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ASSOCIATION BETWEEN GIP GENE POLYMORPHISM AND OBESE ADULTS IN BABYLON GOVERNORATE, IRAQ

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ABSTRACT

The aim of this study was to describe the association between GIP gene polymorphism with obese adults in Babil, Iraq who do not suffer from chronic diseases. This study was performed on (50) Samples, for age ranged (19-40 year). The results of the sequences showed there was only one mutations in nucleotide position 110 stretches for comparisons of SSCP variants of *Homo sapiens* in exon 3 in GIP gene, there is no association between AB and BB polymorphisms in genotypes obese with control group, and also no association between A and B allele in obese with control group. Finally absence any significant differences in the mean of CW, TG, Insulin and FBG levels between AB and BB polymorphisms in GIP genotypes of obese.

KEYWORDS: Obesity, BMI, GIP, polymorphism, Sequences.

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INTRODUCTION

Obesity is a complex, multifactorial, chronic disease involving environmental (social and cultural), genetic, physiologic, metabolic, behavioral and psychological components, which characterized by an increase in body weight that results in excessive fat accumulation, that it may cause a negative effect on health. [1] In adults, overweight and obesity have been defined or classified through a number of different systems, including the National Institutes of Health (NIH) and the national heart, lung and blood institutes (NHLBI) that are based on a body mass index (BMI) classification system, which is the measured by ratio of weight in kilograms divided by the height in meters squared. [2] Where overweight is defined as a BMI between 25.0 kg/m2 and 29.9 kg/m2 and at risk of becoming obese, while obesity is defined as a BMI higher than 30.0 kg/m2, whereas those with BMI of 18.5 kg/m2 to 24.9 kg/m2 are considered at low risk for morbidity. The waist circumference (concentration of fat in the abdominal region) it is measured in overweight and obese adults in order to assess the abdominal obesity also to measuring BMI where waist circumference (WC) ≥40 in 102 cm for men and ≥35 in 88 cm for women are considered elevated indicates increased risk of metabolic diseases and the disorder of energy balance.[3] Glucose-dependent insulintropic polypeptide (GIP) also known as gastric inhibitory polypeptide is composed of 42-amino-acid hormone secreted from K cells of the upper gastrointestinal tract, mainly in the duodenum and jejunum.[4] GIP has numerous effects such as regulation of adipocyte differentiation, increase in adipocyte glucose uptake, modulation of adipocyte lipolysis, and re-esterification and promotion of the storage of triglycerides by increasing the activity of lipoprotein lipase and considered a key molecule linking over nutrition to obesity.[5] GIP encodes one of the two in cretin hormones (glucagon-like peptide-1(GLP-1) and GIP) in humans and plays a critical role in normal carbohydrate and lipid metabolism, where after ingestion of nutrients, GIP secreted from duodenal and jejuna K cells acts on pancreatic β cells to stimulate the release of insulin, which thereby ensures the prompt uptake of glucose and lipids into the tissues.[6] In addition to effects on glucose homeostasis, GIP has been shown to promote obesity in mice that were fed a high-fat diet, where in humans, genome wide association studies have identified single nucleotide polymorphisms (SNPs) in the GIPR gene that are strongly associated with BMI, and it was as found a genetic association between GIP and visceral fat accumulation in Japanese adults, and a common SNP located near GIP, rs9904288, was associated significantly with visceral fat area (VFA) and in linkage disequilibrium with an single nucleotide polymorphisms (SNPs) proximal to an enhancer element active in the duodenum mucosa, where it is possible that GIP contributes to obesity by acting on visceral adipose tissue, despite the fact that, under conditions of insulin resistance, GIP signaling in subcutaneous adipose tissue is blunted.[7]

