Scientific Research & Studies Center-Faculty of Science- Zagazig University- Egypt

Biochemistry Letters

Journal home page:

Role of gene polymorphisms in obesity incidence in Egypt

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ARTICLE INFO

Obesity
Wnt10a
β-catenin
Egyptian population

ABSTRACT

Background: As Egypt's current policy is heading towards a comprehensive renaissance. Therefore, determining the risk factors of obesity, which represents the key factor for most chronic diseases and disability, is the right way to promote the health, social and economic aspects of Egyptian citizens. Wnts are secreted glycoproteins and comprise a large family of nineteen proteins in humans hinting to a daunting complexity of signaling regulation, function and biological output. Recent studies suggest that WNT10a may play a role in the negative regulation of adipocyte differentiation. ***Objectives:*** The goal of our study is to investigate the association between Wnt10A gene polymorphisms and obesity risk in Egyptian population. In addition, we aimed to determine the participation of Wnt/β-catenin signaling pathway in adipogenesis by determining the serum β-catenin level. It is worth noting, that it is the first study to discuss Wnt10A gene polymorphisms and serum β-catenin level in Egyptian population. ***Methods:*** our study included 48 obese and 48 non-obese of Egyptian volunteers. All of them are unrelated persons without any chronic disease. All the subjects underwent the same investigations, including CBC, serum glucose level , lipid profile, serum β-catenin level, and genotyping for Wnt10A gene polymorphisms (rs121908119- rs141074983) using RFLP-PCR method.

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Results: our results showed that (rs141074983) SNP is associated with obesity risk. There was high significant difference between obese and non-obese volunteers under codominant, dominant, heterozygote, and allelic models (P<0.05). The more interesting result was the association between obesity risk and minor allele carrier (T allele carrier; CT and TT) (OR=6.8 (1.3-47.6), p=0.007). Regarding (rs121908119) SNP, there was a relatively difference in genotypes distribution between obese and non-obese volunteers but with no statistical power (p>0.05). Similarly, the decrease in serum β-catenin level in obese than non-obese volunteers lacked the required statistical confidence (p>0.05). ***Conclusion:*** The Wnt10a SNP (rs141074983) is strongly associated with increased risk for obesity in Egyptian population and larger studies can confirm our results.

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Introduction

Obesity is a remarkable risk to health in Egypt. High percentage of Egyptians are obese. This represents a high risk for sevral diseases such as heart disease and diabetes. More than 17 million Egyptians, or 33 percent of the adult population, are obese which is the highest percentage across the world [1]. The regulation of pre-adipocytes differentiation is done by a different factors that either mediate or stop this process [2]. Wingless-type MMTV integration site family members (WNTs) are a group of growth factors which are glycoproteins rich in cysteine and they control several cellular processes, including differentiation [3]. The name is driven from the name of the first 2 members of the family to be identified: int-1 (mouse) and wingless (Drosophila) [4]. The Wnt proteins function is signal transduction through canonical (Wnt/β-catenin) dependent pathway and the non-canonical (β-catenin-independent) pathway which can be classified into (PCP) the Planar Cell Polarity, and the Wnt/Ca2+ pathways [5]. The Wnt-1 gene was characterized in 1982 as a proto-oncogene activated by the mouse mammary tumor virus (MMTV) integration in mammary solid tumors. By the discovering of Drosophila wingless, it became known that Wnt genes are important factors for many developmental process. The embryonic mouse Wnt-1 gene mutations cause the loss of the cerebellum and midbrain. More-ever a number of processes including limb development and segment polarity are observed in Drosophila wingless mutants [6]. Wnt gene family members have similarity in their sequence to that of mouse Wnt-1 and Wingless in

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Drosophila. They express proteins of ~350-400 residues. Vertebrates have nineteen major Wnt gene families, with several subtypes within some classes [7]. Wnt-signaling is thought to proceed through binding to frizzled family receptors of cell surface; the signal is transported by several cytoplasmic factors to β-catenin, which pass in the nucleus and triggers the transcription of genes important in development [8].

