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# PPARG POLYMORPHISMS AND THEIR ASSOCIATION WITH TYPE2 DIABETES IN SOUTH INDIAN POPULATION

**A.Krishna sailaja1*, P.Bhatnagar**

1 Research scholar, Pharmacy Department, Faculty of technology,Osmania University, Hyderabad

2 Professor, Pharmacy Department, Birla Institute of technology, Mesra, Ranchi.

**Email:** *shailaja1234@rediffmail.com*

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**ABSTRACT**

Type2 Diabetes is highly prevalent in India. Several studies on Asian Indians have shown that they are characterized by higher insulin resistance, early onset type2 diabetes and hypertension. For a given BMI Indians have a higher percentage of body fat and more visceral fat than members of other population. The tendency of Indians to have higher percentage of body fat and central adiposity compared with other races may be programmed in utero. This thin fat phenotype is present at birth. Small size at birth coupled with subsequent obesity increases risk for insulin resistance syndrome in later life.Dietary modifications play an important role in initiation of insulin resistance syndrome. This may also lead to Type2 diabetes in Indian population.The oil preferred for cooking India is considerably changes the ratio of W6/W3 fatty acids. A number of free fatty acids are PPARg activators. When individual with thrifty genotype are supplied energy rich food with reduction in physical activity there will be a greater prevalence of obesity, impaired glucose tolerance and type2 diabetes. PPARg activation enhances adipocyte differentiation make the people more obese. PPARg coordinates the thrifty response and urges the need for studying PPARg in Indians. Because this could explain the heterogeneity of insulin resistance and type2 diabetes in Indians. Aim of this study is to find out PPARg polymorphisms and their association with type2 diabetes in south Indian population.

With the help of PCR and Sequencing the exonic regions were screened for PPARg polymorphisms. Statistically their association with type2 diabetes was studied.

**KEYWORDS:** PPARg, BMI, RXR(retinoid X receptor), PPRES.

**INTRODUCTION**

Peroxisome proliferator activated receptors constitute a distinct sub family of the nuclear receptors that are activated by naturally occurring fatty acids[1]. Three subtypes of PPARS have been identified. PPARα., PPARβ, PPARγ. PPAR subfamily is involved in many cellular processes including lipid and glucose homeostasis, cellular proliferation, differentiation and control of inflammation. PPARg is located on chromosome 3.PPARg has two isoforms. They are a result of alternative mRNA splicing. PPARg1is expressed in most tissues. PPARg 2 is specific for adipose tissue[1,2,3]. When PPARg binds to a natural ligand or synthetic molecule such as thiazolidinedione, it becomes activated and complexes with another transcription factor known as retinoid X receptor (RXR).PPAR-RXR heterodimers bind to DNA motifs termed as Peroxisome proliferative response elements(PPRES) in the promoters of target genes. The whole PPRE consensus sequence exhibits a pattern specific for PPAR-RXR heterodimer and is indistinguishable from the responsive elements of other nuclear hormone receptors belonging to oestrogen,vitaminD of thyroid hormone. The functional PPRES have been identified in several adipocyte specific genes such as phosphoenol pyruvate carboxykinase, fatty acyl synthase,fatty acid transport protein, lipoprotein lipase,glucokinase, GLUT4 glucose transporter. Insulin resistance likely to occur when excess fat is deposited within the abdominal cavity [4,5]. This reduces the insulin sensitivity of fat cell also of other tissues including skeletal muscle and liver [6,7]. Expanding adipose stores alter the availability of free fatty acids and modify the PPAR ligand binding interaction. PPARg is considered to be a candidate gene for type2 diabetes. Because it is involved in adipocyte differentiation and lipid storage, it makes the person obese[8,9]. Obesity is

the major risk factor for type2 diabetes. A study was performed on type2 diabetic patients to find out the role of PPARg in the pathogenesis of type2 diabetes.

**MATERIALS AND METHODS**

Type2 diabetic samples were collected from sahaya diabetic center hyderabad. Total 103samples were used for this study, which represents the south indian population. The study was performed under informed consent from all subjects and it was approved by Ethics committee.