MATERIALS AND METHODS

The study was performed in AL-Qasim green university / College of Agriculture/ Laboratory bio-technology with department of nutrition at the Murjan teaching hospital / in babil province/Iraq for the period from June 2015 to January 2016 (The approval of the institutional research ethics committee and signed written consent of every patient included in the study was obtained. Ethical committee Iraq: Ethics Committee (University of Babylon/ College of Science), Reference number of approval: 215) where the excluded patients with diabetes, hypertension, liver disease, disorder of some hormones,(thyroid, testosterone, LH and FSH), heart failure, renal failure, malignant disease, smoking and alcohol intake, but only suffering from obesity and all obese and control were from the same ethnic group (Arabic) for age ranged (19-40 year). Where sampling was done by asking different questions about age, sex, individual's life style, diet, routine work and family background. Blood samples were collected from 28 obese and 22 control adult individuals from different areas of province of Babylon, and all the obese adults had the BMI above 30 kg/m2 and control adults had the BMI less than 25 kg/m2. DNA extraction was done by kit (FAVORGEN), then applied agarose gel electrophoresis to identified DNA, as show in (Fig 1). after that the polymerase chain reaction (PCR) applied on designing primer (GIP gene) using computer based software Programs, Primer3 plus by online reference through address http://www.ncbi.com, where type of primers used in our work to amplify exon 3 of GIP gene of Homo sapiens is a designed one, as show (Table 1). and because there is no previous study designed such sequences, thus a gradient PCR must be performed to identify the optimum temperature of annealing these primers to the template. Accordingly, gradient PCR experiments were used, the first (49.9 – 62) °C was succeed in picking up the proper annealing temperature and each PCR amplicon before its being subsequently applied for SSCP its purity should be confirmed ,as show in (Fig 2). Only one clean and sharp bands used for the subsequent SSCP and this notion relied on since the non-obvious PCR bands for reduce the sensitivity of SSCP.

Table 1
Forward and reverse primers were purchase from Bioneer (Korea)

Primer gen name	Sequences	Temp.	location	Product size
GIP	F:5'GCCTGTTGGATCTCATGCAT -3'	53.4°C	Exon 3	155 bp
	R:5'CATTCTTCTTCCCCTTTTGG C-3'			

Figure 1

The electrophoresis pattern of DNA extracted from sample study with electrophoresis conditions, 1% agarose, 75 V, 20 Am for 1h. (10 µl in each well)

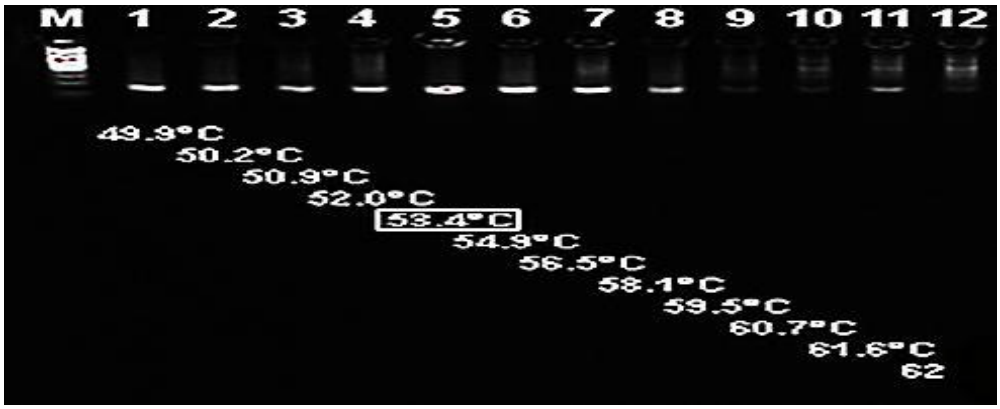

Figure 2

Agarose gel electrophoresis of Peptide GIP gene, exon 3 gradient PCR fragments. Gradient PCR (49.9°C – 62°C) applied on GIP, exon 3 to identify the optimum primer annealing temperature. M; refers to DNA size marker lane 1 into lane 12 refers to the variable gradient annealing temperature applied. Electrophoresis conditions: agarose concentration 1.5%, power applied: 135V (7V / cm), time of run: 45 min. staining method; precast ethidium bromide