β-Catenin (Armadillo in *Drosophila*) is a protein with multiple tasks, and it is conserved molecule that in metazoans exerts an important role in the developmental and homeostasis processes. β-catenin is is expressed in the human by the CTNNB1 gene, and it is the key nuclear effector of Wnt/ β-catenin signaling in the nucleus. More-ever, β-catenin is involved in the harmonization and complementation of cell–cell adhesion. Abnormality of the structure and properties of β-catenin usually results in several disease and poor growth [9]. Beta-catenin (β-catenin) which is a 90 kD protein contributes to cell development under normal conditions , and its aberrant expression induces malignant transformation of the cells, and this attitude was reported in many cancer forms[10].

WNTs have recently been implicated in the regulation of adipogenesis [11–16] .More-ever, few studies discuss the role of Wnt10a in suppressing adipogenensis and stabilization of β-catenin [17,18].

The goal of this study is to evaluate the association between obesity in the Egyptian population with two novel gene polymorphisms of wnt10a gene which is an important member of wnt proteins family, and to correlate the obesity incidence with the level of serum β-catenin which is the key of wnt signaling.

Subject and method

Subjects

This study is composed of two groups; the first included 48 of unrelated obese volunteers while the second group included 48 of age matched non-obese volunteers. All the volunteers are chosen from patients out clinic in zagazig university hospitals in the period of October 2015 till March 2016. BMI was the measure used to differentiate between obese and non-obese subjects. BMI is the most commonly used measure for body fat. It is calculated by dividing a person's weight in kilograms by the square of his/her height in meters (kg/m2). Current guidelines from the World Health Organization define a normal BMI range as 18.5 to 24.9. Overweight is defined as a BMI of 25.0 to 29.9; obesity is defined as a BMI over 30.0; and severe obesity is defined as BMI 35 or higher [19]. We excluded the overweight subjects, pregnant women, children, patients with any chronic or inflammatory disease, patients with malignant disease and patients with thyroid gland dysfunction. All the volunteers were unrelated and in good health. They are all subjected to complete full history about their living standards and life style and family history of obesity.

All volunteers signed consent for their participation in this study. More-ever, an approval was taken from the ethical committee in Zagazig university under the

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ethical consideration of Helenski declaration 1964 [20] .

Blood collection

Five ml of blood was taken from every participant under complete aseptic condition and was divided into 2portions:2ml of whole blood was collected in sterile EDTA containing tube for DNA extraction, and the rest was left for 30-60 minute for spontaneous clotting at room temperature then centrifuged at 3000 rpm for 10 minutes.serum samples were separated into another set of tubes and kept at -20 for chemical and enzyme –linked immunosorbent assay (ELISA)

Routine laboratory tests

All participants underwent routine laboratory tests for CBC, liver function tests including (ALT, AST, and GGT), and kidney function tests including creatinine and BUN.

Glucose and Lipid profile Determination

The concentration of FBS and different parameters of lipid profile including (TG, HDL-Ch, and total-Ch) were measured using BS300 system auto-analyzer. All the parameters are measured using manufacture instructions and according to the suitable method [21–23] for TG, total-Ch, and HDL-Ch respectively. Regarding LDL-Ch, it was calculated using the Friedewald's Formula "LDL-C=Total cholesterol-(HDL-C+TG/5)" [24,25].

Beta catenin Determination

Serum β-catenin level was measured by ELISA reader plate (Synergy HT, Biotec.) using sandwich ELISA technique [26,27] according to the manufacture protocol.

DNA Extraction

DNA was isolated using Genomic DNA spin column purification kit (Fermentas, Germany) according to a previous study protocol [28].

Genotyping of Wnt10a c.321C>A (rs121908119) p.Cys107Ter

Isolated DNA was genotyped for the detection of rs121908119 (C/A) polymorphism allele for each volunteer. The genotyping step has been made in ZSMRC using RFLP-PCR technique [29]. In this method, the amplification for the region containing the desired SNP is done by PCR reaction followed by digestion with suitable restriction enzyme which gives digested products can be identified by gel electrophoresis technique. Each PCR reaction is done in a total volume of 20 µl containing 5 µl of template DNA with concentration of 1 µg, 10 µl of master mix, 3 µl of nuclease free water, and 1 µl of each primer of the two primer used in the reaction. The primers were designed by BLAST software in NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).
The sequence of forward primer was 5'CAGCCATACAGGGCATCCAG3' and that of the reverse primer was 5'ACAGATGGGTGTGTGGGAT3'. The thermal cycling condition were 95^0c/5min for activation followed by 35 cycles of 95^0c for 30 second for denaturation then 57^0c for 35 second for annealing, then 72^0c for elongation and a final cycle 72^0c for 7 min for final elongation. The choosing of annealing temperature was done by making gradient PCR. The expected amplicon size was 250 bp. The PCR product was digested

