

# Correlation of SNP (Pro12Ala) in PPARG2 With Type 2 Diabetes in Maharashtrian Population

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**ABSTRACT:** Type 2 Diabetes mellitus (T2DM) is a complex disease with a public health importance. It is characterized by insulin resistance and reduced secretion of insulin. Both genetic and environmental factors are considered to play a significant role in the manifestation of this diseases. SNP is the most basic variation in the genome of individuals, it can help in identification of many diseases. Genome wide association studies (GWAS) have identified some correlation between specific SNPs in genes like PPARG, T7F7L2, CDKAL1, FTO etc and T2DM.

Peroxisome Proliferator Activated Receptors (PPARG) is a member of nuclear receptor expressed through-out the body and consists of 3 subtypes. PPAR $\gamma$ 2 subtype regulates the lipid metabolism and has a role in insulin sensitivity. A cytosine to guanine shift in the coding region at 34<sup>th</sup> position of PPAR $\gamma$ 2 gene results in amino acid change from Proline to Alanine (Pro12Ala). Some studies have shown that this change in amino acid leads to altered sensitivity to insulin and has correlation with T2DM. There are very limited reports of this correlation in Indian population and none in Maharashtrian population.

Current study was conducted with 10 healthy individuals (control) and 22 T2DM patients. PPAR $\gamma$ 2 gene was amplified by polymerase chain reaction. Pro12Ala variation was checked by RFLP technique. Out of studied T2DM population, 40.9% were heterozygote with Pro12Ala variation and 9.09% were homozygote with both alleles carrying Ala. Even in control group, we could find homozygotes with Ala where the individuals were not diabetic but had a very strong family history of the disease. Our study showed a significant correlation with this SNP in PPAR $\gamma$ 2 and T2DM.

**KEYWORDS:** T2DM, PPARG2, Pro12Ala, PCR, RFLP, SNP.

## I. INTRODUCTION

Diabetes mellitus is a metabolic disorder commonly found in older individuals. It is characterized by high glucose levels in the blood. Glucose fuels the cells in body but at the same time the level of glucose in the blood needs to be controlled which is the major role of insulin hormone. It is the predominant anabolic hormone and is synthesized by B cells of pancreas. In the diabetic patients, there is either defect in pancreas which leads to destruction of B-cell and hence enough insulin is not produced or the cells become resistant to insulin.

There are two types of diabetes mellitus, type 1: insulin dependent diabetes mellitus (IDDM), type 2: non-insulin dependent diabetes mellitus (NIDDM). Type 1 is the most prevalent among children and young adult. They suffer from lack of B-cell in the pancreas due to destruction by their own immune system. Whereas Type 2 has varying degree of insulin resistance and hypo-secretion of insulin which leads to increased glucose level in the blood of suffering individual. Other than this, there is also gestational diabetes which is restricted only during pregnancy (Alberti and Zimmet 1999). Type 2 diabetes mellitus (T2DM) is the most common form of diabetes. Millions of people in the world are suffering from this disease. Before development of T2DM, the pre diabetic condition occurs which can be controlled if detected earlier by change in the life style like diet, physical activity, maintaining weight etc. (DePaula et

# International Journal of Innovative Research in Science, Engineering and Technology

(A High Impact Factor, Monthly, Peer Reviewed Journal)

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Vol. 7, Issue 5, May 2018

al. 2008). There are many diseases related to T2DM such as cardiovascular diseases (CVD) leading to blockage of major artery followed by complications of heart. (Haus et al. 2010). Retinopathy, which causes damage to the retina of the eye leading to impairment of vision. Neuropathy is damage of the nerves, which are controlled by the central nerves system in the brain. It causes swelling, muscle weakens & tingling in different parts of the body leading to nerve damage eventually. Nephropathy, damage to the kidneys due to long standing diabetic condition leading to the damage of blood vessels inside the kidneys. (Trevisan 2006)

Both genetic and environmental factors are considered to play a significant role in the manifestation of T2DM. In past few years, genome wide association studies (GWAS) have identified some correlation between genetic variation and complex disease like T2DM. Many of these studies conducted with different ethnic groups have identified common SNPs in selected genes and correlation with T2DM. Single nucleotide polymorphism (SNP) is a predominant form of genetic variation and human genome contains about 10-30 million SNPs. SNP in coding region can induce amino acid change leading to altered protein functionality whereas SNP in promoter region can impact transcriptional regulation. SNP variants and susceptibility for T2DM have been shown in genes like PPARG, T7F7L2, CDKAL1, FTO, KCNJ11 etc. Although these studies have been scattered across the globe with different ethnic groups there are very limited studies carried out in India to identify correlation between SNPs in these genes and T2DM.

