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Association between *SORT1* gene polymorphism studies in obesity population

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ABSTRACT

Obesity is defined the accumulation of extra fat deposited in the body. Based on body mass index (BMI), obesity is classified in children and adults of both men and women. Sortilin 1 (*SORT1*) gene has been documented with the relationship of low-density lipoprotein cholesterol (LDLC) and coronary artery disease. However, the relation between obesity and LDLC is well documented. The aim of this study was to investigate the relation between rs464218 SNP genotype assay in *SORT1* gene among obesity subjects in the Saudi population. One hundred forty samples were categorized as 80 obesity and 60 control subjects. DNA was extracted to perform PCR for rs464218 SNP genotype assay using restriction enzyme. Based on clinical and genotype data, statistical analysis will be performed. The clinical data documents weight, Body Mass Index, High Density Lipoprotein Cholesterol, LDLC and Triglycerides were associated in obese group ($p < 0.05$). Hardy-Weinberg Equilibrium analysis revealed the deviation with rs464218 SNP genotype assay. None of the genotypes, dominant model and allelic frequencies showed the significant association among obese groups ($p > 0.05$). Anova analysis revealed the positive association among weight, BMI and LDLC among obese groups ($p < 0.05$). In conclusion, the rs464218 SNP genotype assay of *SORT1* gene doesn't showed the genotype association but Anova analysis revealed the positive connection between obesity and LDLC. Future studies are required with large number of sample size.

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1. Introduction

Obesity is a simple and complex condition that develops as a result of our sedentary lifestyle patterns. According to the World Health Organization (WHO), 13% of the global population is obese, with men accounting for 11% and women contributing for 15% (Althumiri et al., 2021). Aside from diabetes and cardiovascular disease (CVD), COVID 19 was added as a new risk factor for obesity, according to WHO. Overweight and obesity-related problems claim the lives of 2.8 million people each year (Pugliese et al., 2022). Over the past few decades, the worldwide obesity rate has nearly tripled, with an estimated 1.9 billion persons classified as overweight or obese. Metabolic disorder has a greater correlation with

visceral than subcutaneous adipose tissue in humans. Obesity can diagnose using Body Mass Index (BMI). Body fat mass can be exaggerated in those who have a lot of muscle mass, even if BMI has an association. Furthermore, BMI was discovered because not all obese persons suffer from metabolic problems that are typically linked to obesity. Both environmental and genetic factors plays a major role in development of obesity as well as developing obesity into further complications, which can leads to diabetes, CVD and other chronic metabolic diseases (Alharbi et al., 2021).

There have been numerous variations identified in complex and multifactorial human disorders by meta-analyses, Genome-wide association studies (GWAS), single nucleotide polymorphisms (SNPs), exome sequencing, next generation sequencing, and cohort studies. Obesity is known to be one of inherited complex chronic and metabolic disorder, which describes the disease with the scale of BMI (Alshammmary and Khan, 2021). Understanding the pathophysiology of disease and enhancing diagnosis, prevention, and treatment are critical aspects of genetic studies (Khan et al., 2015). GWAS have revealed the existence of the Sortilin (*SORT1*) gene, which can be found on the chromosome 1p13.3 area. In both neurons and non-neuronal cells, *SORT1* is produced, and it encodes for the sorting protein that plays a crucial role in lipid uptake. Functional variations at this locus may be located inside the *SORT1*

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gene. The risk allele was related with lower SORT1 expression, an increase in plasma LDL-C content, and an increased risk of coronary artery disease (Alharbi et al., 2018). There have been limited studies carried out with SORT1 gene in obesity. This study was aimed to investigate the role of SORT1 gene (rs464218) in obesity subjects in the Saudi population.