Only one clean and sharp bands used for the subsequent SSCP and this notion relied on since the non-obvious PCR bands for reduce the sensitivity of SSCP. However, only 50 PCR amplicons were included in the subsequent SSCP and accordingly, these amplicons are so eligible for downstream SSCP experiments. Where, the amount of each amplicon is determined empirically to suits the amounts of other amplicons that applied for SSCP. Then agarose gel electrophoresis for PCR, agarose gel was visualized in a UV transilluminator and the photos was captured using canon digital camera, as show in (Fig 3). It was reported that it's preferable to run SSCP products on mingles [8]. This method depends heavily on experimental conditions that optimize migration differences of the conformation polymorphs, it was tried to resolve our PCR products on mingles and it was found that the optimum SSCP gel concentration to resolve these bands is 8%. [9] Very simple and rapid silver staining method was relied on this staining method has proved superior sensitivity over other methods considering staining of ssDNA bands, were screened by SSCP analysis of DNA has been used for detection of genetic mutations in GIP gene because it might be difficult to determine the pattern of all resolved SSCP bands using only the gel visualization, then the silver stained and colored were converted into black and white images to get better resolutions. Accordingly these two haplotypes confirmed using sequencing [10].

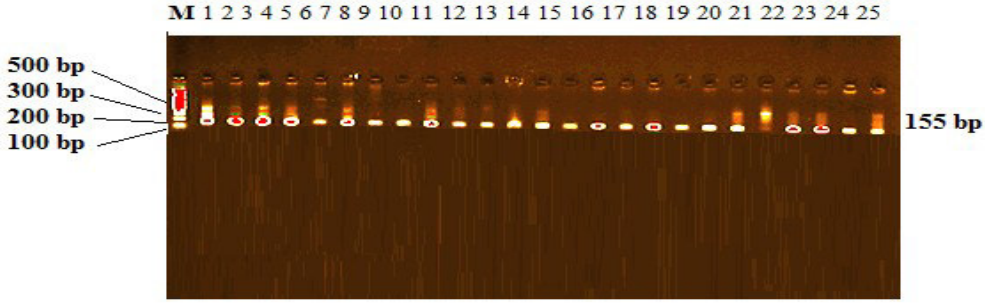

Figure 3

Agarose gel electrophoresis of GIP gene, exon 3PCR fragments. M; refers to DNA size marker lane 1 into lane 25 refers to GIP gene, exon 3 PCR fragments patterns. Electrophoresis conditions: agarose concentration 1.5%, power applied: 135V (7V / cm), time of run: 45 min. staining method; precast ethidium bromide

The waist circumference was measured while the subject standing up , at the narrowest point of the torso widthwise, usually just above the belly button ,which is ≤ 102 cm in male and≤88 cm in female .[11] The Insulin fasting hormones measuring after 12 hours of fasting according to the principle enzyme linked immune sorbent assay is the complete kit from Elascience-China Company for the quantitative determination of insulin serum. where the level of glucose were measured in fasting serum also after 12 hours of fasting according to the principle of enzymatic oxidation presence of an enzyme Glucose _Oxidase (GOD) (Kit) from Audit. Company. Measuring the level of triglycerides (TG) in fasting serum after 12 hours of fasting according to the principle of enzymatic hydrolysis from company BIOLABO and the Rondo Company

RESULTS

The results show the observation of two different haplotypes, where the first AB genotype, in which ssDNA constitute two bands, the second BB genotype constitutes only one ssDNA bands, as it is shown in (Fig 4) and (Fig 5).