PPAR $\gamma$ 2/PPARG (Peroxisome proliferator-activated receptors) is a nuclear hormone receptor and a transcription factor which is related to the nuclear family receptor. It is located on chromosome 3p25 and extended over more than 100 kb of genomic DNA. It regulates lipid metabolism and has a role in insulin sensitivity. Many groups have shown the polymorphism in the coding region of PPAR $\gamma$ 2 gene, a cytosine to guanine shift at codon 12 of this gene results in amino acid change from Proline to Alanine (Pro12Ala) (Bell 2002). This change in amino acid leads to altered sensitivity to insulin and is been shown to have correlation with T2DM.

The focus of the current study is to evaluate the correlation between (Pro12Ala)SNP in PPAR $\gamma$ 2 gene and T2DM in Maharashtrian population. Different age groups and both genders were included in the study. We have chosen this gene because there are very limited studies conducted with this SNP in Indian population. Chandrasekaran et.al (2012) have shown association of this SNP with T2DM among south Indian population whereas Manisha et al. (2016) did not find linkage between this SNP and T2DM in South Indian population. Hence it was important to evaluate if, SNP Pro12Ala in PPAR $\gamma$ 2 has any correlation with T2DM in Maharashtrian population due to ethnicity variation as compared to South Indian population.

## II. MATERIALS AND METHODS

### Sample collection:

In this study 32 individuals from population of Maharashtra were selected randomly (15 woman, 7 male). 22 individuals with T2DM were considered as test samples and 10 healthy individual were considered as control group (5 subjects with no family history of T2DM and 5 with family history).

Buccal cells of all these individuals were collected by scrubbing the inner layer of cheek with sterile swab. The cells were suspended in sterile water in microcentrifuge tubes.

### Genomic DNA extraction and amplification of PPAR $\gamma$ 2 gene:

Genomic DNA was isolated from collected buccal cells of each individual using Quiagen genomic DNA kit following manufacturer's protocol.

The amplification of the gene PPAR $\gamma$ 2 was carried out using the primers (FP/RP) to generate the fragment of length 244 bp encompassing the Pro12Ala site FP 5'CCAATTCAAGCCCAGTCCTTTC-3' and RP 5'-CAGTGAAGGAATCGCTTTCCG-3'. The amplification was carried out using the program as 94°C for 7 min, followed by 35 cycles of 94°C for 30 sec, 30-sec annealing at 55°C, and a 30-sec elongation at 72°C and final extension for 7 min at 72°C. The amplicon was checked on 2% agarose gel.

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RFLP (restriction fragment length polymorphism):

The SNP C-G in PPAR $\gamma$ 2 gene was analyzed by digesting the amplicon with *Bst*UI enzyme. The reaction was incubated at 37°C for 2 hrs and the digested product was checked on 4% agarose gel. Digested amplicon would generate fragments of size 223bp and 21bp whereas undigested amplicon would have 244bp size.

### III. RESULTS

Demographic analysis of Samples:

Table 1 below describes the details of 22 T2DM patients and 10 healthy controls used in this study

Characteristics	Type 2 diabetes patients N=22	Healthy controls N=10
<b>Demographic</b>		
Male /female ratio	7/15	2/8
Age (years )	53.0(30.0 – 76.0)	25.5 (22.0-30.0)
Ethnicity	Hindu	Hindu
Family history	12/22	6/10
<b>Clinical</b>		
Blood sugar test (random)	(97-350) mg/dl	Not checked
(Fasting)	(109 – 200) mg/dl	
Geographic	<b>Pune, Aurangabad, Kolhapur, Mumbai</b>	<b>Pune, Mumbai,</b>

#### III.1: Genomic DNA isolation

Buccal cells collected from 22 test samples and 10 control samples were used for extraction of genomic DNA. As mentioned in figure 1. Genomic DNA quality was checked on 0.8% agarose gel and was found to be suitable for further use in the PCR.

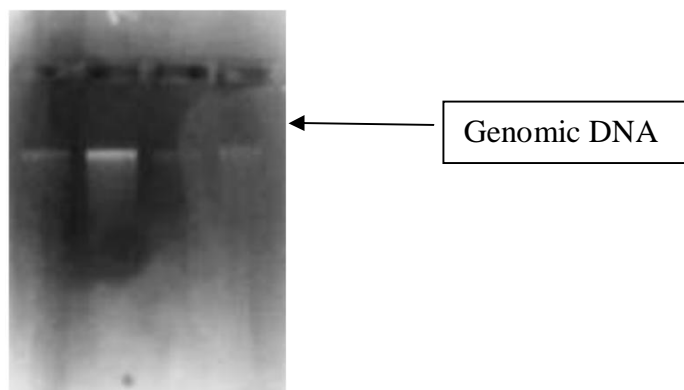


Fig.1. : Genomic DNA isolation from 4 patients of T2DM from buccal cells.

