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Effect of a genetic variant in the *JAZF1* gene among obesity population

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ABSTRACT

Introduction: Obesity is a complex condition characterized by an excess of adipose tissue and a low-grade chronic inflammation. Juxtaposed with another zinc finger protein 1 (JAZF1) is one of the genes identified through genome wide association studies which is associated in type 2 diabetes, obesity and other forms of human diseases. JAZF1 controls the expression of several genes involved in lipid and carbohydrate metabolism. Limited studies have been conducted in the obesity population and none of the studies was documented in the Saudi population. The aim of this study was to investigate the genetic association with rs864745 SNP genotype assay in *JAZF1* gene in the obesity population in Saudi Arabia.

Methods: This study includes 80 obese patients and 50 healthy controls. Polymerase chain reaction was performed in the rs864745 SNP of *JAZF1* gene using isolated genomic DNA from 130 participants. Anthropometric measurements were calculated between cases and controls using t-tests. Hardy-Weinberg Equilibrium (HWE) analysis, genotype and allele frequencies were calculated between obesity cases and controls. Anova analysis was carried out between rs864745 SNP genotype assay and anthropmetric measurements. Statistical analysis was performed using with SPSS software.

Results: Weight, BMI, HDLC, LDLC, and TG were all associated with obesity in the clinical data ($p < 0.05$). The deviation with the rs864745 SNP genotyping assay was identified by HWE analysis. None of the genotypes, dominant models, or allelic frequencies demonstrated a significant connection with obesity ($p > 0.05$). ANOVA analysis was not associated ($p < 0.05$) between genotypes and anthropometric parameters.

Conclusion: In conclusion, rs864745 SNP genotype assay of the *JAZF1* gene did not indicate a genetic association. Anova analysis also revealed the negative association. Future research with a large sample sizes are recommended.

1. Introduction

Obesity and overweight are both defined by high levels of adipocytes and macrophages in the fat tissues, as well as an increase in adipose tissue mass as a result of these cells' continuous activation of inflammatory pathways. Obesity is a complex medical condition that affects multiple organs in the body (Yu et al., 2022). Obesity can be reversible and preventable at any stage of human life. The overweight children are more likely to become obese adults, which can have long-term health consequences (Malacarne et al., 2022). Obesity is defined as the presence of extra fat in the body, and it is measured by a body mass index (BMI), which is beyond 30 kg/m^2 (Alharbi et al., 2020). Obesity has recently been identified as a significant risk factor for COVID-19, and type 2 diabetes mellitus (T2DM) and cardiovascular disease are also thought to be common risk factors for obesity (Guzman et al., 2022). Adipocyte hypertrophy and differentiation, macrophage infiltration, T-cell infiltration at the adipose site, inflammatory cytokine production, and insulin resistance are all affected by obesity (Kiran et al., 2021). Several polygenic genes are involved in multifactorial polygenic obesity, and environmental factors such as nutrition, physical activity, fast food, chemical contamination ultra-processed food with microbiome can modify gene expression (Mayoral et al., 2020). More than 600 genes related with obesity pathways have been found through genome-wide association studies (GWAS) (Alharbi et al., 2021).

Juxtaposed with another zinc finger gene 1 *(JAZF1)* is commonly associated with metabolic disorders such as T2DM, obesity and gestational diabetes. JAZF1 is expected to alter metabolic processes

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such as glucose and fat metabolism and expression of the putative transcription factor (Ustianowski et al., 2021). Several nuclear receptors and protein kinases involved in cell energy metabolism can interact with the *JAZF1* gene. A number of mechanisms are involved in the regulation of these downstream molecules by JAZF1, such as inhibiting the inflammatory response and enhancing glucose/lipid metabolism (Liao et al., 2019). The rs864745 single nucleotide polymorphism (SNP) genotype assay was studied in Obesity (Grarup et al., 2008; Zeng et al., 2021) T2DM (Alharbi et al., 2015; Zano et al., 2020) and GDM (gestational diabetes mellitus) (Ustianowski et al., 2021). There are limited studies have been carried out in the Saudi Arabia and there are no documented studies have been implicated in Saudi Arabia with Obesity. So, this study was aimed to investigate the association of rs864745 SNP genotype assay in *JAZF1* gene in obesity subjects in the Saudi population.