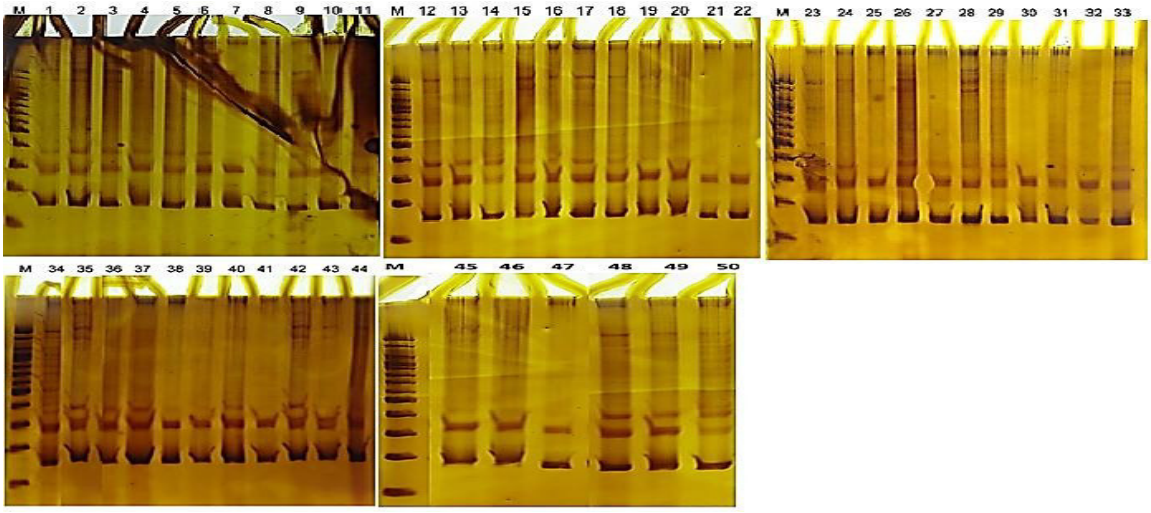

Figure 4

SSCP non-denaturing polyacrylamide gel electrophoresis of Peptide GIP gene, exon 3 PCR fragments. M; refers to GIP gene, exon 3 PCR-SSCP fragments patterns. Electrophoresis conditions: polyacrylamide gel concentration 8%, power applied: 200V (7.5V/cm) – 100mA, time of run: 90 - 120 min. staining method; silver staining

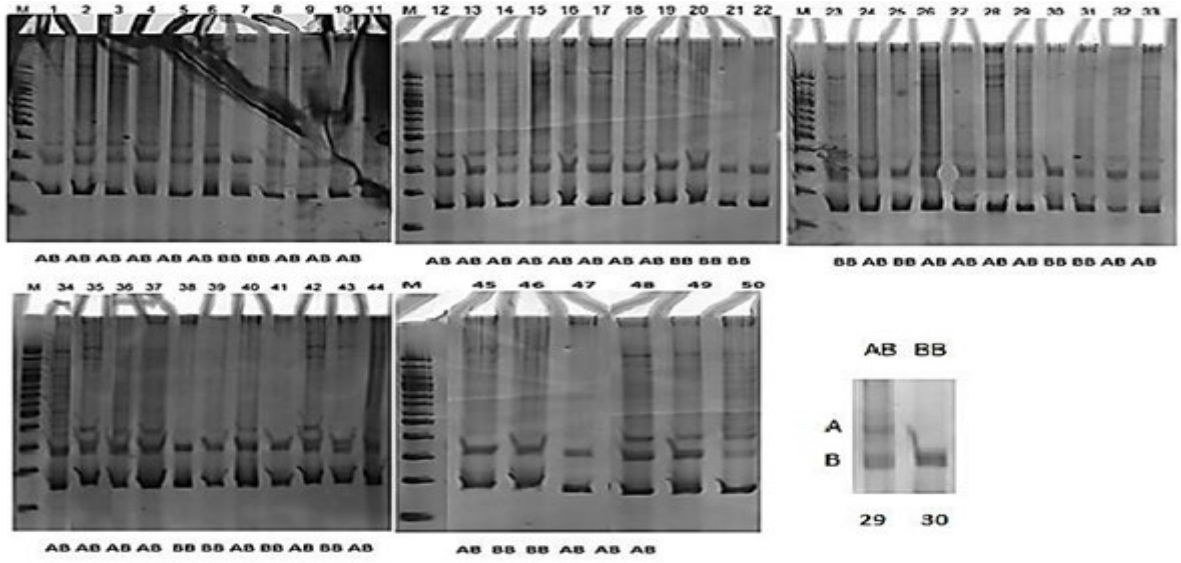

Figure 5

PCR-SSCP Patterns that electrophoresed on non-denaturing polyacrylamide gel electrophoresis for GIP gene, exon 3 PCR fragments. Selected lanes 1 into lane 44 refer to GIP gene, exon 3 PCR-SSCP fragments two observed genotypes (genotype A, B). Lanes 29, 30 refer to the typical differences between genotype A, B, respectively.