2. Materials and methods

The present study included 80 obesity cases and 50 controls attended the King Saud University (KSU) Hospital in the Riyadh city of Saudi Arabia. In this study, a total of 285 subjects were enrolled and based on inclusion and exclusion criteria of this study, we have involved only 80 obesity cases and 50 healthy controls. The inclusion criteria of obesity cases were found to be BMI of 30 and above between the age range of 18–80 years of age. The exclusion criteria of obesity cases were affected with other metabolic disorder or below the BMI of 30 kg/m². The inclusion criteria of control subjects were enrolled based on healthy life style without any complications and with a BMI of 29 kg/m². The exclusion criteria of controls were found to be BMI 31 kg/m². As a result of a wide range of varying etiologies, rates of prevalence, mortality, and anthropometric parameters, the BMI cases were classified to be Class-I (30–34.9 kg/m²), Class-II (35.0–39.9 kg/m²) and Class-III (40 kg/m² and above). This study was carried out between 2020 after approval of ethical grant from Institutional review board at KSU. All the patients recruited in this study has signed the patient consent form. In this study, 155 subjects were excluded for not signing the patient consent form. In this study the sample size was determined based on the reference of Wang et al and Alshammery et al studies with 85% of power and 95% of confidence intervals with the obtained critical value of less than 2.0 (Alshammery and Khan, 2021; Wang and Chow, 2007).

2.1. Data and blood collection

Based on Alharbi et al., 5 ml of EDTA blood was collected and 3 ml was used for lipid profile analysis and 2 ml for separation of DNA (Alharbi et al., 2014). There are 4 types of lipid Profile parameters was obtained which was documented in Table 1. Both coagulant and anti-coagulant tubes were stored at 4 °C after collection of blood and again both serum and DNA were stored at –20 °C after the separation from their specific tubes. Lipid profile analysis was performed with Dimension vista1500 system available in the hospital premises. Based on the recommendations from the kits, lipid profile analysis was measured for obesity and control patients.

2.2. SORT1 genotyping assay

DNA was extracted from 2 ml of EDTA tubes and simultaneously stored at –20 °C (Khan et al., 2015). Genomic DNA was mea-

sured with NanoDrop spectrophotometry for both qualitative and quantitative analysis (Poornima et al., 2019). The genotyping analysis for rs464218 SNP was performed with PCR-RFLP assay as described (Alharbi et al., 2018). The experimental analysis for PCR was optimized for 50 µl using Qiagen-PCR master mix kit, ionized water, primers and DNA templates. Master mix incorporated 10X buffer, dNTPs, MgCl₂ and Taq DNA polymerase. All DNA templates were denatured at 95 °C as initial denaturation for 5 mins, denaturation at 95 °C at 30 s, annealing–60 °C at 30 s, extension–72 °C at 45 s and final extension–72 °C at 10 min (Subramanyam et al., 2016). The amplification was performed for 35 cycles and later on completion of PCR analysis, the unpurified PCR products was run on 2% of agarose gel and then using HhaI restriction enzyme, RFLP analysis was carried out for overnight to digest the restriction enzyme. The digested PCR products were run on 3% agarose gel. The 230 bp (A allele) was the PCR product and after digestion 178 bp (G allele) was formed.

2.3. Statistical analysis

Eight obesity cases and 50 controls were measured the t-tests between both groups. Hardy-Weinberg equilibrium (HWE) analysis was performed for rs464218 SNP assay. Genotyping analysis was performed between cases and control groups using OR and 95%CI with P values. One-way Anova analysis was performed with lipid profile and rs464218 SNP assay. P value was confirmed as <0.05 as a significant analysis (Khan et al., 2019). The statistical analysis was performed with SPSS software.

3. Results

3.1. Demographical features of obesity population

This study involves 140 subjects of obese and non-obese population. The anthropometric features and lipid profile parameters are presented in Table 1. The obesity group had a higher BMI levels when compared with controls ($p < 0.0001$) and it was vice versa in weight ($p < 0.0001$). The elevated levels of HDLC, LDLC and TG showed in obesity with positive association ($p < 0.05$). The mean age of obesity subjects was found to be 58.52 and 55.21 in control subjects. Age, gender, height and TC showed the negative association ($p > 0.05$).