III.2 Amplification of PPAR $\gamma$ 2 gene The region surrounding the SNP site on the PPAR $\gamma$ 2 gene was amplified using specific primers and the amplicon was checked on 2% agarose gel. As shown in fig2 the amplicon size was 244bp.

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Vol. 7, Issue 5, May 2018

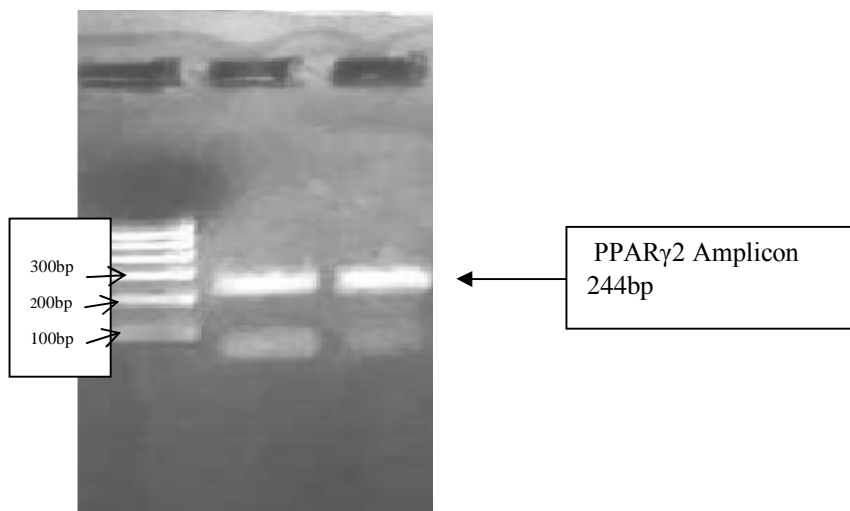


Fig.2. PCR amplification from genomic DNA for 4 different individuals, 1st Lane: M-marker 100bp, 2nd and 3rd Lane: PPAR $\gamma$ 2 amplicons from 2 controls

### III.3 RFLP;

244bp region amplified from PPAR $\gamma$ 2 gene was digested with *Bst*U1 enzyme to check the presence of C-G variation at 34 nucleotide position in PPAR $\gamma$ 2 gene. The digested product was loaded on 4% gel. As seen in figure 3a when the individuals do not have C-G variation, the amplicon is undigested and size remains as 244bp post digestion. If one allele has C-G variation and another is CC then 2 bands are visible 1 is 244bp (undigested) and another 223 bp (digested). This is referred as heterozygote for the SNP (Fig 3b). When the individual is homozygote for the SNP, amplicons of both the alleles get digested with enzyme leading to the shift in the size from 244bp to 223bp (Fig 3c).

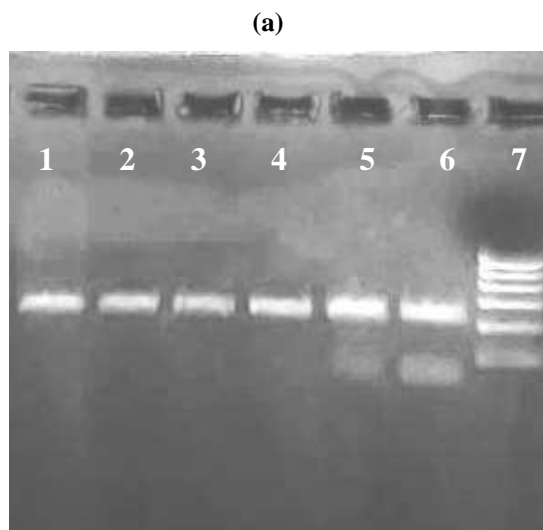


Figure 3a: RFLP: Restriction digestion of amplicon by *Bst*U1 enzyme to detect SNP for healthy individuals (1st lane uncut amplicon, 2nd - 6th lane digested amplicon for control, 7th lane: marker 100bp)