**GENETIC ANALYSIS**

Genomic DNA was extracted from all 103 type2 diabetic blood samples by salting out method and was quantified by agarose gel electrophoresis. Primers were designed to amplify 1st exonic regions of PPARg."Genetool Bar" is the software used for primer design. The designed primers were checked for their stability and band intensity by running electronic PCR.Following primer sequences were used to amplify 1st exonic regions of PPARg

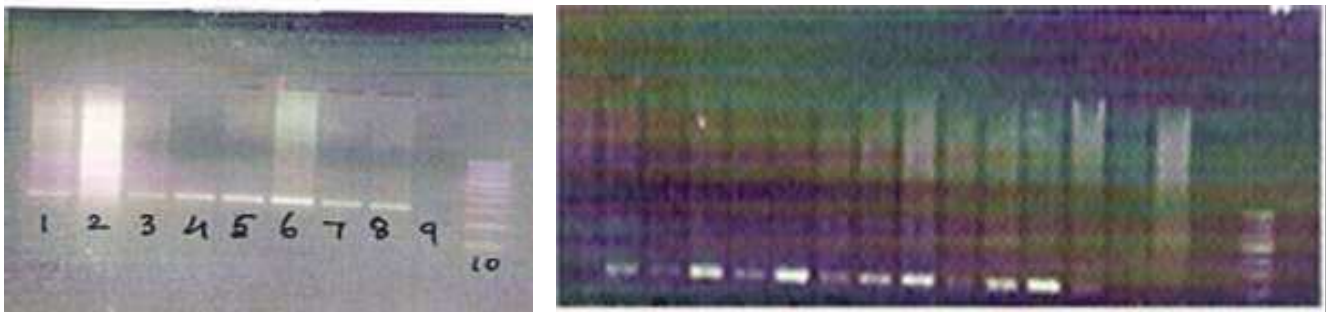
Forward primer: CTGATGTCTTGACTCATGGG

Reverse primer: GCTCTTGTAGTTTGTCTTCC

**THE CONDITIONS FOR PCR WERE**

PCR in a 25 ul reaction mixture contains 100 ng/ul Genomic DNA, 1.25 picomole forward primer, 1.25 picomole reverse primer, 2.5µl 10X buffer, 1µl TaQ polymerase, 250µM dNTP.The reaction mixtures were incubated at 94’ for 3 min followed by 35 cycles of denaturation at 94’ for 1 min,annealing at 55’ for 1 min and extention at 72’ for 90 seconds. The 295 base pair PCR product was checked for the amplification on 2%gel by using 6X xylene cyanol dye.100 base pair ladder was used as a marker to conform the PCR product.

**Figure1: All lanes shows the amplification of 1st exon of PPARg in patient samples**




The amplified samples were sequenced. Sequencing PCR was done in Gene AMP thermacycler. The reaction was performed in a 96 well sequencing plate. In 96 well plates each well contains 3µl big dye, 1µl forward primer and 1µl PCR product. The sequencing PCR was set at 96' for 10' min, 50' for 5 seconds, 60' for 4 min. Sequencing analysis was done in MAC Intosh system with the help of two software's "Auto assembler" and "Assembly set up".