2. Materials and methods

This study was designed as a case-control and recruited 130 subjects from King Saud University (KSU) Hospital after the approval of ethical grant within the hospital premises. All 130 patients were signed the consent form and enrolled in this study. The subjects were not signing the consent form or involved in oral consent form were excluded from this study. In this study, the sample size was calculated before the design of this study. Based on Alshammary et al studies, the sample size was determined with 85% power and 95% confidence intervals with a critical value of less than 2.0 (Alshammary and Khan 2021). This study includes 80 obesity cases and 50 control participants. A total of 285 people was participated in this study, but only 80 of them were obese, and the other 50 were healthy participants. This study had strict inclusion and exclusion criteria. Obesity cases have to have a BMI of 30 kg/m^2 or more and be between the ages of 18 and 80 were included. Obesity cases were excluded if they had a metabolic condition or a BMI of less than 30 kg/m^2. A healthy lifestyle and a BMI of 29 kg/m^2 were used as the inclusion criteria for the control group. BMI of 31 kg/m^2 was found to be the exclusion criterion for controls. Anthropometric characteristics and a wide range of etiologies led to the BMI cases being divided into moderately and extremely obesity.

2.1. DNA and blood collection

From each patient, 5 ml of peripheral blood was collected and 3 ml was used for lipid profile analysis and 2 ml for separation of DNA (Alharbi et al., 2014). After blood was collected, coagulant and anticoagulant tubes were stored at 4 °C, while serum and DNA were stored at −20 °C after being removed from their respective tubes. Both were then stored at 4 °C. The hospital Dimension vista1500 system was used to perform the lipid profile analysis and this study was performed on obese and non-obese patients according to the guidelines.

2.2. JAZF1 genotyping assay

DNA was isolated using the DNA isolation kits and stored at −20 °C from 2 ml EDTA tubes (Khan et al., 2015). NanoDrop spectrophotometry was used to measure genomic DNA for both qualitative and quantitative purposes (Al-Otaiby et al., 2021). Genotyping of the rs864745 SNP was carried out using the PCR-RFLP method in accordance with the instructions (Alharbi et al., 2015). It was determined that 50 µl of Qiagen-PCR master mix kit, ionized water, primers, and DNA templates was the optimal amount of PCR for the experiments conducted. dNTPs, magnesium chloride, and Taq DNA polymerase were included in the master mix. Initial denaturation at 95 °C for 5 min, denaturation at 95 °C for 30 s, annealing-58 °C for 30 s, extension 72 °C for 45 s, and final 72 °C extension for 10 min were used for all of the DNA templates in this experiment (AlQahtani et al., 2021). An overnight restriction analysis was done on agarose gels using the PCR products that had not been purified (Bin Saif et al., 2022), using the SSPI restriction enzyme, after 35 cycles of amplification were completed. On a 3% agarose gel, the digested PCR products were analyzed. The PCR result, 378 bp (A allele), was digested to generate 338/40 bp (G allele).

2.3. Statistical analysis

SPSS was used to conduct the statistical analysis. The *t*-tests were used to compare the two groups of eight obese people and fifty healthy individuals. The rs864745 SNP test was subjected to a Hardy-Weinberg equilibrium (HWE) study. OR and 95% CI with P values were used to do genotyping analysis between cases and control groups. Using the lipid profile and the rs464218 SNP assay, we did a one-way Anova analysis. The significance of the P value being 0.05 was validated by the analysis (Khan et al., 2019).

3. Results

3.1. Demographical features of obesity population

A total of 130 adults, both obese and non-obese, are being explored in this study. Table 1 shows the anthropometric characteristics and lipid profile values. Comparing between obese and control groups, the BMI was significantly higher among cases ($p < 0.0001$), as well as in weight ($p < 0.0001$). Obesity is associated with higher levels of HDLC, LDLC, and TG ($p < 0.05$). Research discovered that the average age of those who were overweight or obese was 58.52 years old, compared to 55.21 years old for those who were not overweight or obese ($p = 0.06$). There was a significant negative correlation between TC and age, gender, height, and sex ($p > 0.05$).