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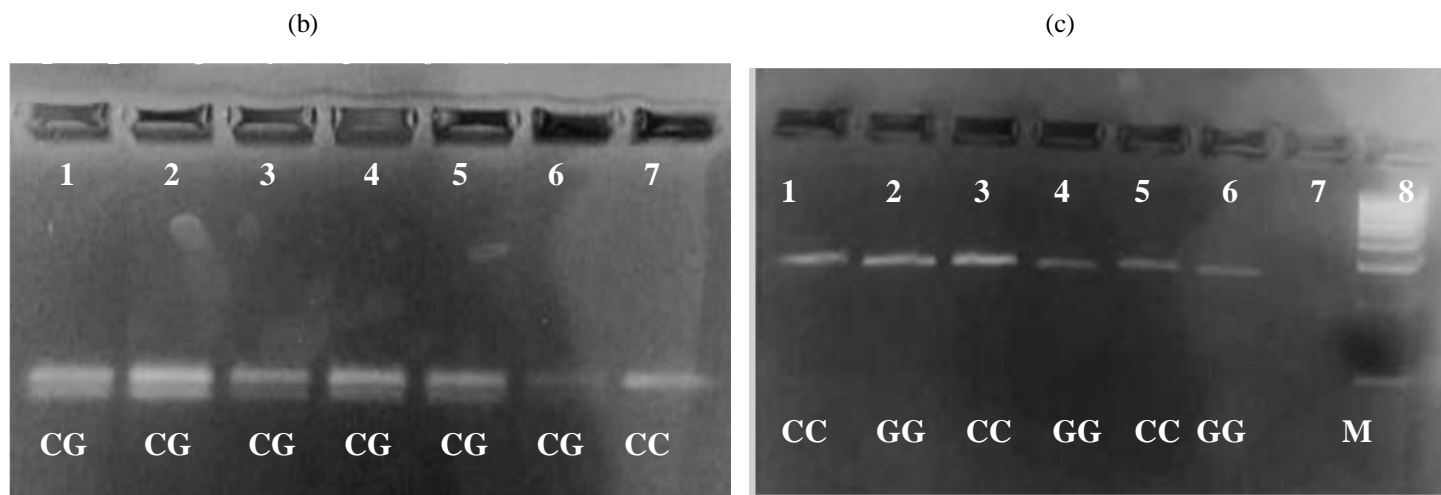


Fig.3b. Restriction digestion of amplicon by BstU I enzyme to detect SNP. 1<sup>st</sup> to 6<sup>th</sup> amplicon of patients digested: upper band is 244bp and lower one is 223bp all are heterozygotes(CG). 7<sup>th</sup> lane control digested 244bp(CC).  
Fig.3c: 1<sup>st</sup> to 5<sup>th</sup> lane 5 patients of T2DM 6<sup>th</sup> is control sample 2<sup>nd</sup>, 4<sup>th</sup> and 6<sup>th</sup> are homozygotes,(GG)223bp and 7<sup>th</sup> lane is blank, 8<sup>th</sup> 100bp marker.

Table 2. Summarizes the results for 22 T2DM patients and 10 healthy individuals based on RFLP analysis PPAR $\gamma$ 2 amplicon.

	Diabetic patient N=22		Control N=10	
	N	%	N	%
Genotype				
CC	11	50	7	70
CG	9	40.9	1	10
GG	2	9.09	2	20

### IV. CONCLUSION

The main aim of this study was to assess if the SNP Pro12Ala in PPAR $\gamma$ 2 gene has any correlation with T2DM. In this study, the Pro12Ala was evaluated for 32 individuals, 22 samples from the cases of T2DM and 10 control (five with diabetic family history). The PCR-RFLP was applied to identify the three different variations in PPAR $\gamma$ 2 gene. The Pro/Pro homozygotes (244bp), Ala/Ala homozygotes (233 fragment), Pro12Ala heterozygotes (two fragments first 244bp, second 233bp).

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It was seen that Pro/Pro homozygotes were (50% in cases) of T2DM and 70% in control. The number of Ala/Ala homozygotes were 2(9.09%) in T2DM cases, 2 (20%) in control, the numbers Pro12Ala were 9(40.9%) in T2DM cases, 1(10%) in control.

Various studies have shown Pro12Ala polymorphism in T2DM in different population (Ciccodicola et al. 2010). In India, Sanghera, D. K. *et al* (2006) found the association of this polymorphism with T2DM in north Indian population. Another study by S. Chandrasekaran *et al* (2012) showed significant association between the Pro12Ala polymorphism of the PPAR $\gamma$ 2 gene and T2DM among the south Indian population. Dharambir K *et al* revealed a significant association Pro12Ala with T2DM in Asian Sikh community from North India (Dharambir K et.al. 2008). On the other hand a study by Radha *et al* (2006) did not observe any reduction in concentration of insulin in Pro12Ala polymorphism carriers of T2DM in South Indian population in Chennai.

In the current study, we could identify statistically significant association of SNP Pro12Ala in PPAR $\gamma$ 2 gene with T2DM population in Maharashtra from different geography for both genders. Although, the population size used in the current study is small, it is worthwhile to explore the correlation of this SNP with T2DM in different ethnic and age groups in India. In future, large population studies can be conducted and this SNP and other SNPs can be developed as screening tools for identification of T2DM predisposition which can help in prevention of this disease through good diet, better lifestyle and physical activities.

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