Primers were designed to amplify 7th exonic region of PPARg

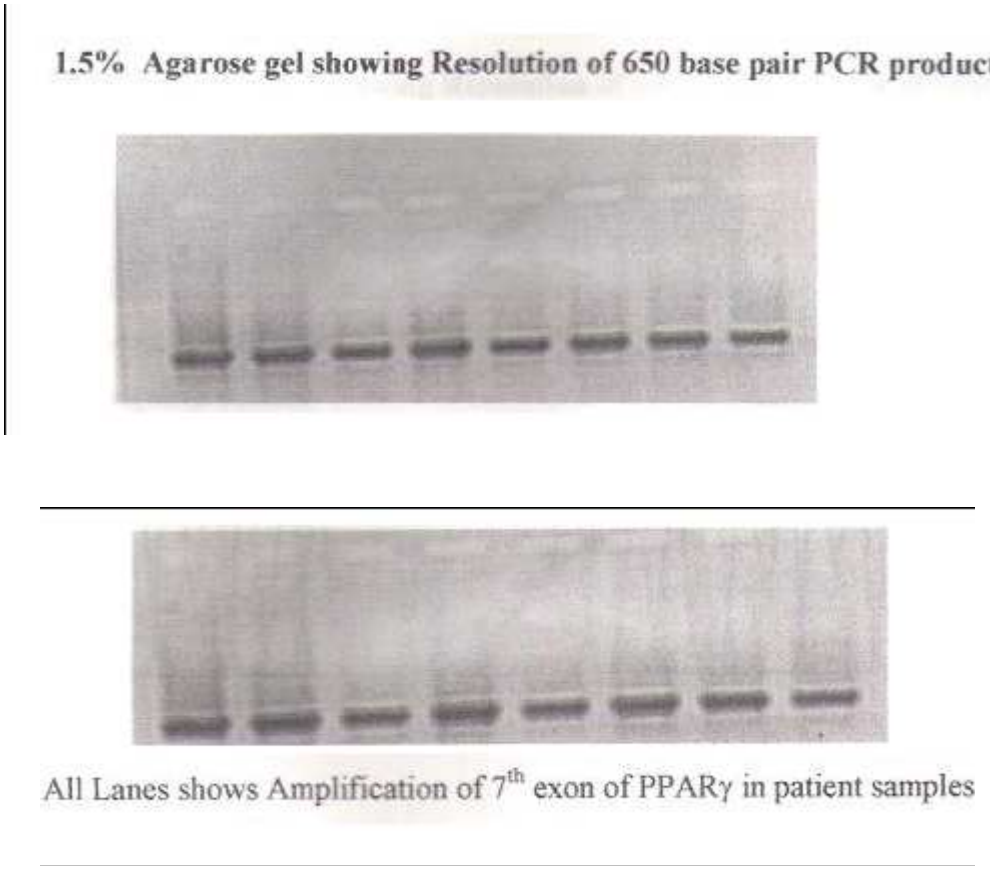
Forward primer: 5 CCATTATCTGCTTACCCTTC 3

Reverse primer: 5 GGAAACACACAAGACTCAGT 3

**The conditions for PCR:**

PCR in 25 µl reaction mixture contains 100ng/µl DNA, 100µM dNTPs, 1.5 Picomole of Forward primer and reverse primer, 1µl TaQ, 2.5µl 10X buffer. The reaction mixtures were incubated at 94' for 3 min, followed by 35 cycles of denaturation at 94' for 1 min, annealing at 54.5' for 1 min and extension at 72' for 90 seconds. Sequencing of the $7^{th}$ exon of PPARg was done in gene AMP 9600 thermacycler. Genotyping was done in MAC Intosh system.