3.2. Distribution of SORT1 genotyping assay

The distribution of rs464218 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 58%, 26% and 16% and 53.75%, 28.75% and 17.5% in obesity groups (Fig. 1). The allele frequencies in obesity group showed 68% of A allele and 32% of G allele, where as in control group, 71% of A allele and 29% 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-1.19 (0.52–2.72); $p = 0.67$, AA vs AG + GG; OR-1.18(0.58–2.42); $p = 0.63$ and G vs A; OR-0.87 (0.50–1.50), $p = 0.62$).

3.3. Analysis of Anova

Table 3 defines the one-way of Anova analysis in obesity groups. Obesity groups has been categorized into 3 groups based on BMI. The Class-I obesity group consists of 59 subjects, Class-II obesity group consists of 15 subjects and Class-III obesity group consists of 06 subjects. The Class-I obesity group subjects showed

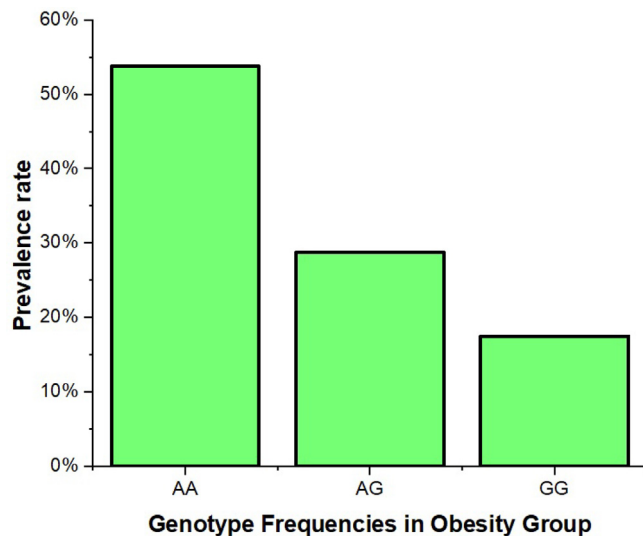
Table 1
Demographical characteristics features in obesity and control subjects.

	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%CI)	P Value
AA	43 (53.75%)	29 (58%)	Reference	Reference
AG	23 (28.75%)	13 (26%)	1.19 (0.52–2.72)	0.67
GG	14 (17.5%)	08 (16%)	1.18 (0.43–3.17)	0.74
AA vs AG + GG	37 (46.25%)	21 (42%)	1.18 (0.58–2.42)	0.63
A allele	109 (0.68)	71 (0.71)	Reference	Reference
G allele	51 (0.32)	29 (0.29)	0.87 (0.50–1.50)	0.62
HWE	0.32	0.29	–	–
X ²	11.42	13.58	–	–
P values	0.0007	0.0002	–	–

**Fig. 1.** Representation of prevalence frequencies of rs464218 SNP genotype assay in obese group.

the elevated levels of TG (4.21 ± 1.74) and LDLC (3.38 ± 0.97), whereas in first group of Class-II obesity group showed height (158.53 ± 6.41) and HDLC (2.90 ± 2.20) as elevated levels. The Class-III obesity group subjects showed Age (60.6 ± 9.54), weight (102.28 ± 6.60), BMI (43.35 ± 2.65) and TC (3.36 ± 1.94) as elevated levels among the three groups. The Anova analysis revealed the positive association in weight ($p = 0.0004$), BMI ($p = 0.0008$) and LDLC ($p = 0.001$) among the three groups.