3.2. JAZF1 genotyping assay

The distribution of rs864745 genotypes and allele frequencies in both the study subjects were shown in Table 2. A deviation was confirmed in HWE analysis in both the subjects ($p < 0.05$). Among control groups, the genotype frequencies of AA, AG and GG were 50%, 30% and 20% and 58%, 32% and 10% in obesity groups (Fig. 1). The allele frequencies in obesity group showed 65% of A allele and 35% of G allele, where as in control group, 74% of A allele and 26% of G allele was confirmed. None of the genotypes including dominant models and allele frequencies showed the positive association between the obesity and control groups (AG vs AA; OR-1.08 (0.49–2.40); p = 0.83, AA vs AG + GG; OR-1.38(0.67–2.81); p = 0.37 and G vs A; OR-1.53 (0.88–2.66), p = 0.12).

Table 1
Demographical characteristics features in obesity and control subjects.

	Obesity (n = 80)	Controls (n = 50)	P Value
Age (Years)	58.52 ± 10.55	55.21 ± 8.76	0.06
Gender (F:M)	35:45	22:28	0.06
Weight (kg)	83.25 ± 10.42	62.14 ± 9.57	<0.0001
Height (cms)	157.2 ± 6.13	156.7 ± 6.16	0.65
BMI (kg/m^2)	33.59 ± 3.68	25.3 ± 3.09	<0.0001
HDL-C (mmol/L)	1.77 ± 1.83	1.21 ± 0.71	0.04
LDL-C (mmol/L)	3.21 ± 1.05	2.24 ± 0.84	0.001
TG (mmol/L)	2.60 ± 1.89	1.72 ± 0.65	0.001
TC (mmol/L)	4.02 ± 1.72	4.03 ± 0.84	0.96

Table 2
Genotype and allele frequencies for rs464218 SNP genotype assay in obesity.

Genotype	Obesity Cases (n = 80)	Controls (n = 50)	OR (95%CIs)	P Value
AA	40 (50%)	29 (58%)	Reference	Reference
AG	24 (30%)	16 (32%)	1.08 (0.49–2.40)	0.83
GG	16 (20%)	05 (10%)	2.32 (0.76–7.05)	0.13
AA vs AG + GG	40 (50%)	21 (42%)	1.38 (0.67–2.81)	0.37
A allele	104 (0.65)	74 (0.74)	Reference	Reference
G allele	56 (0.35)	26 (0.26)	1.53 (0.88–2.66)	0.12
HWE	0.35	0.26	–	–
X^2	11.60	2.83	–	–
P values	0.0006	0.092	–	–

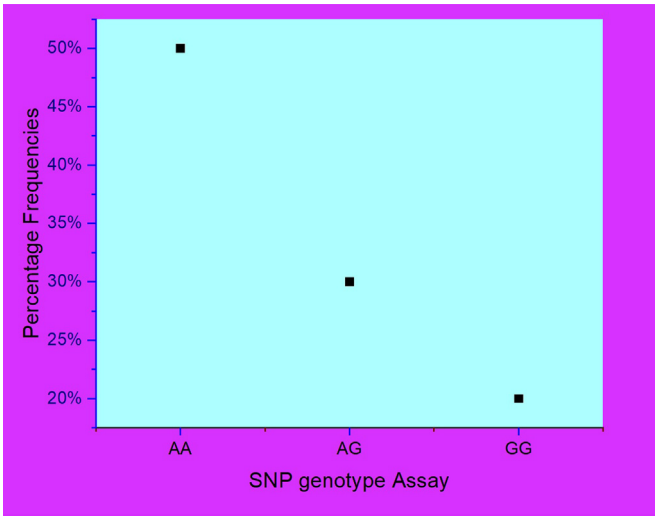

Fig. 1. Genotype frequencies of the rs864745 SNP genotyping assay in the obese group.

3.3. Anova analysis

Obesity groups has been categorized into 3 groups based on rs864745 genotypes. Table 3 defines the one-way of Anova analysis in obesity cases. The AA, AG and GG consists of 40, 24 and 16 genotypes. Age (59.30 + 10.75), LDLC (3.31 + 0.97) and TC (2.75 + 1.80) were found to have elevated levels in AA genotype, while, weight (85.47 + 10.21), height (157.62 + 5.95), BMI (34.35 + 3.41) and HDLC (2.06 + 1.89) levels were found to be in heterozygous genotypes. In GG genotypes, only TG (4.23 + 1.76) levels were found to be high and none of the genotypes showed the significant association ($p > 0.05$). From this Anova analysis, we conclude as rs864745 SNP assay has no role in BMI in our studied population ($p > 0.05$).