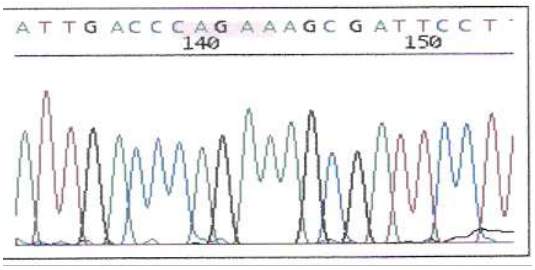
**Figure-2:**

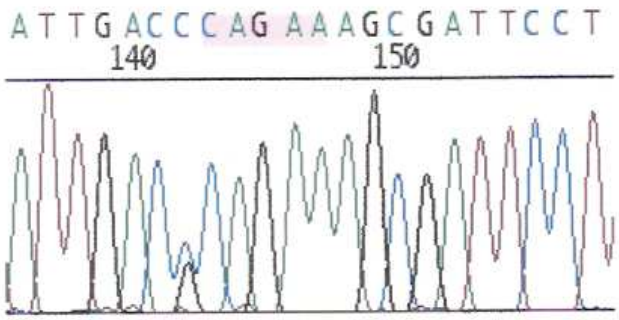


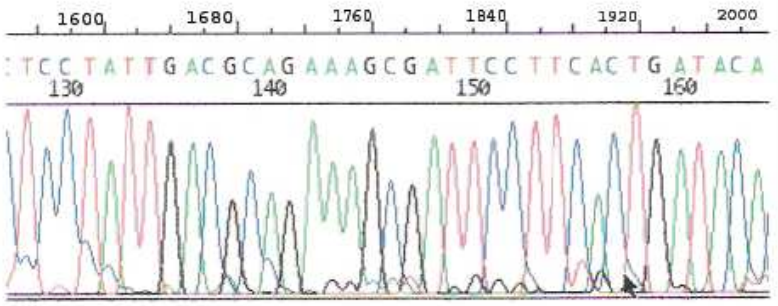

**Results**

The aim of this study was to investigate PPARg polymorphisms and their association with type2 diabetes in south Indian population. All 103 type2 diabetic samples were screened for PPARg polymorphisms. Two functional mutations were reported in 1st and 7th exons of PPARg[10].There was a proline to alanine substitution in codon 12 in PPARg2 which was reported to be associated with decreased risk of type2 diabetes[11,12]. In a study on a healthy Indian population the prevalence of pro12Ala polymorphisms was found to be 6%. Out of 103 diabetic patients 3 were homozygous, 18 were heterozygous and 82 were wild for pro12 Ala polymorphisms. The allelic frequency of G&C allele was found to be 0.116 and 0.883 respectively. The prevalence of Ala 12 polymorphism in diabetic patients was 11.6%. The genotype frequencies for each group were within Hardy- Weinberg expectations. With the help of Graff-pad statistical package by using chisquare test and Fisher test the data was analyzed.

**Figure 3:** Chromatogram showing wild condition Heterozygocity and homozygocity for Pro12Ala polymorphism in exon 1 of PPARg

