Metabolic syndrome and influence of pro12ala polymorphism

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Metabolic syndrome and influence of pro12ala polymorphism
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Abstract

The Pro12Ala polymorphism of the Peroxisome Proliferator-Activated Receptor-Gamma 2 (PPAR gene) is known to be associated with metabolic syndrome. However, studies reported in south Asian population are rare. Hence, it was thought of interest to carry out this research. The study was registered in Clinical Trial Registry of India (CTRI) and Ethical Approval was obtained from Institutional Human Ethical Committee. The target populations of the study were young adults (65 male subjects) in the age group of 18 – 24 years. The aim of the study was to identify the frequency of pro12ala polymorphism and investigate possible association with the presence of various parameters of metabolic syndrome. The Pro12Ala genotypes were determined by PCR-restriction fragment length polymorphism (RFLP). The parameters for metabolic syndrome were assessed based on ATP III criteria. The anthropometric measurements such as height, weight, BMI, waist hip ratio were measured. Total cholesterol, HDL cholesterol and triglycerides were determined by spectrophotometry method. LDL cholesterol was calculated by the Friedewald equation. The fasting blood glucose and blood pressure were also measured. The subjects were grouped based on BMI latest Asian cutoff. The frequencies of PPAR gamma 2 genotypes were found to be PP type, 83 Per cent; PA type, 17 Per cent; and AA type, 0 Per cent. Genotype frequencies were found to obey Hardy-Weinberg equilibrium. The various parameters of metabolic syndrome such as fasting blood glucose, waist circumference, triglycerides, HDL cholesterol and blood pressure were correlated statistically with PPAR Gamma genotype.

Keywords: Metabolic syndrome, PPAR gamma, Pro12Ala polymorphism

Introduction

Metabolic syndrome is a major public health problem of the world including India. It is a complex disorder characterized by central obesity, dyslipidaemia, abnormal glucose tolerance and hypertension. Metabolic syndrome increases the risk of developing diabetes mellitus by 5 fold and CVD by 2 fold. Globally, prevalence of metabolic syndrome ranges from 20-25 % of the world’s adult population. People with metabolic syndrome are 2 times more likely to suffer from heart attack.

Prevalence of the metabolic syndrome as defined by National Cholesterol Education Program, Adult Treatment Panel III (NCEP, ATP III) and other criteria like IDF, WHO ranges from about 11 to 41 per cent in different regions of India. The prevalence of metabolic syndrome in urban Indian population has been reported as 22.9% in men and 39.9% in women and the age-adjusted prevalence was 24.9%, 18.4% in men and 30.9% in women.
The prevalence of metabolic syndrome was found to be 6.7% among subjects in the age group of 20-29 years, while it was 43.5% among subjects aged 60-69 years and 42% among subjects aged 70 years and above (Ford et al). The underlying cause of the metabolic syndrome continues to challenge the experts but both insulin resistance and central obesity are considered significant factors. Central obesity and insulin resistance are considered as the main underlying significant factors behind metabolic syndrome. In India, obesity has grown into a global epidemic. Prevalence ranges from 30-65% among urban adults. Genetic strategy is considered as an important clue for envisaging the pathological mechanism behind the development and progression of metabolic syndrome.

Peroxisome Proliferator Activated Receptors (PPAR) are ligand activated transcription factor that belong to the nuclear receptor protein family that is known to regulate gene expression by influencing PPAR responsive elements. The PPAR family is known to consists of three subtypes: PPAR α, PPAR β and PPAR gamma. Of the three subtypes, rather than PPAR α and PPAR β, PPAR gamma is extensively researched as it possess highest adipogenic activity than other subtypes. Moreover, it is expressed at a very early stage of adipocyte differentiation. It plays a vital role in control of various activities involved in fat. It is also a well known key transcriptional factor as a fat specific direct regulator and therefore known as the master gene that trigger the program of adipogenesis. Two isomers of PPAR gamma are PPAR Gamma1, and PPAR gamma2. Various polymorphism has been reported to be exhibited by PPAR gamma2. Among them, pro 12 ala polymorphism has found to be associated with variations of BMI and insulin sensitivity depending upon difference in ethnicity. PPAR gamma 2 is known to possess affinity towards specific proliferators and expressed in adipose tissue of human. It also modulates expression of gene in adipocytes, insulin sensitivity, angiogenesis and inflammatory process. Central obesity is largely associated with Metabolic syndrome. Beamer reported that Pro 12 Ala polymorphism was associated with increased BMI and the possible role of ala 12 isoform in contributing to the susceptability for metabolic disorders in obesity. However the genetic influence with regard to specific polymorphism in south asian population is not yet clearly exhibited. Further, Metabolic syndrome in young population is gaining attention since early identification will foster better health and preventive measures. So far, data regarding metabolic syndrome among the young Asian adult are scarce. This kind of study can lead to personalised nutrition based on SNP. Hence, this study was aimed to determine the prevalence of metabolic syndrome among young adult of age group 19-24 years and to find out the influence of pro12ala of PPAR Gamma 2 on the same.

