of age and sex on the prevalence of masked hypertension determined by home blood pressure monitoring was examined in 630 Japanese people not taking antihypertensive drugs. Older (≥ 40 years, $n=231$) and male ($n=413$) subjects showed a significantly higher prevalence of masked hypertension than younger (<40 years, $n=399$) and female ($n=217$) subjects. Therefore, the prevalence of masked hypertension evaluated by home BP was influenced by age and sex.

In the gene analysis for single nucleotide polymorphism (SNP) using ACTN3 gene

primer showed varied results. After PCR amplification, the agarose gel electrophoresis studies showed that no band amplification in control samples, where as amplification could be seen in SNP carrying genes were this specific primer amplified the gene. This could be due to mutation in the particular gene, Liu *et al.*, 1997 also gave similar for other hypertension studies using specific primers, and Chikh Bengra *et al.*, (2002) reported human hypertension is a complex, multifactorial disease with a heritability of more than 30-50%. A genetic screening test based on analysis of multiple single nucleotide polymorphisms (SNPs) to assess the likelihood of developing hypertension would be helpful for disease management. Tailed allele-specific primers were designed to amplify by PCR six biallelic SNP loci. We developed six homogeneous PCR assays that were set up, performed and fluorometric analysis in 96 well microplates.

ACTN 3 gene has specific sites for different restriction enzymes, but in the present study, we have tested ACTN3 gene for carrying any site for Dde I, the DNA extraction in the present study digested with Dde I which shows four bands, this could be due to the presence of ACTN3 gene in the blood sample. Dominiczak *et al.*, 2000 also observed same type results.

In our findings, we failed to demonstrate the presence of ACTN3 gene in all patients, even though they were hypertension patients based on their clinical studies. So, we conclude that the amplification PCR SNP analysis is a convenient. Cost effective and useful method to significantly improve the reliable SNP detection in specimens containing very low concentrations. This approach is an accurate sensitive high through out genotyping of SNPs associated with hypertension patients.



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