While the result discovered by sequencing was listed in (Table 2). there was only one mutations when comparisons of SSCP variants in exon 3 of Homo sapiens GIP for the references sequence and genotype AB with BB were discovered this mutation at BB genotype in nucleotide position (110), but it is not translated to protein resulting from a gene variant thus probably that was relation with obesity and may not, because this gene is discovered the first time between people in Babil Province and the first on the level of people in Iraq, as show in (Fig 6).

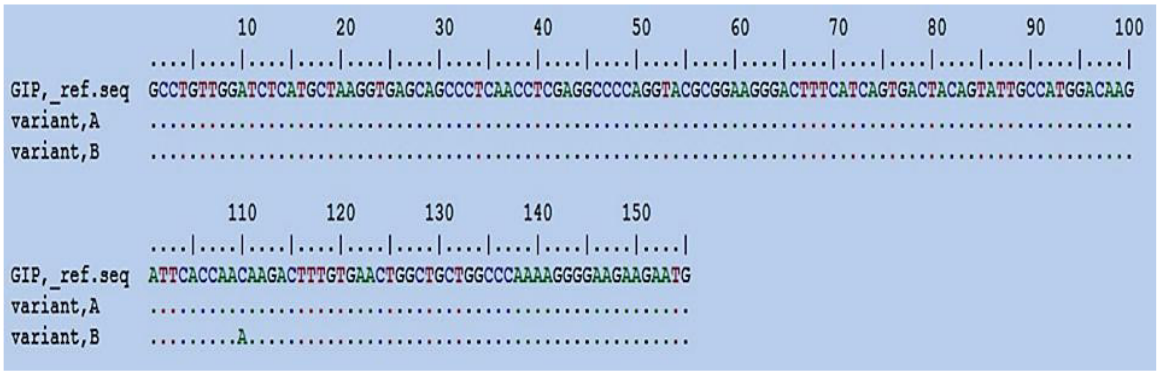

Figure 6

Sequences alignment results for Homo sapiens GIP Gene fragment, one SSCP variants with their reference sequence (Bank accession number M31676.1) using DNA STAR, Editseq software. By applying ClustalW alignment in the same software, only one point mutations (SNPs) are discovered between the GIP references sequence and the two genotypes AB, BB.

Table 2

The differences of nucleic acids patterns between Genotype AB and Genotype BB, in exon 3 of Homo sapiens GIP Gene

Nucleotide position	Comparison s of SSCP Variants in exon 3 of Homo sapiens GIP	
	Variant AB	Variant BB
110	.....	A

In this study show there is the distribution of the genotype AB and BB of GIP gene polymorphism were 75.0%, 25.0% respectively in the patient with obese group, and 63.6%, 36.4% respectively in the control group. While the allele frequency of the allele A and B of GIP gene polymorphism where 37.55, 62.5% respectively in the patient with obese group and 31.82%, 68.18% respectively in the control group, as it is shown in (Table 3).

Table 3

The distribution genotype of GIP gene polymorphism with Allele frequency between the two groups (obese vs control)

Genotype	Obese group N (%)	Control group N (%)	Allele frequency	
AB	21 (75.0)	14 (63.6)	**Obese (%)**	**Control (%)**
BB	7 (25.0)	8 (36.4)	**A** (37.5)	**A** (31.82)
			B (62.5)	**B** (68.18)
Total	28	22		

Where this study show there is no association (OR = 0.58 [0.17-1.97], (P = 0.39) between AB and BB polymorphisms in obese with control group, and there is also no association (OR = 0.78 [0.34-1.79], (P = 0.55) between A and B allele in obese with control group, as it is shown in (Table 4). while Table 5 refers into the absence any significant differences (P> 0.05) in the mean of CW (cm), TG (mmol/L) , Insulin and FBG (mmol/L) levels between AB and BB polymorphisms in GIP genotypes of obese patients.

Table 4

The association of genotype and allele frequencies between the two groups (obese vs control)

GIP Polymorphism	obese group N (%)	Control group N (%)	χ2	P-value	OR	95% CI
Genotype						
AB	21 (75.0)	14 (63.6)				
BB	7 (25.0%)	8 (36.4)	0.74	0.39	0.58	0.17-1.97
Allele						
A	21 (37.5)	14 (31.82)	0.35	0.55	0.78	0.34-1.79
B	35 (62.5)	30 (68.18)				

Table 5
GIP genotypes of obese patients in association with biochemical parameters

GIP Polymorphism	Mean ± SE			
	WC	Insulin	Sugar	TG
AB	106.90±3.05	25.56±2.46	4.90±0.17	1.12±0.08
BB	106.43±6.46	24.49±3.93	4.70±0.10	1.11±0.07
P-value	0.73 NS	0.75 NS	0.09 NS	0.06
* (P<0.05), NS: Non-significant.				

