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# The relationship between Fatty acid binding protein 2 gene and human obesity in the elderly population

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Global studies carried out as case-control and meta-analysis studies confirmed all forms of associations in *FABP2* gene within obesity. Saudi Arabia is recording as one of the high prevalence of obesity in the gulf region and this studies principle objective is to investigate the role of A54T genotype in FABP2 gene in obesity subject. This work was designed by involving 102 obesity confirmed cases and 102 controls. PCR analysis was carried out after extracting the genomic DNA from all the subjects and then RFLP analysis was performed. Anthropmetric measurements and lipid profile showed the higher frequency among obesity. T allele, TT genotype and dominant model showed the positive association (p=0.05). Anova analysis revealed the higher frequency of TT genotypes among WC, weight and BMI (p=0.05). This study confirms T allele and TT genotype including dominant model showed the positive association with A54T genotype in FABP2 gene in the obese subjects.

**Keywords: A54T genotype, FABP2 gene, obesity and Anova analysis**

## INTRODUCTION

Among human diseases, obesity is confirmed as one of the multiple metabolic disorders that represent a major health issue in the worldwide population (Alharbi et al. 2021). Obesity and overweight have been demonstrated to be factors that increases the risk of human diseases, including type 2 diabetes mellitus (T2DM), cardiovascular disease, and insulin resistance (Pramono et al. 2021). Obesity is caused by a number of interactions involving genetic, metabolic, environmental, economic, behavioral, and sociodemographic factors. According to WHO, the obesity prevalence has tripled between 1975 and 2018. Between 2016 and 2017, almost 650 million adults were proven to be obese, while 41 million children were either overweight or obese. One of the projections for the year 2025 is that one billion people will be obese, with 177 million of them being morbidly obese as per body mass index (BMI) calculation. Obesity prevalence among Arab adults has increased over three decades, from 6.5% to 20% from 1975 to 2016, and prevalence rates vary throughout 22 Arab countries, ranging from 7.8% in Comoros to 37.9% in Kuwait (Younes et al. 2021). The overall prevalence of obesity in Saudi Arabia is estimated to be 35% (Al-Nozha et al. 2005). BMI is a standard method for calculating a person's adiposity. Obesity is defined as having a BMI more than the 95$^{th}$percentile, and overweight is defined as having a BMI greater than the 85$^{th}$ percentile. Obesity has become a major health concern in Saudi Arabia, with 44% of females and 28% of males becoming obese at some time in their life (Alshammary et al. 2021). According to WHO data, the prevalence of obesity in adults is 13%, and the prevalence of obesity in children aged 5 to 19 years is 18% (Alharbi et al. 2020).

Obesity is influenced by genetic characteristics such as heredity, family history, and single nucleotide polymorphism (SNP). Fatty-acid binding protein 2 (FABP2) is a candidate gene implicated to type 2 diabetes (Alharbi et al. 2014), metabolic syndrome, obesity (Han and So, 2019), and other human diseases. FABP2 is a member of the superfamily of small intracellular lipid binding proteins. It has 3.4 kilobases and is found on chromosome 4q28-31. It has four exons with 700bp and three introns with 2650bp. FABP2 comprises 131 amino acids and a significant β-strand structural composition (Liu et al. 2015). Alanine-54-Threonine (Ala54Thr) is a SNP shows rs1799883 polymorphism situated at exon-2 and substitutes nucleotide from G-A. When compared to Ala protein, Thr protein has a two-fold greater affinity for long chain fatty acids. The A54T SNP may impair the protein's ability to transport dietary fatty acids, increasing serum saturated fatty acid levels (Alharbi et al. 2014). Previous studies have identified the A54T variant in *FABP2* as a potential gene in metabolic diseases involving obesity. There have been no studies that have screened A54T SNP in Saudi Arabia with obesity, hence the purpose of this study was to explore FABP2 gene at A54T SNP in obese patients in the Saudi population.

## MATERIALS AND METHODS

### 2.1. Study plan

This case-control study was carried out in a local community of 204 participants, who were classified into 102 obesity patients and 102 controls. In this study, an equal number of obesity cases and controls were chosen for each gender. The study participants with obesity cases were between the age range of 40-80 years of old. Both obesity cases and controls were collected from diverse regions within the Kingdom. The sample size for both Obesity cases and healthy controls was estimated using survey system criteria and an online tool, with 97 subjects in the obesity cases and controls groups participating (Alfaifi, 2021). Based on World Health Organization (WHO) criteria, 102 obesity cases were recruited. The inclusion criteria for obesity subjects were based on BMI, which should be >30kg/$m^2$, and participants who were found to be <30kg/$m^2$ or even overweight were excluded from this study. Healthy controls (n=102) were selected based on normal BMI levels (<30kg/$m^2$) without any history of any form of obesity and diabetes. The exclusion criteria were categorized as patients were obese and diagnosed with other metabolic disorders. Ethical grant was obtained for this study. Signed informed consent form participants were involved in this study. This study has followed the Declaration of Helsinki.

