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Association of SNP rs7181866 in the nuclear respiratory factor-2 beta subunit encoding GABPB1 gene with obesity and type-2 diabetes mellitus in South Indian population

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ABSTRACT

GABPB1, known as nuclear respiratory factor 2 (Nrf2), activates mitochondrial genes that are responsible for oxidative phosphorylation. Earlier studies on GABPB1 reported that two single nucleotide polymorphisms (SNPs) such as rs7181866 and rs8031031, to be associated with increased endurance in athletes. In the present study, a cohort of 302 South Indians, including normoglycemic healthy controls, T2DM with and without obesity were genotyped for the two SNPs by PCR-RFLP method and correlated with serum adipokines. The 'G' allele of rs7181866 was found to be associated with obesity whereas rs8031031 didn't show any significant association with obese individuals. The increased levels of adipokines such as Leptin, IL-6 and TNF-α and decreased adiponectin were found among obese-T2DM, when compared to non-obese T2DM subjects. Further, Factor analysis on metabolic components revealed four factors which accounts for 71.5% for non-obese control and 88.3% for obese T2DM of variance. The bias-corrected and accelerated bootstrap analysis revealed GG genotype to have significant positive and negative correlation with both TNF-α and adiponectin. In conclusion, the G allele of (rs7181866 A/G) was found to be significantly associated with risk for obesity among T2DM subjects.

1. Introduction

Obesity is defined as an excess accumulation of adipose tissue that impairs both physical and psychosocial health and well-being [1]. According to the World Health Statistics Report by the year 2012 had reported that globally, 1 in 6 adults become obese and almost 2.8 million individuals die every year due to obesity or overweight. In addition, obesity is also found to be strongly associated with other metabolic disorders such as diabetes, dyslipidemia, cardiovascular disease and hypertension. Diabetes Mellitus is a chronic disorder that is characterized by impaired glucose regulation. It has been estimated by International Diabetes Federation (IDF) in the year 2015, that 415 million people have been diagnosed with diabetes globally and this figure is expected to rise to 642 million by 2040 [2]. A study conducted in Chennai city of Tamil Nadu reported that the prevalence of generalized obesity to be 45.9% [3] and the rise in obesity prevalence in India could be attributed to the increasing urbanization, increasing availability of processed foods, use of mechanized transport [4], adoption of less physically active lifestyles and consumption of more energy-dense and nutrient-poor diets [5]. The peptides or hormones released from the adipose tissue are called the adipokines or adipocytokines and these adipokines are known to play a crucial role in insulin resistance, type 2 diabetes mellitus (T2DM) and obesity induced inflammation [6]. Enlargement of adipose tissue seen in obesity causes dysregulation of adipokines released and considered as a crucial causative factor in obesity related T2DM [7]. Adipokines such as adiponectin, leptin, Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) are known to play a crucial role in manifestation of obesity and T2DM. TNF-α is a proinflammatory

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cytokine that plays a crucial role in inflammatory cell activation and recruitment [8]. IL-6 is a proinflammatory cytokine that induces insulin resistance and found to be increased in obesity and T2DM [8,9]. TNF-α and IL-6 are known to reduce adiponectin levels [8]. On the other hand, Leptin has an inhibitory effect on insulin secretion and exerts anti-diabetic effect; it stimulates lipolysis, reduces lipogenesis and is crucial for regulating body weight [8,10]. Adiponectin was found to enhance insulin sensitivity and has an anti-diabetic effect [11]. Further, adiponectin and TNF-α were reported to have an inhibitory effect against each other [8,10].

Several genome wide association studies have identified variants or Single Nucleotide Polymorphisms (SNPs) in genes such as FTO [12], MC4R [13], TCF7L2 [14], KCNK3 [15], RARB [16], ENPP1 [17], TNF-α [18] conferred significant risk to obesity and diabetes. On the other hand, Nuclear respiratory factor-2 (Nrf2), also known as the GA-binding protein (GABP) transcription factor is an obligate multimeric protein in Ets (E26 transformation-specific) family transcription factors, which plays crucial roles in progression of cell cycle and mitochondrial biogenesis [19,20]. The gene *GABPB1* encodes the β-subunit of Nrf2. It acts as a major transcriptional activator for several nuclear genes that encode mitochondrial enzymes, complex-IV subunits and assembly factors, and TOM complex receptors [21–23]. Mitochondrial dysfunction characterized by reduced mitochondrial mass and/or function is known to be associated with obesity, insulin resistance and T2DM pathophysiology, due to impaired fatty acid oxidation, adipokine secretion and glucose homeostasis [24–27].

In the present study, we explore the association of SNPs (rs7181866 and rs8031031) in *GABPB1* gene among T2DM subjects with obesity and its correlation with adipokines between the genotypes. Further, we employed bootstrapped statistics for estimating and validating the distributions.

2. Materials and methods

2.1. Study population

For the present study, a total of 302 unrelated subjects from South Indian origin (Dravidians) were recruited, which included non-obese normoglycemic healthy individuals as controls ($n = 116$; M = 65, F = 51), non-obese individuals with T2DM ($n = 93$; M = 53, F = 40) and obese individuals with T2DM (n = 93; M = 53, F = 40). The study subjects were selected from the outpatient department of M.V. Hospital for Diabetes, Royapuram, Chennai, India. The non-obese normoglycemic subjects were randomly selected healthy volunteers with no history of diabetes and renal or cardiovascular diseases (CVDs). The study protocol was approved by the institutional ethics committee of Prof. M. Viswanathan Diabetes Research Centre (Ref No-IHEC/N-007/09/2016) and the written informed consent was obtained from each subject in accordance with principles of the Declaration of Helsinki. All anthropometric and demographic details like age, weight, height, duration of diabetes were recorded. Fasting blood samples were drawn from all the subjects for the biochemical analysis and the genomic DNA was extracted.