DISCUSSIONS

Where the observed high population differentiation in GIP polymorphisms could be associated with the adaptations of GIP physiology to changes in diets and cultures in select human populations , Such that the mutation can be observed based on represents a functional mutation because the GIP effect induced by the glucose levels that represents a critical endocrine circuit to monitor exogenous energy intake and regulate subsequent storage .[12] Where previous reports about GIP receptor knockout mice resistant to obesity suggest that inhibition of GIP signaling might be a novel target for anti-obesity drug development , where founded there was no statistically significant association was observed between any of the of the total SNPs analyzed and type 2 diabetes in our population, through two SNPs namely rs2291726 and rs937301 in south Indian population .[13]The animal studies have demonstrated that glucose-dependent insulin tropic polypeptide (GIP) and GIP receptor (GIPR) contribute to the etiology of obesity and in humans, genome wide association studies have identified single nucleotide polymorphisms (SNPs) in the GIPR gene that are strongly associated with BMI .[7] In addition a genetic association exists between GIP and visceral fat accumulation in Japanese adults, where a common SNP located near GIP, rs9904288, was associated significantly with visceral fat area (VFA) and in linkage disequilibrium with a single nucleotide polymorphisms (SNPs) proximal to an enhancer element active in the duodenum mucosa. The Genome wide association studies (GWAS) discovered GIPR variants (rs2287019 and rs55669001) on obesity traits, such as VFA, WC, and BMI. These results provided new evidence supporting the roles of GIP and GIPR in the development of obesity in humans and it is possible that GIP contributes to obesity by acting on visceral adipose tissue, despite the fact that, under conditions of insulin resistance, thus GIP signaling in subcutaneous adipose tissue is blunted .[14] Although recent advances in GWASs have identified multiple genes, including GIPR, that are associated with human obesity .[15] the genetic architecture underlying depot-specific adiposity remains unclear. Limited numbers of genetic variants have been reported for the specific control of visceral adipose tissue (VAT) area .[16] Expression levels of GIPR are higher in VAT than in subcutaneous adipose tissue, suggesting that genetic variants of GIP may affect visceral fat accumulation rather than whole-body adiposity .[14] thus the genetic variants of the GIP/GIPR axis exhibit stronger associations with VFA and WC, and obesity traits are influenced by expansion of visceral adipose tissues. So we though probably there is no relationship between GIP gene in the levels of genotype or allele in the obese group may be because the sample size to detect an association with Obesity was low, furthermore, the obese group they do not suffer from any health problems from chronic diseases such as problems in the liver, kidney, thyroid ,sex hormones, but the cause of obesity overeating and lack the physical activity ,while the obesity rate of Class III (excessive obesity) was in the number is small thus may be the reasons the absence any significant differences in the mean of CW (cm), TG (mmol/L), Insulin and FBG (mmol/L) levels between AB and BB polymorphisms in GIP . So we need future studies of potential genotype–phenotype relationships for the selected GIP variants could provide a better understanding of which and how these variants contribute to phenotypic variation in energy-balance regulation among individuals or human populations, we suggest a detailed genetic analysis of SNPs in GIP in other Arabian populations to obesity treatment, at the levels of physiology and genetically.

CONCLUSION

Thus there was one mutation founded in GIP genotypes of obese patients and there was no association between AB and BB polymorphisms of GIP gene with obese adults in Babylon, Iraq, and there was no association between A and B allele with obese, with absence any significant differences in the mean of CW, TG, Insulin and FBG levels between AB and BB polymorphisms in GIP genotypes of obese patients. We suggesting future studies should examine for relationship between genetic variants of GIP genotype with visceral fat accumulation that causes disease rather than whole-body adiposity in healthy obesity.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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