### 2.2. Measurements of participants and blood sampling

For obesity cases and controls, age is recorded in years, gender is documented as male or female, height is measured in centimeters (cms), and weight is recorded in kilograms (kg). BMI was determined using weight and height using the formula BMI=kg/$m^2$ (Alharbi et al. 2020). Normal, overweight, and obese levels were classified based on BMI values. In this study, 5ml of venous blood was collected for biochemical and molecular analysis in an anticoagulant EDTA vacutainer and also in coagulant tubes. Serum was separated for biochemical processes, and human DNA was isolated using EDTA blood (Alharbi et al. 2014).

### 2.3. Biochemical Assays

Serum was extracted from the coagulant tubes and used for biochemical analysis of lipid profiles for total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDLC), and low-density lipoprotein cholesterol (LDLC). Biochemical analysis was performed with an automated assay (Alharbi et al. 2017).

### 2.4. Genotype determination

Two hundred and four EDTA blood samples were used to extract human DNA using a kit-based procedure that followed the manufacturer's recommended protocol (Qiagen, USA). The isolated DNA was diluted in 200µl of TE buffer before being placed onto a 1% agarose gel. The samples were afterwards maintained at -20°C until the polymerase chain reaction analysis process was completed. The PCR process is initiated with a genotyping examination of the rs1799883 SNP in the *FABP2* gene. The target SNP locus for rs1799883 was amplified in a single system utilizing a PCR machines thermal cycler. The PCR process used 50µl of reaction, which included 20µl of PCR master mix, 1µl of diluted forward/reverse primers, 23µl of purified water, and 5µl of genomic DNA. Both forward 5'-CTACCGAGTTTTCTTCCCACC-3' and reverse primer 3'-AATTAAACCATCCAATGAAATAGAGC-5' sequences were adapted from the previous studies (Han and So, 2019). The PCR program for the A54T SNP was as follows: initial denaturation at 95°C for 10 minutes, 40 cycles of total reaction with the remaining processes as denaturation at 94°C for 1 minute, 56°C for 1 minute as annealing temperature, 72°C for 1 minute as extension, and 10 minutes to complete the final extension at 72°C. Following the end of the genotyping process, a 376bp PCR product was obtained, and a 2% agarose gel was utilized to check the PCR product using a 100bp DNA ladder. The amplified PCR products were digested using the HhaI restriction enzyme. The restriction digestion analysis lasted 18 hours at 37°Celsius, and the digested products were 200 and 175 bp for AA genotypes, 375 bp for the GG genotypes, and 375, 200, and 175 bp for the heterozygous genotypes. The digested PCR products were run on a 2% agarose gel stained with ethidium bromide once again. Validation was confirmed with Sanger sequencing analysis (Al-Otaiby et al. 2021).

### 2.5. Statistical analysis

SPSS software was used for the statistical analysis ($25^{th}$ version, USA). All clinical and genotype data were gathered in Excel and then converted to SPSS files for the convenience of performing the student t-test, Hardy Weinberg Equilibrium (HWE), and genotyping analysis for the rs1799883 SNP between obesity cases and controls. Furthermore, in obesity cases, multiple logistic regression analysis was performed using BMI as the reference. Additionally, Anova analysis was also performed with 3 genotypes of *FABP2* gene analysis. Continuous variables are represented by the mean and standard deviation, while categorical variables are represented by percentages. When comparing two groups, a p value of <0.05 indicates a significant correlation (Khan et al. 2019).

## RESULTS

### 3.1. Clinical features in obese and non-obese subjects

In this study, 102 obese individuals and 102 healthy adults participated. Obesity and healthy controls were recruited based on BMI. Table 1 shows the clinical characteristics of obese cases and healthy controls. When compared to controls, the obese group was elder and showed a significant association with t-tests ($p<0.0001$). Genders were found to be similar in both groups. When

compared to controls, anthropometric parameters such as weight, BMI, and waist circumference showed elevated levels in obesity cases and a significant association ($p<0.05$). Furthermore, lipid profile measurements revealed that the obese group had high levels of HDL-C, LDL-C, TG, and TC ($p<0.05$). Height was found to be nearly identical in both groups ($p=0.22$).