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using suitable restriction enzyme “BCll” (NEB enzyme) which makes cutting at “A” allele and not “C” allele. This enzyme was chosen by using NEB restriction enzyme finder and cutter (https://enzymefinder.neb.com/#!#nebheader) (http://nc2.neb.com/NEBcutter2/). The digested amplicon was subjected to gel electrophoresis with 2% agarose stained with ethidium bromide under ultraviolet light. Three genotypes were given; CC genotype with one band with size of 250 bp, CA genotype with three bands with size (250 bp, 201 bp, 49 bp), and AA genotype with two bands with size (201 bp, 49 bp).

Genotyping of Wnt10a c.337C>T rs141074983 p.Arg113Cys

Similarly, the genotyping of rs141074983(C>T) was done using RFLP-PCR method with the above mentioned steps30. The sequence of forward primer was 5’GCACCCAATGACATTCTGGAC3’and that of the reverse primer was 5’GCTTGAGGCAGTTGGGTTAGAA3’. The thermal cycling condition were 950c/5min for activation followed by 35 cycles of 950c for 30 second for denaturation then 560c for 35 second for annealing, then 720c for elongation and a final cycle 720c for 7 min for final elongation. The choosing of annealing temperature was done by making gradient PCR. The expected amplicon size was 351 bp. The PCR product was digested using “Hinfl” (NEB enzyme) which makes cutting at both “C” allele and “T” allele but giving bands with different size. The digested amplicon was subjected to gel electrophoresis with 2% agarose stained with ethidium bromide under ultraviolet light. Three genotypes were given; CC genotype with four bands with size of (216/73/38/24) bp, CT genotype with five bands with size (240/216/73/38/24) bp, and TT genotype with four bands with size (240/73/38/) bp.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS version 20.0). Qualitative date was represented as number and percentage, quantitative data were represented by mean ± SD, and the following tests were used to test for significance of mean differences between different groups. Chi square test (X^2) was used for estimating significance of difference and association of qualitative variable. Odds ratio (OR) was used for estimating significant association and to find strength of association or risk. Differences between quantitative independent groups were tested by t test or Mann Whitney. Correlation between parameters was done by Pearson's correlation or Spearman’s. ROC analysis was used to estimate the sensitivity and specificity of betacatenin as aprognostic biomarker for obesity Kappa for agreement and logistic regression for independent predictors were used. .P value was set at <0.05 for significant results &<0.001 for high significant result.

Results

Our study included two groups of obese and non-obese Egyptian volunteers. They are all subjected to the same measurements including anthropometric measurements, routine laboratory analysis, fasting blood sugar determination, lipid profile determination, serum β- catenine

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determination, genotyping for two SNPs in Wnt10a gene. Our data using statistical analysis showed a variety of the results. Some of the parameters were associated with obesity risk while others were not.

Anthropometric measurements in both obese and non-obese groups

The anthropometric measurements show significant difference between obese and non-obese subjects in weight, BMI, Waist circumference, and waist to hip ratio ($p<0.001$). While, there was no significant difference between obese and non-obese subjects in age and height ($p>0.05$) **(Table 1).**

The percent of female was higher (72.9%) than that of male in obese group. On the contrast, the percent of female was lower (45.8%) than that of male in non-obese group. There was significant difference between obese and non-obese groups in the sex distribution ($p= 0.007$). The female sex is associated with high obesity risk (OR =3.18; CI 95% (1.35-7.47) **(Table 1).**

Glucose, lipid profile and serum β-catenin concentrations

Our results showed significant differences between obese and non-obese groups in FBS and all lipid profile parameters ($p<0.001$). All lipid profile parameters increased in obese group except for HDL **(Table 2)**

The serum β-catenin level was lower in obese than non-obese but with no significant power (p>0.05) **(Table 2).**

Receiver operating curve analysis

Using Roc analysis, the sensitivity of β-catenin as a prognostic biomarker was 77.1%. while the specificity was 16.7%. The area under curve was 0.567 with cut off value of <17.9 **(Fig 1).**