2.2. Analysis of clinical parameters and serum biomarkers

Anthropometric measurements including Height, weight, and blood pressure were measured by use of standardized methods [28]. Height (m) and weight (kg) were measured in clinical centers by a research staff. Standing height was measured to the nearest 0.1 cm with the participant looking straight ahead in bare feet and with his/her back against a wall. Weight was measured to the nearest 0.1 kg in light clothing. Blood pressure was recorded in the sitting position in the right arm to the nearest 2 mmHg with a mercury sphygmomanometer (Diamond Deluxe BP apparatus, Pune, India). Two readings were taken 5 min apart and the mean of the two was taken as the blood pressure. The BMI was calculated as weight (kg) divided by the square of height (m). Biochemical analysis of all the serum biomarkers was carried out in Hitachi-912 Autoanalyzer (Hitachi, Ltd., Tokyo, Japan), using commercial kits supplied by Roche Diagnostics, Mannheim, Germany. Measurements were made as per the manufacturer's instructions. The various biochemical parameters were measured using standard protocols, as described earlier [29], Glucose oxidase-peroxidase method for fasting and postprandial glucose, glycerol phosphate oxidase-peroxidase-amidopyrine method for triglycerides, cholesterol oxidase-peroxidase-amidopyrine method for total serum cholesterol, HDL-cholesterol by direct method, using polyethylene glycol-pretreated enzymes, urea and Jaffe's method for creatinine. LDL-cholesterol was estimated using Friedewald formula [30]. Levels of glycated hemoglobin (HbA1c) was estimated by HPLC (Bio Rad, Hercules, CA), and the intra- and inter-assay coefficient of variation was <5%. HOMA-IR was calculated as described previously [31]. The clinical characteristics of control subjects and T2DM patients are summarized in Table 2.

Plasma levels of adipokines such as Leptin, Adiponectin, IL-6 and TNF-α were measured using quantitative sandwich enzyme-linked immunosorbent assay (ELISA), as per the manufacturer's instructions (eBioscience, CA, USA). The lower detection limits for IL-6 was 1.9 pg/ml, TNF-α was 1.5 pg/ml, adiponectin- 0.01 pg/ml and leptin-1.9 pg/ml respectively. The intra- and inter-assay coefficients of variation were <5%. In brief, capture antibody specific for each adipokines was pre-coated in the microplate prior to the experiment, sealed and incubated overnight at 4 °C. On the next day, the plates were washed with wash buffer and standards or samples were added to the appropriate wells. After 2 h incubation, the unbound antibody was washed. Then, the detection antibody followed by avidin-HRP was added, incubated and washed. Finally, the color was developed after the addition of substrate solution. The reaction was stopped by stop solution and read at 450 and 570 nm. The intra and inter assay coefficients of variations were <10.0%.

2.3. Sample size calculation and power of study

Pilot study using 50 subjects per group was conducted and based on the preliminary results, with an estimated p value of <0.05, 95% confidence interval (CI), and a power of 80%, the present sample size was derived. The formula used for calculating the sample size is as follows [32]

$$N = \frac{r+1}{R}\ \frac{(p^*)(1-p^*)(Z_\beta + Z\alpha/2)^2}{(P1-P2)^2}$$

2.4. PCR-RFLP genotyping

Genomic DNA from all the study cohorts were extracted from peripheral blood using phenol-chloroform method as described earlier [33]. Genotyping of the *GABPB1* gene intronic polymorphism rs7181866 (A/G) and rs8031031 (C/T) was performed by polymerase chain reaction (PCR) and amplification was accomplished by initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, primer annealing at 59 °C for 30 s, and chain extension at 72 °C for 1 min, final extension at 72 °C for 10 min. The amplified PCR products were digested by *Rsa*I enzyme in conditions recommended by the manufacture (New England Biolabs). The digested products were visualized by 1.8% in agarose gel electrophoresis (Fig. 1). The primer sequences, amplicon and digested product sizes are given in Table 1. Quality of genotyping was confirmed by duplicating 20% of the randomly selected samples. Furthermore, a few variants were confirmed by direct sequencing with SeqStudio Genetic Analyzer (Applied Biosystems).