**Table 1: Clinical and demographical characteristics between control subjects and diabetic cases**

| | Controls (n=102) | Cases (n=102) | P Value |
|---|---|---|---|
| **Age (Years)** | 50.1±7.63 | 58.1±10.68 | <0.0001 |
| **Gender (F:M)** | 51:51 | 51:51 | 1.00 |
| **Height (cms)** | 157.6±6.96 | 157.4±5.88 | 0.22 |
| **Weight (kg)** | 70.1±10.63 | 84.2±10.98 | <0.0001 |
| **BMI (kg/m$^2$)** | 28.2±3.13 | 33.8±3.62 | <0.0001 |
| **Waist Circumference** | 81.2 ± 7.81 | 92.4 ± 9.51 | <0.0001 |
| **HDL-C (mmol/L)** | 1.1±0.34 | 1.5±1.68 | 0.01 |
| **LDL-C (mmol/L)** | 2.1±0.81 | 3.3±0.98 | <0.0001 |
| **TG (mmol/L)** | 1.2±0.57 | 3.1 ±1.97 | <0.0001 |
| **TC (mmol/L)** | 4.7±0.89 | 5.4±1.9 | 0.009 |

### 3.2. A54T analysis

Genotype of the control group was found to be consistent with HWE analysis in the *FABP2* gene ($p=0.006$). In this study, Table 2 documents the genotype and allele frequencies of A54T SNP and HWE analysis.

**Table 2: Genotype frequencies for controls subjects and diabetic cases**

| Genotype | Controls (n=102) | Obesity Cases (n=102) |
|---|---|---|
| **AA** | 74 (72.6%) | 39 (38.3%) |
| **AT** | 21 (20.6%) | 43 (42.1%) |
| **TT** | 07 (6.8%) | 20 (19.6%) |
| **A allele** | 169 (0.82) | 121 (0.59) |
| **T allele** | 35 (0.18) | 83 (0.41) |
| **HWE** | 0.17 | 0.41 |
| **X$^2$** | 7.47 | 1.62 |
| **P values** | 0.006 | 0.20 |

The AA, AT and TT genotype levels in obesity cases were found to be 38.3%, 42.1% and 19.6%, whereas in controls 72.6%, 20.6% and 6.8% genotype levels were confirmed. The T allele was found to be high in obesity cases and Allele was found to be high in control subjects. Table 3 shows the confirmed genotype and allele frequency results, which reveal a substantial association between AT and TT genotypes (AT vs AA; OR-3.88; 95%CI: 2.02-7.44; $p=0.0002$, TT vs AA; OR-5.42; 95%CI: 2.11-13.93; $p=0.0001$), dominant mode (AA vs AT+TT; OR-3.59; 95%CI: 1.97-6.54; $p=0.0001$), and allele frequency distribution (T vs A; OR-3.31; 95%CI: 2.09-5.24; $p<0.0001$) of the *FABP2* gene.

**Table 3: Statistical association between controls and diabetic cases**

| | P value | 95%CI | ORs |
|---|---|---|---|
| 1/2 vs 1/1 | 0.0002 | 2.02-7.44 | 3.88 |
| 2/2 vs 1/1 | 0.0001 | 2.11-13.93 | 5.42 |
| 1/1 vs 1/2+ 2/2 | 0.0001 | 1.97-6.54 | 3.59 |
| 1/1 + 2/2 vs 1/2 | 0.0004 | 0.19-0.66 | 0.35 |
| 1/1 + 1/2 vs 2/2 | 0.003 | 0.12-0.75 | 0.32 |
| 2 vs 1 | <0.0001 | 2.09-5.24 | 3.31 |

### 3.3. ANOVA Analysis

In this study, one-way ANOVA was used to compare A54T genotypes in the *FABP2* gene among obese people. With AA, AT, and TT genotypes, age, weight, height, BMI, and waist circumferences were measured. The AA genotype had the highest maximum age of 59.1 ±9.78, while the TT genotype had the highest maximum weight, height, BMI, and waist circumference. The Anova analysis results confirmed the positive relationship between weight and BMI ($p=0.0001$). Age, height and waist circumference was found not to be associated ($p<0.05$) with Anova analysis.

**Table 4: One-way Anova analysis for A54T genotypes in obesity cases**

| ANTHROPOMETRIC | AA (38.3%) | AT (42.1%) | TT (19.6%) | P values |
|---|---|---|---|---|
| Age [Years] | 59.1 ±9.78 | 57.1 ±11.46 | 58.4 ±10.92 | 0.61 |
| Weight [Kilograms] | 77.8 ±5.69 | 84.1 ±10.02 | 95.6 ±11.13 | 0.0001 |
| Height [Centimeters] | 157.4 ±5.34 | 157.3 ±6.13 | 157.5 ±6.53 | 0.54 |
| BMI [kg/m$^2$] | 31.3 ±1.32 | 33.5 ±3.08 | 38.4 ±3.11 | 0.0001 |
| Waist Circumference [cms] | 91.5 ±9.57 | 92.8 ±9.51 | 93.3 ±9.71 | 0.74 |