Methods

Subjects

A total of 65 male subjects in the age group of 19-24 years were included for the study based on random purposive sampling. The nature of study were explained to all the participants. Patient information sheet explaining the study protocol were prepared in English as well as Tamil language and distributed to the participants. Oral as well as written consent was obtained from all the participants. The study were presented before the Human Ethics Committee of the University and obtained ethical approval. The Study was also registerd in Clinical Trial Registry of India (CTRI) and trial registration number was obtained. The ethical committee approval number
The CTRI registration number is CTRI/2014/11/005222. The inclusion and exclusion criteria were strictly followed for the recruitment of subjects.

**The inclusion criteria were**

- Subjects willing to participate and sign consent.
- Subjects between 18-24 yrs of age
- Subjects with BMI above 23
- Subjects with sound mental health

**The exclusion criteria were,**

- Subjects not willing to sign consent.
- Subjects below 18 and above 23 yrs of age.
- Subjects enrolled in weight loss programs.
- Subjects enrolled in other clinical trials
- Subjects with complication of DM or CVD

The subjects in each category were carefully scrutinized for participation with the above inclusion and exclusion criteria

**Anthropometric measurement, biophysical and biochemical parameters**

Anthropometric measurements such as height and weight were measured and Body Mass Index (BMI) was calculated based on WHO latest BMI cut off points for Asian population. Waist circumference was measured according to the WHO guidelines in midway between the iliac crest and the lower most margins of the ribs and at the end of normal expiration. Blood pressure was measured with the help of digital blood pressure monitor in the right arm with the subject comfortably seated after a rest of 5 minutes. At least two readings at 5 min intervals were taken and if any deviation was obtained, another reading was taken after rest of 30 min. Blood sample was collected after 12 hours of fasting and blood glucose levels were estimated GOD-POD method and lipid profile namely Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) were measured using spectrophotometry technique. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald's formula. 

\[
LDL = \text{Total cholesterol}(mg/L) - \text{HDL Cholesterol}(mg/L) - \text{Triglyceride}(mg/L/5)
\]

Friedewald equation used for calculation of LDL cholesterol is most accurate when triglyceride level is less than 400 mg/dL. Therefore LDL cholesterol was directly measured if triglyceride level were greater than 400 mg/dL.

**Diagnosis of Metabolic syndrome**

Metabolic Syndrome was diagnosed using the criteria proposed by National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III). The revised NCEP criteria require at least three of the following components: Abdominal obesity (waist circumference
≥90 cm for Asian men, triglycerides ≥150 mg/dL, HDL cholesterol ≤40 mg/dL for men, systolic/diastolic blood pressure ≥130/85 mmHg or receiving drug treatment, and fasting plasma glucose ≥100 mg/dL.

**Genotyping**

Peripheral blood samples were collected in EDTA tubes and genomic DNA was extracted from peripheral blood samples. The DNA extracted were checked nanocount for its purity and stored at -20°C till analysis. The Pro12ala PPAR gamma 2 sequence variant rs 1801282 was carried out by Polymerised Chain Reaction (PCR) followed by Restriction Length Polymorphism(RFLP).