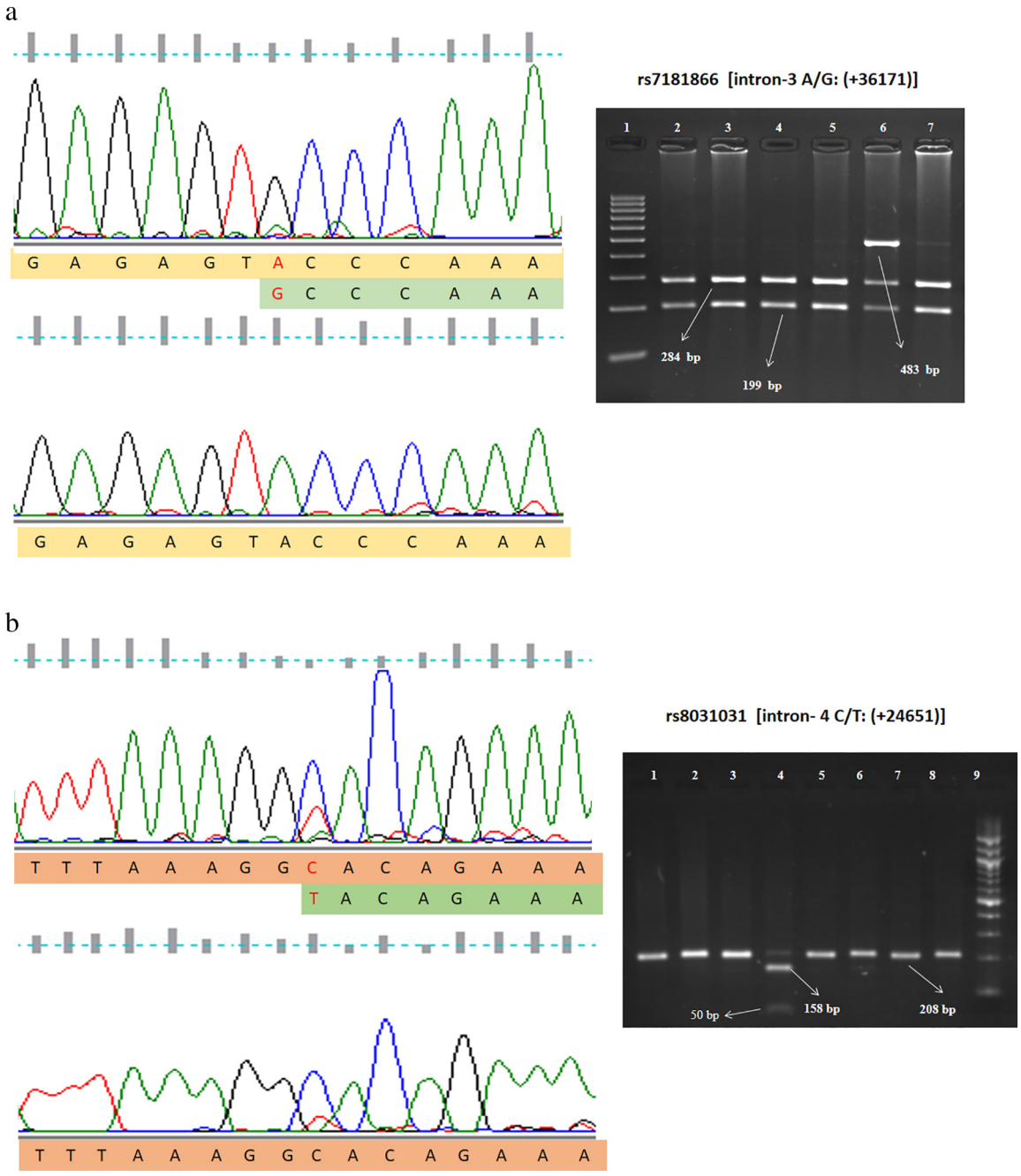

Fig. 1. PCR-RFLP results of Nrf2 gene intron-3 A/G: (+36171) (a) and intron-4 C/T: (+24,651) (b) with direct sequencing.

2.5. Statistical analysis

Statistical calculations were performed using SPSS (version 20.0; SPSS, Chicago, IL, U.S.A.). Normally distributed data are presented as mean ± S.D. The Hardy-Weinberg equilibrium was tested with the $\chi 2$ test. Genotype distribution and allele frequencies were compared among groups using a $\chi 2$ test of independence with 2×2 contingency and z statistics. A Student's *t*-test and a Mann-Whitney *U* test were used to determine the statistical significance. Wherever appropriate, the odds ratio (OR) with 95% CI were calculated. A two-tailed type I

Table 1
Details of the genotyped SNPs: the position of the polymorphism, sequences of the specific primers used, and restriction enzymes used for genotyping are given.

S.No.	Gene	SNP position	dbSNP ID	Primer Sequence	PCR product size	Restriction Enzyme	Expected band pattern
1	*GABPB1*	Intron-3 A/G (+36171)	rs7181866	5′-AGT TTA GTG TCT CCC AGT GT-3′ 5′-CTT AGT TTT CTT GTA TCC GT-3′	483 bp	Rsa 1	483 bp, 284 bp & 199 bp
2	*GABPB1*	Intron-4C/T (+24651)	rs8031031	5′- CTA AAA TGT GAG GGA AGG AAG A-3′ 5′- ATA GAG AGA TAG GAC TAA GGA C-3′	208 bp	Rsa 1	208 bp, 158 bp & 50 bp

error rate of 5% was considered statistically significant. The Exploratory principle component (EPC) analysis was performed to determine the underlying latent factors. EPC analysis was conducted in both non-obese T2DM and Obese T2DM subjects. Primarily, the adequacy of samples was tested by the Bartlett's Chi square test and the significant value of the test showed the adequacy of samples in EPC analysis. Factor analysis was done for all eligible subjects in three steps: 1) extraction of factors followed by rotation of factors to help interpretation; and finally by naming and interpretation of each factor based on the estimated values for the factor loadings. Factors with eigenvalue >0.9 were selected and then orthogonal (varimax rotation) method was used for rotation of factors. Factor loading of 0.4 and above was considered to determine which original variables represent primary constituents of each factor. [34]. To validate the Pearson's Correlation Coefficient and to check the internal validity of the model, we have employed bootstrap sampling to refine the models by excluding variables with unreliably included as necessary for prediction [35].