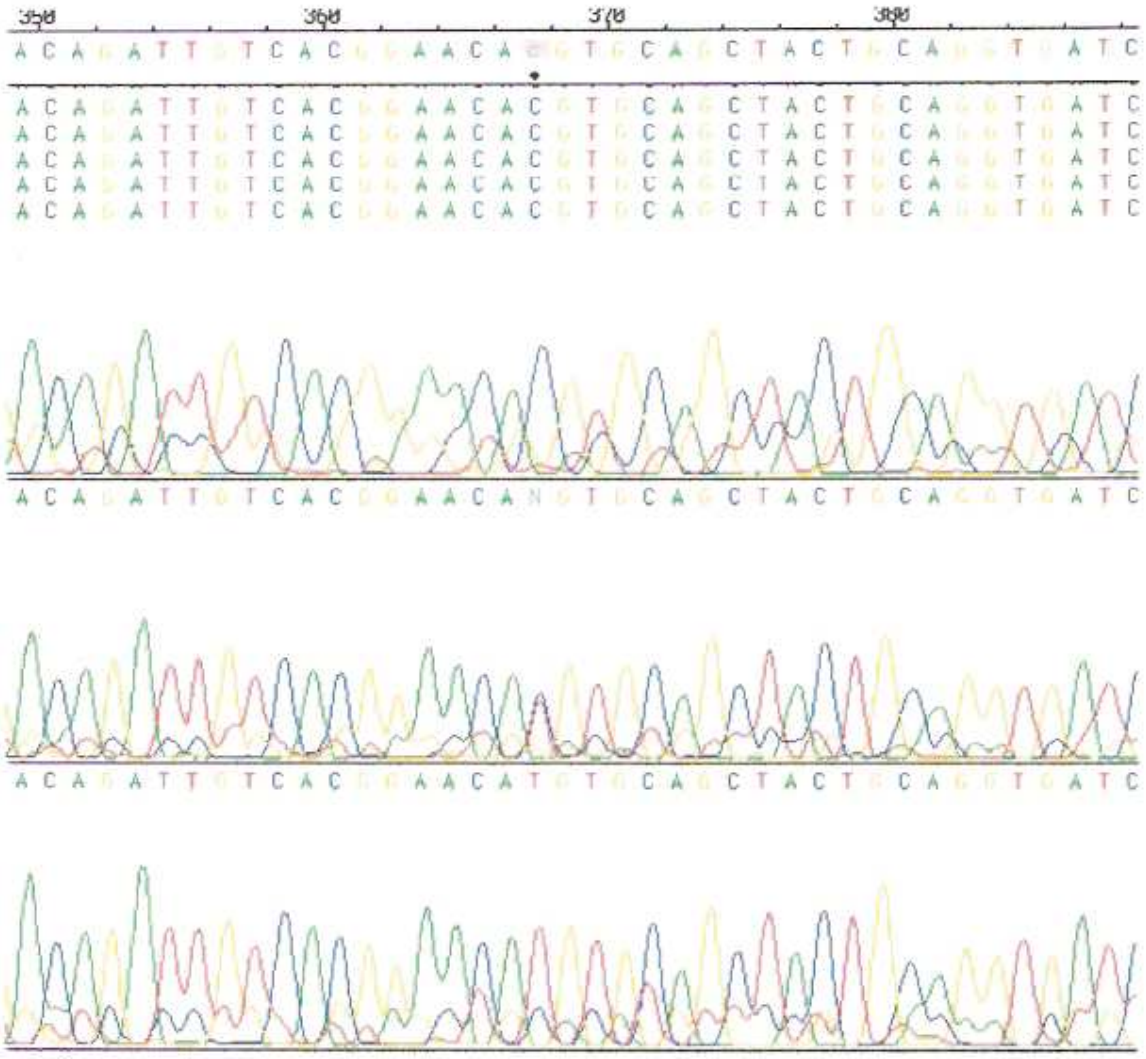



A second polymorphism was detected in exon7 at nucleotide 1431of PPARg2 resulting in silent substitution from C toT[13]. In our study out of 103 patients 3 were homozygous, 24 heterozygous and 76 were found to be wild for C161T polymorphism. The allelic frequency of T allele was found to be 0.145 and C allele was 0.854.When genotypic frequencies of both Proo12 Ala and C161T were analyzed it was found that the patients who were homozygous mutant for pro12Ala were also showing homozygosity for C161T polymorphism. So out of 18 heterozygotes of Pro12 Ala, 60% of them were carriers for the second mutations

**Figure 4:**




**Discussions**

In this study two functional mutations were reported in the coding region of PPARg2 gene, which were already reported in several populations. The Pro12Ala mutation in PPARg2 was identified in 1997[13,14,15].The rare allelic frequencies are 12% in Caucasians, 10% in Native Americans, 8% in Samoans, 4% in Japanese, 3% in African-Americans, 2% Nauruans, 1% in Chinese[16,17]. This polymorphism was extensively studied in different populations to find its association with type2 diabetes, effect on obesity and

insulin sensitivity. First evidence for an association between Pro12Ala polymorphism and type2 diabetes came from Japanese-Americans. The frequency of Ala allele in controls and type2 diabetic patients were found to be 9.3 and 2.2% respectively in their population[18]. A case-control study of Pro12Ala PPARg2 polymorphism in Japanese diabetic and non-diabetic subjects reported that the frequency of Ala allele was significantly lower in diabetic group. In their study genotypic distribution of Pro12Ala polymorphism was in Hardy-Weinberg equilibrium. A second polymorphism has been reported in exon7 at nucleotide 1431 of PPARg2 resulting in a silent substitution from C to T.This polymorphism was associated with obesity. As obesity is the major risk factor for type2 diabetes this polymorphism was studied for its association with type2 diabetes. Studies on this polymorphism concluded that obese subjects bearing at least one T allele had higher plasma leptin levels than subjects who did not[19]. Since C/T exon7 polymorphism is silent, this suggests that this polymorphism is in linkage disequilibrium with a functional mutation in PPARg locus. The frequencies of C and T allele in French population were reported to be 0.860 and 0.140 respectively[20,21]. In our study out of 103 patients 3 were homozygous, 18 were heterozygous and 82 were wild for Pro12Ala polymorphism and out of 103, 3 were homozygous, 24 were heterozygous and 76 were found to be wild for C161T polymorphisms. The allelic frequencies of G&C C&T were 0.116,0.883,0.854 and 0.145 respectively. Our results were very similar to the results of French population. When genotypic frequencies of both Pro12Ala and C161T were analyzed it was found that the patients who were homozygous mutant for Pro12Ala they were also showing homozygosity for C&T.Among 18 heterozygotes of Pro12Ala, 11 patients were showing heterozygosity for C161T polymorphism. So out of 18 heterozygotes of Pro12Ala, 60% of them are carriers for second mutation. In our study we found Pro12Ala and C161T are associated with type2 diabetes. The prevalence of these polymorphisms in type2 diabetic patients were 11.6% and 14.5% respectively. But statistically these polymorphisms could not reach the significant level. In this we tried to

find out the contribution of PPARg in pathogenesis of type2 diabetes. We reported 11-14% contribution towards type2 diabetes.

Further research can be done to find out the effect of Pro12Ala and C161T polymorphisms on BMI, obesity, insulin sensitivity and plasma leptin levels. Knowledge of the genetics of type2 diabetes would increase our understanding of the complex gene–gene and gene-environment interplay causing the disease and will serve to facilitate early diagnosis, treatment and intervention.

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**Corresponding Author:**

**Krishna Sailaja*,**
Ousmania Unversity,
**Email:** *shailaja1234@rediffmail.com*