**DNA PURITY CHECK IN NANO COUNT**

**Statistical analysis**

Pro 12 Ala genotype distribution were tested for deviation form Hardy-Weinberg equilibrium. Kolmogorov-Smirnov test, Mann whiney U test, Chisquare test were used for statistical analysis. Continuous data are expressed as Mean and Standard deviation. P value corresponding to P<0.05 was considered as statistically significant. Prism 6.0 software was used for statistical analysis.
Results and Discussion

Distribution of genotype

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N(%)</td>
<td>%</td>
<td>N(%)</td>
<td>N(%)</td>
<td>N(%)</td>
</tr>
<tr>
<td>Pro 12 Pro</td>
<td>54(83)</td>
<td>F(Ala) 91.5</td>
<td>18 (78.3)</td>
<td>17 (77.27)</td>
<td>19 (95)</td>
</tr>
<tr>
<td>Pro 12 Ala</td>
<td>11(17)</td>
<td>F(Pro) 8.5</td>
<td>5 (21.7)</td>
<td>5 (22.72)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>100</td>
<td>23</td>
<td>22</td>
<td>20</td>
</tr>
</tbody>
</table>

\( p=0.2312, (<0.05) \)

The distribution of genotype revealed that 83% of the subjects carried pro 12 pro genotype while 17% of the subjects carried pro 12 ala genotype. Ala 12 ala, a rare genotype were absent among the selected individuals. The frequency of ala allele was found to be 91.5% while pro allele was 8.5%. Based on BMI, almost 78.3%, 77.27 % and 95 % of obese, overweight and normal subjects were carriers of pro12 pro genotype while 21.7%, 22.72% and 5% of obese, overweight and normal subjects were carriers of pro 12 ala polymorphism. Thus, it is evident that pro12ala polymorphism were common among obese and overweight than normal individuals. It may be attributed to the increased BMI of the subjects. Pereira et al\(^ {12} \), 2013 opines that obese individuals possess a 1.196 more chance of carrying the polymorphism than non-obese

Prevalence of Metabolic Syndrome based on phenotype

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolic syndrome</td>
<td>15(23)</td>
<td>8(35)</td>
<td>6(28)</td>
<td>1(5)</td>
</tr>
<tr>
<td>Non - Metabolic syndrome</td>
<td>50(77)</td>
<td>15(65)</td>
<td>16(72)</td>
<td>19(95)</td>
</tr>
<tr>
<td>Total</td>
<td>65</td>
<td>23</td>
<td>22</td>
<td>20</td>
</tr>
</tbody>
</table>

\( P = 0.0586 \)
The prevalence of metabolic syndrome was found to 23% among selected subjects. Prevalence of metabolic syndrome reported by Apurva S\textsuperscript{13} et al, 2011 among 20 – 40 years male were found to be 20.16% and Chow\textsuperscript{14} et al reported the prevalence of metabolic syndrome as 26.9% among male south Indian population. According to BMI category, metabolic syndrome prevalence was 35%, 28% and 5% among obese, overweight and normal individuals respectively. It is observed that obese and overweight subjects had greater possibility to develop metabolic syndrome than individuals with normal BMI. Apurva S\textsuperscript{13} et al., 2011 elucidated that BMI is the key factor for progression of metabolic syndrome and found that about three fourth of the subjects who had metabolic syndrome were overweight/obese.

### Prevalence of Metabolic syndrome based on Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Metabolic Syndrome</th>
<th>Non – Metabolic syndrome</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro 12 Pro</td>
<td>8(15)</td>
<td>46(85)</td>
<td>54(100)</td>
</tr>
<tr>
<td>Pro 12 Ala</td>
<td>7(64)</td>
<td>4(36)</td>
<td>11(100)</td>
</tr>
<tr>
<td>Total</td>
<td>15(23)</td>
<td>50(77)</td>
<td>65(100)</td>
</tr>
</tbody>
</table>

$p=0.0018^*$ ($<0.005$)

Prevalence of metabolic based on genotype revealed that pro 12 ala carriers are prone to develop metabolic syndrome while carriers of pro 12 pro had less chance of developing metabolic syndrome among selected subjects. 63% of pro 12 al carriers had metabolic syndrome while only 14% of pro 12 pro carriers had metabolic syndrome among selected individuals. The increased prevalence of metabolic syndrome among pro 12 ala carriers is probably due to increased waist circumference (central obesity) among selected subjects.