3. Results

3.1. Clinical characteristics and serum biomarker profile of the study subjects

A total of 302 subjects were subdivided into three cohorts-non-obese healthy individuals, non-obese individuals with T2DM and obese individuals with T2DM. The non-obese healthy individuals were matched with age, sex and ethnicity (hereafter referred to as 'controls') in the study. The clinical and biochemical parameters depicted in Table 2, were taken into consideration for inclusion and grouping of the study subjects. The controls consists of normoglycemic individuals with normal BMI (21.2 ± 1.2 kg/m^2) and biochemical parameters within the normal range. Those control individuals whose parameters deviated from the normal range, were excluded from the study. BMI was a crucial factor in grouping the individuals with T2DM either as obese or non-obese. The BMI in the obese T2DM individuals and in controls was significantly higher than that in the non-obese T2DM individuals (29.8 ± 3.6 kg/m^2; $p < 0.001$). Irrespective of the BMI, the individuals with T2DM were found to be hyperglycemic and hypertensive ($p < 0.001$); the glycated hemoglobin (HbA1c) levels were significantly higher in both, obese and non-obese individuals T2DM, when compared to the control individuals ($p < 0.001$). Similarly, increased levels of serum urea and creatinine were observed in the T2DM individuals, compared to the healthy controls ($p < 0.05$ & $p < 0.001$, respectively). However, the HOMA-IR values were significantly increased in the non-obese T2DM cohort (3.7 (1.1–9.0); $p < 0.001$) than in the controls and showed higher in the obese T2DM cohort (4.6 (1.3–15.1); $p < 0.001$). In the obese T2DM cohort, significant increase in the levels of serum triglycerides, total serum cholesterol and LDL were observed; however, the HDL levels were found to be significantly lower than that in the healthy controls and non-obese T2DM cohort.

3.2. SNP genotypes and their association with obesity in the study cohort

The study subjects from the three cohorts (controls, non-obese T2DM and obese T2DM) were genotyped for the two SNPs - rs7181866 & rs8031031 in the gene *GABPB1*. Genotyping was carried out using PCR-RFLP approach, with specific primers and corresponding restriction enzymes, as shown in Table 1 and the results of the restricted products for both the intronic regions along with the sequencing results are shown in Fig. 1a & b. The allele and genotypic frequencies with respect to the two SNPs rs7181866 & rs8031031, observed among study subjects form the three cohorts is given in Table 3. We found that both the SNPs were at Hardy-Weinberg equilibrium in our cohort. With respect to rs7181866, the frequency of the minor allele 'G' was significantly higher in the obese T2DM cohort (25.3%; 47/186 alleles), than that observed in the non-obese T2DM cohort (12.4%) and controls (10.3%). Similarly, the 'GG' genotype was more common among the obese-T2DM (10.8%; 10/93 individuals), compared to that in the non-obese T2DM cohorts and controls (4.3% and 2.6%, respectively). An increased frequency of the 'AG' was observed in the obese T2DM individuals (29%; 27/93 individuals), compared to that in the non-obese T2DM (15%; 16/93 individuals) and controls. Further, stratification based on sex revealed that the frequency of GG genotype (13.4%) and G allele (28.8%) were found to be higher among male individuals when compared to females of Obese T2DM cohort (S.Table 1a). However, the allelic and genotypic frequencies observed in this study with respect to rs8031031 in the three cohorts were comparable.

Odds ratio (OR) was calculated using multivariate logistic regression analysis to study the association of the SNP alleles and their respective genotypes in T2DM associated with obesity in the three cohorts (Tables 4a and 4b). For rs7181866, AA genotype is considered as reference genotype. When non-obese T2DM subjects were taken as control and compared with obese T2DM subjects, we found that both AG (OR: 2.16; 95% CI: 1.0–3.9; $P = 0.001$) and GG (OR: 3.71; 95% CI: 1.2–10.8; $P = 0.01$) genotypes conferred significant risk over AA genotypes, even after adjusting for the potential confounders such as age, gender and BMI. Also, the minor allele 'G' was found to be associated with obesity, when computed with obese T2DM against controls (OR 2.44; 95% CI 1.55–3.83; $p < 0.0001$) and obese T2DM against non-obese T2DM

Table 2
Summary of clinical parameters of the study subjects in the three cohorts.

Clinical Parameters ($n = 302$)	Non-Obese Healthy controls (n = 116)	Non-Obese T2DM[a] (n = 93)	Obese T2DM[b] (n = 93)
Gender (M/F)	65/51	53/40	53/40
Age (Years)	48.4 ± 5.5	51.3 ± 9.7	50.1 ± 9.6
Body mass index (kg/m^2)	21.2 ± 1.2	21.5 ± 0.9*	29.8 ± 3.6***
Systolic BP (mm Hg)	110.7 ± 8.6	127.8 ± 19.4***	128.7 ± 15.1
Diastolic BP (mm Hg)	81.4 ± 8.8	88.1 ± 19.6**	85.6 ± 8.8
Fasting plasma glucose (mg/dL)	94.5 ± 6.9	165.6 ± 22.8***	169.8 ± 46.7
Postprandial plasma glucose (mg/dL)	110.9 ± 9.9	208.2 ± 34.5***	223.2 ± 47.1
Glycated hemoglobin (%)	5.2 ± 0.2	9.3 ± 1.7***	9.2 ± 2.0
Total serum cholesterol (mg/dL)	171.8 ± 26.3	178.5 ± 34.5	193.8 ± 38.5**
Serum triglycerides (mg/dL)	122.7 ± 24.8	128.7 ± 19.6	168.3 ± 32.8***
HDL-cholesterol (mg/dL)	47.9 ± 5.3	47.2 ± 8.5	40.1 ± 9.2***
LDL-cholesterol (mg/dL)	78.2 ± 11.4	81.9 ± 17.8	100.7 ± 21.8***
Urea (mg/dL)	22.3 ± 7.0	24.3 ± 4.6*	23.9 ± 6.0
Creatinine (mg/dL)	0.8 ± 0.1	1.0 ± 0.3***	1.0 ± 0.2
HOMA-IR	1.4 (0.4–3.2)	3.7 (1.1–9.0)***	4.6 (1.3–15.1)***

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] Indicates comparison was made with NGT-non-obese.
[b] Indicates comparison was made with KDM-obese.

Table 3
Allelic and genotypic frequencies observed with respect to the 2 SNPs, among the study cohort.