## DISCUSSION

Genetic factors play an important role in the development and progression of obesity disease by passing down inherited genes from parents to offspring and from one generation to the next. Obesity can be caused by genes, and the disease can be further subdivided into morbid obesity, Bardet-Biedl, and Praderwilli syndromes. Approximately 118 candidate genes were caused by obesity, which was further classified as Mendelian and polygenic obesity. Multiple gene configurations can range from polygenic to oligogenic. Limited genes with substantial quantifiable effects are frequently expressed in the presence of a residual polygenic background is defined as oligogenic (Martínez-Hernández et al. 2007). In this study, obesity subjects were analyzed using FABP2 A54T with the risk of obesity in the Saudi assorted population, and the results revealed substantial differences in genotypes (AT vs AA; OR-3.88; 95%CI: 2.02-7.44; p=0.0002, TT vs AA; OR-5.42; 95%CI: 2.11-13.93; p=0.0001) and allele frequencies between obesity cases and controls. Furthermore, the AT+TT genotype of dominant model (AA vs AT+TT; OR-3.59; 95%CI: 1.97-6.54; p=0.0001), was associated with an increased risk of obesity. Both co-dominant (OR-0.35; 95%CI: 0.19-0.66; p=0.0001) and recessive genotype models (OR-0.32; 95%CI: 0.12-0.75; p=0.003) reveal no risk of developing obesity in the general population of Saudi Arabia. Additionally, Anova analysis revealed positive association with weight and BMI in AA, AT and TT genotypes in FABP2 gene ($p<0.05$). The clinical characteristics revealed When compared to controls, age, weight, BMI, circumference, and lipid profile were all strongly associated with obesity ($p<0.05$).

The Island country of Nauru has the highest prevalence of obesity in the adult population, with 61%, while Vietnam has the lowest incidence, with 2.1% of obesity cases. According to WHO, 1.9 billion people worldwide are overweight, with 650 million obese. The prevalence of obesity has more than tripled since 1975. Based on Procon.org, the prevalence of obesity in Saudi Arabia ranks at 14$^{th}$ position with 35.4%. One of the important risk factors for human beings are increase in weight, which leads to T2DM, HTN, cardiac and bone diseases and many more (Organization, 2020). According to statistics from the Global Health Observatory (GHO), the prevalence of obesity was 31.7% in Saudi Arabia, 34.2% in Kuwait, 28.4% in the UAE, 23.7% in Oman, 26.4% in Bahrain, and 33.4% in Qatar.

The FABP2 gene encodes a 15kDa protein that is released by epithelial cells and binds to fatty acids. FABP2 has been connected to fasting insulin concentrations and human insulin levels (Takakura et al. 2005). FABP2 is made up of 3382 nucleotides, 700 exons, and 2650 introns, and it encodes a small-bowel fatty acid that belongs to a protein family that affects lipid transport and metabolism. The rs1799883 SNP is a missense mutation that appears in the exon-2 region and converts adenine to guanine at 54 codon locations. Furthermore, this will have an impact on the transportation of dietary fatty-acid levels as well as enhanced serum fatty-acid levels, resulting in endothelial dysfunction (Alharbi et al. 2014, Hasnain, 2015). Obesity was shown to be associated with A54T genotypes in FABP2 in the current study, which were in agreement with the prior studies (Albala et al. 2004, Berthier et al. 2001, Fisher et al. 2006, Han and So, 2019, Liu et al. 2015, Martínez-Hernández et al. 2007, Takakura et al. 2005), and that some of the studies revealed a detrimental effect (Tavridou et al. 2009, Torres et al. 2020). In addition, a few meta-analysis studies were conducted, which revealed both positive (Hasnain, 2015) and negative associations (Liu et al. 2015). The similar genotype studies were conducted in T2DM disease in the Saudi population and documented with negative association (Alharbi et al. 2014). The lipid profile was associated with Cases and controls in this study, and earlier studies were documented with the current studies (Leońska-Duniec et al. 2021, Nakanishi et al. 2004). This study has documented limited strengths and limitations as one of the limitations was performed with single SNP without documenting the physical activity and diet. Incorporating the Saudi community subjects with obese and morbid obese was one of the strengths of this study.

## CONCLUSION

This study confirms T allele and TT genotype individually and in combination with heterozygous genotype showed the significant association with A54T genotype in FABP2 gene in the Saudi population with obese subjects. Moreover, TT genotype was significantly associated with weight and BMI in obese subjects. Future studies are recommended to explore the influence of modifications in anthropometric measurements and lipid profile parameters with the risk of cardiac diseases in the obese patients.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

## AUTHOR CONTRIBUTIONS

NA designed, performleed the experiments, data analysis and also wrote the manuscript.

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