4. Discussion

GWAS was found to be a powerful method for identifying the genetic loci most closely connected with obesity because of the huge cohorts of samples and the specific genes and genetic diversity it is associated with (Ke et al., 2021). There has been a recent flurry of new GWAS discoverers as a result of increased sample size and improved sequencing techniques (Loos and Yeo, 2021). Obesity was linked to 750 loci, according to the findings (Sun et al.,

2020). *SORT1* gene was discovered by GWAS and has been studied in numerous human disorders, including obesity (Alharbi et al., 2018). This study was designed to investigate the role of rs464218 SNP genotype assay in *SORT1* gene in the obesity subjects in the Saudi population. There were no studies between obesity and the rs464218 SNP in Saudi Arabia, although Alharbi et al reported a comparable SNP genotype in Stroke patients (Alharbi et al., 2018). This study confirmed the negative association with genotype, dominant model and allele frequencies ($p > 0.05$). Anthropometric measurements such as weight and BMI were strongly associated between cases versus controls ($p < 0.05$). Lipid profile parameter revealed the significant association between HDLC, LDLC and TG among the cases ($p < 0.05$). Anova analysis also revealed a strong association among obesity such as weight and BMI as well as LDLC ($p < 0.05$).

SORT1 gene was studied in obesity subjects with the limited population (Al-Eitan et al 2020). Apart from obesity, *SORT1* gene was studied in other human diseases (Alharbi et al., 2018; Luay et al., 2021; Møller et al., 2021; Zeng et al., 2013; Zhou et al., 2015). Till now there were no meta-analysis studies have been discovered between obesity and *SORT1* gene. Apolipoprotein B100 (ApoB100) binds to Sortilin, which is produced by *SORT1*. As a result, plasma LDL-C is regulated, as is the production and hepatic export of lipoproteins containing ApoB100 (Zhou et al., 2015). This indicates there is a relation between *SORT1* gene and lipid profile, specifically towards LDLC. In this study, LDLC levels were strongly associated with controls as well as within the obesity groups which was documented in Table-3 ($p < 0.05$). One of the recent studies has reconfirmed the relation between lipid profile parameter and *SORT1* gene as this gene is a confirmed as a potential biomarker for CVD (Møller et al., 2021).

Average height is used as a proxy for nutritional well-being, and the term 'biological standard of living' is used instead of 'standard of living' in this context. The latter is evaluated from a financial standpoint. Those who are taller than their peers are also viewed as having an edge. Obesity is more likely to strike children who are taller than average for their age (Rosario et al., 2018). Obesity and morbid obesity are likely to occur in the future as a result of the combination of shorter people with elevated BMI levels (Sahoo et al., 2015). Genotyping errors were ruled out with 10% of genotyping with Sanger sequencing analysis (Al-Otaiby et al.,

Table 3

Variations between three different modes of obesity using Anova analysis.

	Class-I Obesity (n = 59)	Class-II Obesity (n = 15)	Class-III Obesity (n = 06)	P value
Age (Years)	59.08 \pm 10.01	55.46 \pm 13.37	60.6 \pm 9.54	0.44
Weight (kgs)	79.02 \pm 6.93	93.35 \pm 8.12	102.28 \pm 6.60	0.0004
Height (Cms)	157.48 \pm 6.13	158.53 \pm 6.41	153.63 \pm 5.16	0.25
BMI (kg/m ²)	31.81 \pm 1.35	37.09 \pm 1.34	43.35 \pm 2.65	0.0008
TG (mmol/L)	4.21 \pm 1.74	3.31 \pm 1.47	3.90 \pm 1.85	0.19
TC (mmol/L)	2.55 \pm 1.78	2.47 \pm 2.05	3.36 \pm 1.94	0.56
HDLC (mmol/L)	1.63 \pm 1.80	2.90 \pm 2.20	1.39 \pm 0.60	0.051
LDLC (mmol/L)	3.38 \pm 0.97	2.31 \pm 1.25	3.04 \pm 0.52	0.001

2021; Bogari et al., 2021). DNA sequencing analysis is very accurate analysis to identify the specific SNPs but this technique is expensive. Genotyping errors may be caused due to the handling of pipettes, human errors, low quality of DNA and many more.

One of the strengths of this study was selection of cases and controls from a single tertiary hospital and low sample size of cases and control were one of the limitations of this study. This study concludes as rs464218 SNP genotype is not associated in obesity subjects. This is due to the low sample size and future studies recommend to perform the large sample size in the global population studies.

Conflict of interest

None.

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