Genotype/allele	NGT (n = 116)	T2DM (*n* = 186)	
	Non-obese	Non-obese (n = 93)	Obese (n = 93)
rs7181866 (Intron-3 A/G: (+36171))			
Genotype			
AA	95 (81.9%)	74 (79.6%)	56 (60.2%)
AG	18 (15.5%)	15 (16.1%)	27 (29.0%)
GG	3 (2.6%)	4 (4.3%)	10 (10.8%)
Allele			
A	208 (89.7%)	163 (87.6%)	139 (74.7%)
G	24 (10.3%)	23 (12.4%)	47 (25.3%)
rs8031031 [(Intron-4 C/T:(+24651)]			
Genotype			
CC	82 (70.7%)	59 (63.4%)	53 (57.0%)
CT	24 (20.7%)	28 (30.1%)	32 (34.4%)
TT	10 (8.6%)	6 (6.5%)	8 (8.6%)
Allele			
C	188 (81.0%)	146 (78.5%)	138 (74.2%)
T	44 (19.0%)	40 (21.5%)	48 (25.8%)

Values are numbers (percentage).

(OR 2.40; 95% CI 1.29–3.22; $p < 0.001$) (Table 4a). Next, the OR was computed for rs8031031 and the results of our study showed that CT genotype (OR: 1.91; 95% CI: 1.0–3.4; $P = 0.03$) alone showed a significant risk for obese T2DM when compared with non-Obese control as reference group even after adjusting for various confounding factors, however minor allele 'T' didn't show any significant risk between groups (Table 4b).

3.3. Serum adipokines profiling among the study subjects

The serum levels of adipokines such as leptin, adiponectin, IL-6 and TNF-α were studied among the three cohorts in the study; the results are plotted in Fig. 2. The levels of leptin was significantly increased (18.3 pg/ml (4.2–24.2); $p < 0.001$) in the obese T2DM cohort when compared to that in the non-obese T2DM (11.3 pg/ml (6.1–15.9)) and controls (9.9 pg/ml (8.1–12.9)). Similarly, the IL-6 levels in the obese T2DM cohort was significantly higher (67.6 pg/ml (21.8–213.3); $p < 0.001$), when compared to the controls. The TNF-α level were higher among the individuals with T2DM than in controls. Further, the levels were significantly higher in the obese T2DM individuals (107.8 pg/ml (45.84–194.5); $p < 0.001$) than in the controls (16.57 pg/ml (11.44–53.67)). However, an altered trend in the levels of adiponectin was observed, when compared to that in non-obese T2DM and controls, the levels were significantly reduced in obese T2DM cohort (299 pg/ml (132.9–1202.1); $p < 0.01$ & $p < 0.05$, respectively). Also, we have stratified the levels of adipokines based on sex and found that the levels of adipokines such as TNF-α (112.8 ± 14.8; $p < 0.01$), IL-6 (74.5 ± 51.5; $p < 0.01$) and adiponectin (384.7 ± 211.0; $p < 0.04$) were found to be significantly decreased among female when compared to male obese T2DM individuals, whereas the levels of leptin didn't show any significant difference between sex of obese T2DM individuals (S.Table 1b).

3.4. Factor analysis using Varimax rotation

EPC analysis on metabolic syndrome components revealed four factors with eigenvalues ≥0.9 (Table 5), accounting for 71.5% and 88.3% of variance in non-obese control and obese T2DM respectively. In non-obese controls, the first factor is characterized with negative loading for FPG and positive loading for LDL-c, the second factor had positive loading only for DBP, the third factor had positive loading for both BMI and cholesterol and the fourth factor had negative loading for cholesterol and positive for HDL. However, in non-obese T2DM subjects, the first factor is characterized with positive loading for both SBP and DBP, factor 2 had a negative and positive loading for cholesterol and LDL-c respectively, the third factor had a positive loading for both BMI and FPG, the fourth factor had positive loading for only HDL.

3.5. Estimating Pearson's correlation coefficient with bootstrapping confidence interval approach

The results of our study had showed that both AG and GG genotype of rs7181866 SNP have significant positive correlation with TNF-α level. Even after the bias-corrected and accelerated (BCa) bootstrap analysis remained the same with positive confidence interval. Also, the GG genotype of rs7181866 SNP showed a significant negative correlation with adiponectin, which was further validated with BCa bootstrapping (Table 6).

3.6. Comparison between the adipokines level and the genotypes observed in the study subjects

The levels of the adipokines leptin, adiponectin, IL-6 and TNF-α, were compared among the three cohorts, in individuals with the genotypes 'AA' and 'GG', for the SNP rs7181866 are plotted in Fig. 3. Among the individuals with 'AA' genotype, the leptin levels were significantly higher among the obese T2DM cohort (22.53 ng/ml; p value 0.01 & 0.001, respectively) than in the controls (10.37 pg/ml) and the non-obese T2DM cohort (8.58 ng/ml). However, the adiponectin levels were lower in obese T2DM individuals (290.4 ng/ml) than in the other two cohorts. The IL-6 level was higher among the obese T2DM than the non-obese counterparts and controls. Levels of TNF-α were significantly higher in the non-obese T2DM (71.94 pg/ml; $p < 0.001$) when

Table 4a
Multivariate logistic regression for the association of the rs7181866 (Intron-3 A/G) with obesity and T2DM.

rs7181866 (Intron-3 A/G: (+36171))	non-obese T2DM vs non-obese control		Obese T2DM vs non-obese control		Obese T2DM vs non-obese T2DM	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]	Unadjusted OR (95% CI)	Adjusted OR (95% CI) [a]	Unadjusted OR (95% CI)	Adjusted OR (95% CI) [a]
Genotype						
AA	Ref		Ref		Ref	
AG	1.04 (0.55–1.94); 0.12	1.38 (0.63–2.31); 0.24	1.87 (1.10–3.17); **0.02**	2.98 (1.43–5.29); **0.01**	1.80 (1.02–3.15); **0.04**	2.16 (1.01–3.97); **0.001**
GG	1.66 (0.38–7.2); 0.49	1.73 (1.0–8.4); 0.36	4.15 (1.17–14.67); **0.02**	3.84 (1.3–15.35); **0.01**	2.5 (0.81–7.68); **0.02**	3.71 (1.2–10.86); **0.01**
Allele						
G	1.19 (0.69–2.04); 0.64	–	2.44 (1.55–3.83); **0.0001**	–	2.04 (1.29–3.22); **0.001**	–

Figures in bold were significant ($P < 0.05$).
[a] Odds ratio (OR) adjusted for confounding factor (age, gender & BMI).