4. Discussion

The aim of this study was to investigate the genetic association between rs864745 SNP genotype assay in *JAZF1* gene in Saudi subjects confirmed with obesity. The purpose of this study was to examine the role of the rs864745 SNP genotyping analysis in the *JAZF1* gene in obese Saudis. In Saudi Arabia, no studies on obesity and the rs864745 SNP were conducted, while Alharbi et al identified a comparable SNP genotype in T2DM patients (Alharbi et al., 2015). The negative association with genotype, dominant model, and allele frequencies was confirmed in this study ($p > 0.05$). Anthropometric parameters such as weight and BMI differed between cases and controls ($p < 0.05$). The lipid profile parameter demonstrated a positive correlation between obesity and control participants among HDLC, LDLC and TG ($p > 0.05$). ANOVA analysis couldn't identify a connection between rs864745 genotype and obesity ($p > 0.05$). This study found that the rs864745 SNP genotyping assay plays no role on obesity in the Saudi population.

Obesity is caused due to the failure of burning excess calories which was consumed in the form of fat and sugar. When it comes to understanding human obesity, knowledge beyond the simplistic assumption that it's all due to poor eating habits. The accumulation of lipids, particularly triglycerides, in skeletal muscle, liver, and other organs is commonly accepted to be the outcome of an imbalance between energy expenditure and energy intake (Younes et al., 2021). Obesity has been associated to the aging process and has a strong genetic component. Many patients with obesity and T2DM develop metabolic syndrome (Mets), which includes dyslipidemia, central obesity, hypertension, and insulin resistance (IR). Obesity is frequently associated with IR, which is a risk factor for the development of MetS (Sheikhpour et al., 2020). Many genes have been associated to the combination of IR, obesity, T2DM, and MetS. Among these, the *JAZF1* gene was discovered to be frequently related with IR and other human diseases. JAZF1 controls the expression of several genes involved in lipid and carbohydrate metabolism (Ustianowski et al., 2021). JAZF1 encoded 27 kDa protein with three C2H2-type ZnFs represses DNA response element 1-dependent transcription of NR2C2, TR4, and JAZF1. Transactivation of numerous metabolically important genes may be inhibited by JAZF1 encoded transcription factor that interacts with protein NR2C2. Susceptibility alleles for impaired beta cell activity have been linked to JAZF1 locus variations as a gene transcriptional repressor that has a detrimental impact on the metabolism of glucose (Kobiita et al., 2020; Zano et al., 2020). T2DM has already been connected to rs1635852, rs849133, and rs849142, rs1635852, and

Table 3
Variations between three different modes of obesity using Anova analysis.

	AA (n = 40)	AG (n = 24)	GG (n = 16)	P value
Age (Years)	59.30 ± 10.75	57.37 ± 10.93	58.31 ± 9.95	0.77
Weight (kgs)	81.77 ± 9.16	85.47 ± 10.21	83.61 ± 13.41	0.38
Height (Cms)	157.58 ± 6.40	157.62 ± 5.95	155.53 ± 5.79	0.48
BMI (kg/m^2)	32.96 ± 3.65	34.35 ± 3.41	34.02 ± 4.09	0.30
HDLC (mmol/L)	1.54 ± 1.59	2.06 ± 1.89	1.93 ± 2.28	0.51
LDLC (mmol/L)	3.31 ± 0.97	2.99 ± 1.16	3.30 ± 1.07	0.46
TG (mmol/L)	4.18 ± 1.69	3.61 ± 1.74	4.23 ± 1.76	0.38
TC (mmol/L)	2.75 ± 1.80	2.38 ± 2.03	2.52 ± 1.99	0.74

rs864745. Because it has been discovered that SNP rs864745 is more widespread in the global population, we have included it in our study. The rs864745 SNP was associated with T2DM (Alharbi et al., 2015; Liao et al., 2019; Muacevic et al., 2021; Ng et al., 2008; Omori et al., 2009; Rong et al., 2009; Stančáková et al., 2009; Zano et al., 2020), GDM (Ustianowski et al., 2021)and Obesity (Grarup et al., 2008; Zeng et al., 2021). Our results had no association with any form of genotype inheritance or allelic association.

The strength of this study was confirmed as all the samples were collected from single care hospitals and opting only 80 cases and 50 controls were one of the limitations of this study.

5. Conclusion

This study concludes as rs864745 SNP was not associated in our study. It might be due to low sample size and future studies recommend to carryout this study in large sample size with additional SNPs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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