### Comparison of Various components of Metabolic Syndrome between groups of Genotype

<table>
<thead>
<tr>
<th>Criteria</th>
<th>ATP III VALUES</th>
<th>Pro 12 Pro N=54</th>
<th>Pro12ala N=11</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Waist Circumference(Cm)</td>
<td>102</td>
<td>99.83±8.51</td>
<td>101.64±9.70</td>
<td>0.6303</td>
</tr>
<tr>
<td>Blood glucose(mg/dl)</td>
<td>&gt;110</td>
<td>90.00±8.33</td>
<td>90.64±9.91</td>
<td>0.9807</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>&gt;150</td>
<td>99.72±38.09</td>
<td>166.18±60.22</td>
<td>0.0093*</td>
</tr>
<tr>
<td>HDL Cholesterol(mg/dl)</td>
<td>&lt;40</td>
<td>44.74±7.43</td>
<td>34.36±4.80</td>
<td>0.0006*</td>
</tr>
</tbody>
</table>

\* Indicates statistical significance.
The various metabolic components between groups of genotype were compared. Mean waist circumference were found to higher among subjects with pro 12 ala carriers than pro 12 pro carriers. However, it was found to be less than ATP criteria. The difference in fasting blood glucose level between groups of genotype was negligible and it was also below ATP III criteria in both the groups. Carriers of pro 12 ala group had higher triglycerides to the tune of 166mg/dl while it was only 99mg/dl among pro 12 pro group. The difference was found to be statistically significant. Comparison of HDL cholesterol level between groups of genotype revealed a favourable trend towards carriers of pro 12 pro. The mean HDL cholesterol was less than ATP III criteria (40gm/dl) among pro 12 ala carriers. The difference was found to statistically significant. Blood pressure (systolic and diastolic) in both groups of genotype were found to be less than of ATP III criteria and the difference between groups were also found to be negligible and statistically insignificant.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Pro/Pro (N=54)</th>
<th>P</th>
<th>Pro/Ala (N =11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal</td>
<td></td>
<td></td>
<td>Abnormal</td>
<td></td>
</tr>
<tr>
<td>WC(Cm)</td>
<td>23(42.59%)</td>
<td>0.27</td>
<td>7(63.63%)</td>
<td>0.60</td>
</tr>
<tr>
<td>FBG(mg/dl)</td>
<td>4(7.40%)</td>
<td>0.09</td>
<td>1(9.09%)</td>
<td>0.20</td>
</tr>
<tr>
<td>Triglycerides(mg/dl)</td>
<td>7(12.96%)</td>
<td>0.72</td>
<td>7(63.63%)</td>
<td>1.58</td>
</tr>
<tr>
<td>HDL Cholesterol(mg/dl)</td>
<td>11(20.37)</td>
<td>0.68</td>
<td>10(90.9%)</td>
<td>1.50</td>
</tr>
<tr>
<td>Systolic BP(mmHg)</td>
<td>3(5.55%)</td>
<td>0.41</td>
<td>4(36.36%)</td>
<td>0.90</td>
</tr>
<tr>
<td>Diastolic BP(mmHg)</td>
<td>7(12.96%)</td>
<td>0.78</td>
<td>1(90.09%)</td>
<td>1.32</td>
</tr>
</tbody>
</table>

All the individual components of metabolic syndrome were taken into consideration with view of higher contributing factor. With regard to Pro 12 ala carriers, increased waist
circumference were the major contributing factor followed by elevated triglyceride level and diastolic pressure. The least contributing factors were systolic pressure. However, the pro 12 al carriers exhibited a different trend towards the same criteria. Lower HDL C was the major contributing factor, followed by increased waist circumference and elevated triglycerides. The least contributing factors were diastolic pressure and fasting blood glucose.

**Conclusion**

83% of the subjects carried pro 12 pro genotype while 17% of the subjects carried pro 12 ala genotype while ala 12 ala genotype were absent among selected subjects. Carriers of pro 12 ala had higher BMI, waist circumference when compared with pro 12 pro carriers. The overall prevalence of metabolic syndrome was found to be 23%. pro 12 ala carriers are prone to develop metabolic syndrome than pro 12 pro carriers among the selected subjects. The ala allele might be a contributing factor for development and progression of metabolic syndrome among selected subjects. However studies with large sample size are to be carried out to confirm the results.

**References**