Table 4b
Multivariate logistic regression for the association of the rs8031031 (Intron-4C/T) with obesity and T2DM.

rs8031031 (Intron-4C/T: (+24651))	non-obese T2DM vs non-obese control		Obese T2DM vs non-obese control		Obese T2DM vs non-obese T2DM	
	Unadjusted OR (95% CI)	Adjusted OR (95% CI)[a]	Unadjusted OR (95% CI)	Adjusted OR (95% CI) [a]	Unadjusted OR (95% CI)	Adjusted OR (95% CI) [a]
Genotype						
CC	Ref		Ref		Ref	
CT	1.45 (0.90–2.33); 0.11	1.62 (0.76–3.04); 0.09	1.66 (1.05–2.61); **0.02**	1.91 (1.0–3.43); **0.03**	1.14 (0.75–1.73); 0.53	1.43 (1.1–2.33); 0.62
TT	0.74 (0.28–1.98); 0.56	0.89 (1.7–2.27); 0.51	0.99 (0.41–2.42); 0.99	1.14 (0.6–3.62); 0.24	1.33 (0.48–3.69); 0.57	1.67 (0.91–5.02); 0.48
Allele						
T	0.65 (0.40–1.03); 0.06	–	1.36 (0.94–1.95); 0.10	–	1.08 (1.32–3.28); 0.22	–

Figures in bold were significant ($P < 0.05$).
[a] Odds ratio (OR) adjusted for confounding factor (age, gender & BMI).

compared to the controls (17.68 pg/ml), and it was found to be further increased in the obese T2DM cohort (126.1 pg/ml; $p < 0.001$).

In individuals with 'GG' genotype, the obese T2DM cohort (23.80 ng/ml; $p < 0.001$) showed significantly higher levels of leptin, those individuals with 'AA' genotype from the same cohort; further, it was significantly higher than the control and non-obese T2DM individuals with 'GG' genotype ($p < 0.05$ & 0.001, respectively). The adiponectin levels were significantly decreased among the T2DM individuals with GG genotype, compared to the controls. However, in obese T2DM individuals, the adiponectin levels (260.4 ng/ml) were significantly lower than that observed in the 'GG' genotype non-obese T2DM and 'AA' genotype obese T2DM cohorts ($p < 0.01$ & 0.05, respectively). Higher levels of IL-6 and TNF-α were observed among obese T2DM individuals with 'GG' genotype. IL-6 was significantly increased in the 'GG' genotype obese T2DM individuals (106.9 pg/ml) than in the 'GG' genotype non-obese T2DM and 'AA' genotype obese T2DM individuals ($p < 0.05$ & 0.01). We observed that TNF-α level was significantly higher in the 'GG' genotype obese T2DM individuals (208.8 pg/ml), than that in the 'GG' genotype non-obese T2DM and 'AA' genotype obese T2DM individuals ($p < 0.001$ & 0.01, respectively).

4. Discussion

The link between T2DM and obesity is well known; BMI is known to be associated with diabetes and insulin resistance. Obesity associated with hyperinsulinemia and insulin resistance is a crucial factor in

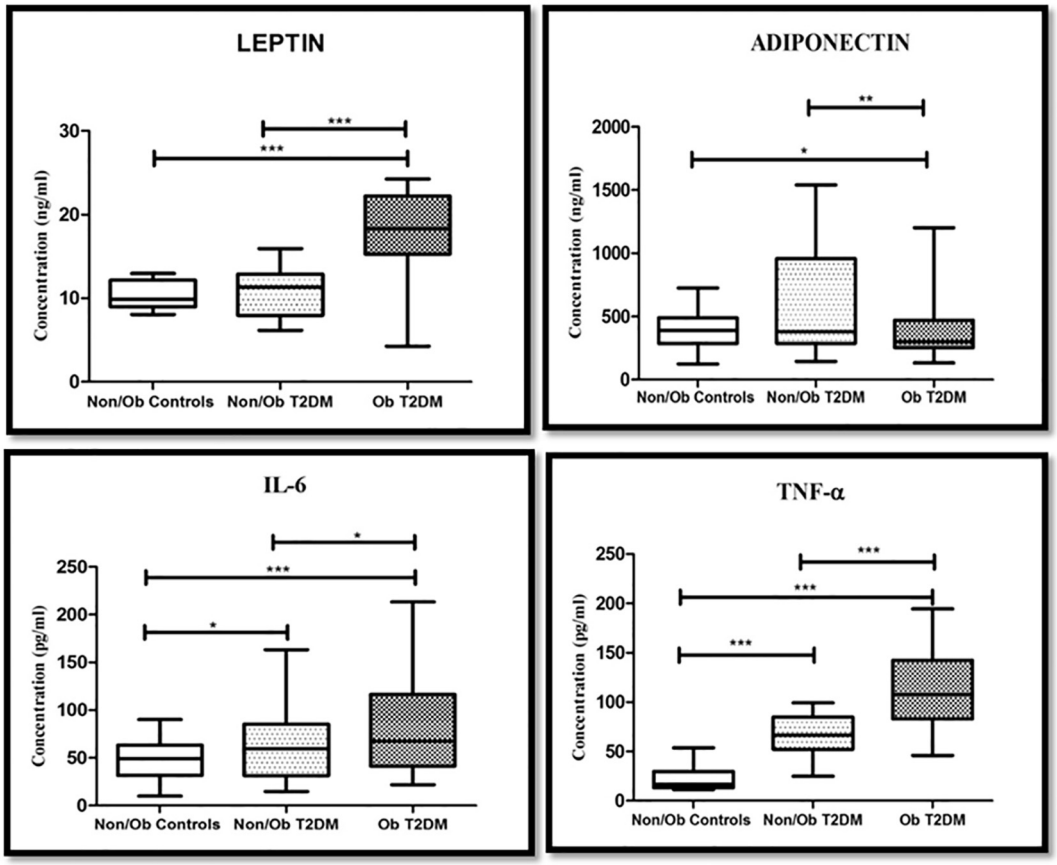

Fig. 2. Circulatory levels of adipokines such as Leptin, Adiponectin, IL-6 and TNF-α in the study cohorts were measured using ELISA. The lower detection limits for IL-6 was 1.9 pg/ml, TNF-α was 1.5 pg/ml, adiponectin- 0.01 pg/ml and leptin-1.9 pg/ml respectively. The intra- and inter-assay coefficients of variation were <5%. All data are reported as geometric mean with 95% Confidence Interval; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 5
Factor loadings for components of metabolic syndrome with rotated factors after varimax rotation.

Variables	non-obese control				Obese T2DM			
	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3	Factor 4
BMI	−0.054	0.019	**0.713**	0.127	−0.019	−0.002	**0.66**	−0.318
FPG	**−0.626**	−0.015	0.113	0.11	0.021	0.002	**0.627**	0.36
Total Cholesterol	0.173	−0.071	**0.461**	**−0.452**	0.067	**−0.636**	−0.06	−0.226
HDL-c	0.022	−0.022	0.065	**0.676**	0.01	0.01	−0.012	**0.769**
LDL-c	**0.505**	−0.021	0.153	0.16	0.065	**0.662**	−0.057	−0.217
SBP	0.069	0.576	0.158	0.302	**0.566**	0.063	0.11	0.022
DBP	−0.051	**0.639**	−0.117	−0.223	**0.565**	−0.062	−0.109	−0.022
% of Total Variance	23.29	19.09	15.68	13.44	39.556	20.616	17.418	10.82

Factor loadings ≥0.40 are in bold.

Table 6
Association between SNPs of rs7181866 and its correlation with serum adipokines levels.

Genotypes	TNF-α			IL-6			Leptin			Adiponectin		
	Pearson correlation		Adjusted Bca 95% (CI)	Pearson correlation		Adjusted Bca 95% (CI)	Pearson correlation		Adjusted Bca 95% (CI)	Pearson correlation		Adjusted Bca 95% (CI)
	r	*P*		r	*P*		r	*P*		r	*P*	
AA			Ref			Ref			Ref			Ref
AG	0.244	**0.01**	**(0.039–0.428)**	0.13	0.176	(−0.322–0.053)	0.136	0.109	(−0.017–0.275)	−0.113	0.174	(−0.251–0.057)
GG	0.564	**0.001**	**(0.427–0.685)**	0.105	0.334	(−0.159–0.368)	0.074	0.429	(−0.068–0.211)	−0.344	**0.001**	**(−0.463–0.223)**

Figures highlighted in bold shows significance less than $P < 0.05$.

occurrence of T2DM [36]. Several genetic variants and genes have been associated with T2DM and obesity [12–14,16,17]. Nrf2 is an obligate multimeric Ets factor that consists of two subunits, the GABPα and GABPβ. They occur as hetero-tetramers that are transcriptionally active ($GABP\alpha_2\beta_2$). The GABPβ is involved in transactivation [23,37]. It is involved in transcription of several nuclear genes for mitochondrial enzymes and complex-IV factors [21,23] and in mitochondrial biogenesis [20]. Mitochondrial dysfunction and reduced mitochondrial mass are known to occur in obesity and T2DM [24,25].

In the present study, the genetic susceptibility to obesity and T2DM in South Indian population was assessed. The individuals from the three cohorts were genotyped for two SNPs - rs7181866 and rs8031031, in the gene *GABPB1* that encodes the β-subunit of Nrf2. These SNPs have been previously reported to be associated with physical performance

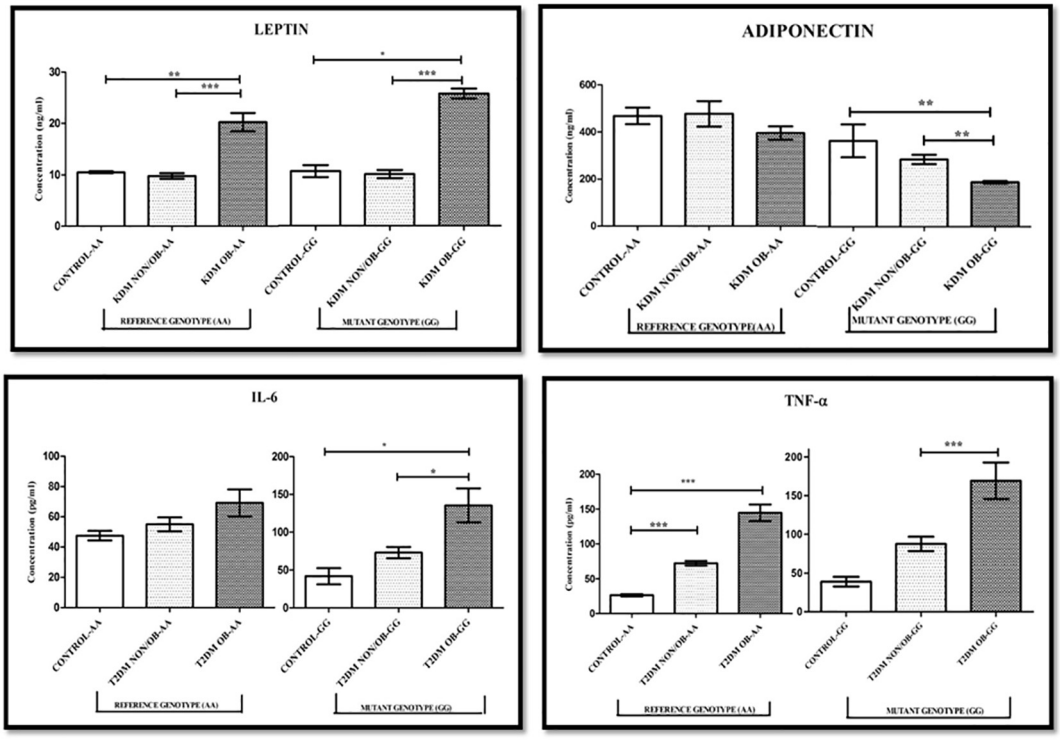

Fig. 3. Association of various genotypes observed with the adipokine such as Leptin, Adiponectin, IL-6 and TNF-α levels in the study cohorts. All data are reported as mean ± SD; *P < 0.05, **P < 0.01, ***P < 0.001.

and elite endurance in various studies among people from physically demanding and athletic professions [38,39]. Especially, the 'AG' genotype of rs7181866 was reported to induce increased mRNA expression in Israeli endurance athletes, and the 'G' allele was reportedly associated with higher oxygen uptake [40]. To the best of our knowledge, this is the first study that deals with association of *GABPB1* SNPs with obesity and T2DM. Here, the study subjects included hyperglycemic obese individuals with T2DM, hyperglycemic non-obese individuals with T2DM and normoglycemic non-obese healthy individuals as controls; BMI, fasting glucose levels, and other bio-clinical parameters were measured before grouping the subjects into these three cohorts. Glycated hemoglobin (HbA1c) levels were higher among obese and non-obese T2DM individuals than in the controls. HOMA-IR values were higher among the T2DM individuals, suggesting increased insulin resistance. Similarly, the serum levels of urea and creatinine were increased among the T2DM individuals (both obese and non-obese). The serum triglycerides, cholesterol and LDL levels were increased among obese T2DM, than in the non-obese T2DM and controls. They were found to be proportional to obesity, as reported in previously [41]. However, the HDL levels were reduced among obese individuals with T2DM. These findings suggest that the obese individuals with T2DM have a higher risk of developing comorbidities such as CVD, than the non-obese T2DM and control individuals. Both the SNPs tested were in Hardy-Weinberg equilibrium. The 'G' allele of the SNP rs7181866 was the most common allele among the obese-T2DM individuals; further, the 'GG' genotype was found at a higher frequency (10.8%) among this cohort. Odds ratio estimates showed that the 'G' allele and 'GG' genotype of the SNP rs7181866 was associated with the obesity and T2DM. These results show that the individuals with the 'GG' genotype are predisposed to obesity and T2DM. However, the 'AG' genotype reported to contribute to endurance in athletes [42] was also occurring in a higher percentage among the obese T2DM individuals, suggesting that it does not impart any favorable characteristic in our study cohort.

Adipokines are known to play a crucial role in obesity induced inflammation, insulin resistance and T2DM [6]. The levels of adipokines leptin, adiponectin, IL-6 and TNF-α were recorded in the three cohorts of subjects in the present study. The levels of leptin, IL-6 and TNF-α was increased among obese T2DM individuals when compared to that in non-obese T2DM individuals and controls. Further, the levels of these adipokines were higher in non-obese T2DM cohort, than in controls. However, the levels of adiponectin were reduced among the obese T2DM cohort, than of that in the non-obese T2DM and control individuals. Bootstrap is one of a plethora of estimation techniques based on the empirical distribution function of the data. The problem of non-normality data for estimating the correlation coefficient has been considered in the present study. The percentile bootstrap confidence interval can be employed, when there is a uniform distribution of samples and the sample sizes are larger than or equal to 50 [43]. Since our data has a sample size of 93 in both non-obese and obese T2DM subjects we have employed bootstrapping methodology for the present study. The results of the present study have shown that GG genotype of rs7181866 SNP showed a significant negative correlation with adiponectin. These results are in accordance with the adipokine levels reported in previous studies. When the genotypes with respect to the SNP rs7181866 in the study subjects was correlated with their adipokine levels, we found that the leptin, IL-6 and TNF-α levels were increased in obese T2DM individuals with 'GG' genotype, than in those with 'AA' genotype, non-obese T2DM and controls with 'GG' genotype. Adiponectin levels were lowest among the obese T2DM individuals with 'GG' genotype than those with 'AA' genotype, non-obese T2DM and controls with 'GG' genotype. The levels of adipokines leptin, IL-6 and TNF-α were significantly increased among the obese T2DM cohort and adiponectin was significantly decreased among this cohort. These findings suggest that the obese T2DM individuals have increased insulin resistance, which results in increased T2DM-related morbidity among this cohort; this is evident from the various clinical parameters tested such as plasma glucose levels and HOMA-IR. In summary, these results together suggest that the SNP rs7181866 was associated with obesity and T2DM among South Indians. The minor allele 'G' of the SNP is probably a predisposing factor in obesity associated T2DM.

Conflict of interest

None declared.

Author contributions

DU, RKM and VV designed the experiment, DU, PV drafted the manuscript. DU, PV, BP, TR and KE performed the experiment. DU, PV, TR, KE, PN and RKM analyzed and interpreted the data. DU, VV and RKM contributed to the discussion and reviewed the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.03.125.

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