Correlation analysis

Our results showed positive significant correlation between weight and both total cholesterol and LDL. While, there was negative significant correlation between height and both BMI and FBS. Regarding BMI, it was positive correlated with FBS, total cholesterol, TG, and LDL. Both FBS and TG is positive correlated with total cholesterol. Both total cholesterol and triglyceride is positive correlated with LDL. The only negative significant correlation was between HDL and LDL. There was no significant correlation between serum β-catenin and any of the other parameters **(Table 3).**

Distribution of genotypes and alleles in the Wnt10a C>A (rs121908119) and C>T (rs141074983) SNP

In Wnt10a (C/A) (rs121908119) SNP, the highest frequency was for CA genotype in obese subjects (52.1%) and for CC genotype in non-obese subjects (66.7%). The minor allele "A" was with the lower frequency in both obese (28.1%) and non-obese (17.7%). Using chi-square test for comparing the genotype and alleles distribution showed no significant difference in either genotypes or alleles frequencies between both obese and non-obese subjects ($p>.05$) **(Table 4) (Fig 2).**

In Wnt10a (C>T) (rs141074983) SNP, there was a significant difference in genotype and allele frequency between obese and non-obese subjects (p<0.05) **(Table 4) (fig 3)**.

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Association between Wnt10a C>T (rs141074983) SNP and obesity risk

Our results showed significant difference in genotype distribution under co-dominant, dominant, heterozygote and allelic models. More-ever, the minor allele carrier (CT+TT) is associated with obesity risk 6.8 (1.3-47.6) (*p*=0.007) **(Table 5).**

Association between β catenin and different genotypes of Wnt10a (rs121908119) and (rs141074983) SNP

Our results showed no significant association between serum β catenin level and any genotype of the two SNPs of Wnt10a gene (*p<0.05*) **(Table 6).**

Multivariate logistic regression for independent predictors for obesity

Allele T in rs14107983 were the only independent predictors for obesity (p<.05) **(Table 7).**

Discussion

Mutations in wnts genes have been linked to several diseases and defects in humans, highlighting the importance of the wnt signaling pathway [30–34]. Several wnts ligands, addition to wnt10b, have been discussed in the context of obesity such as wnt1,wnt3a [14,35],wnt5b [36,37], wnt5a and wnt4 [38]. However, the link between the polymorphism differences of these wnts genes with either obesity or adipogenesis is not yet fully addressed.

In this study, we genotyped two SNPs in wnt10a gene in obese and non-obese volunteers. The first polymorphism was non-sense (stop coding) mutation ***c.321C>A (rs121908119) p.Cys107Ter*** in which cysteine (TG**C**) is substituted to termination code (TG**A**) at position 107 in the functional protein. While the second SNP was missense mutation ***c.337C>T rs141074983 p.Arg113Cys*** in which arginine (**C**GC) is substituted to cysteine (**T**GC) at position 113 in the functional protein.

Our results showed a non-significant difference in the genotypes and alleles distribution in Wnt10a (C/A) ***p.Cys107Ter*** (rs121908119) SNP, the highest frequency was for CA genotype in obese subjects (52.1%) and for CC genotype in non-obese subjects (66.7%). The minor allele "A" was with the lower frequency in both obese (28.1%) and non-obese (17.7%). The non-significant effect for the substitution in amino acid located at 107 position in wnt10a protein is probably because that cysteine amino acid is used in the disulfide linkage formation, and not affects the wnt10a protein function. Wnts proteins are glycoprotein rich in cysteine amino acids. Hence, losing one of the cysteine residues may not much affect structure and function of wnt10a protein. This was not in agreement to the previous report of tooth agenesis which demonstrated the pathological effect of this non-sense mutation[39].

Regarding the missense mutation of Wnt10a (C>T) ***Arg113Cys*** (rs141074983) SNP, there was a significant difference in genotype and allele frequency between obese and non-obese subjects (p<0.05)**.** There was significant difference in genotype distribution under co-dominant, dominant, heterozygote and allelic models. More-ever, the minor allele carrier (CT+TT) is associated with obesity risk 6.8 (1.3-47.6) (*p*=0.007).

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The pathological effect of Arg to Cys missense mutation was reported in tooth agenesis and oligodontia disease [40]. The mutagenesis of arginine to cysteine is frequent, and was discussed in different proteins, other than wnt10a, associated with several diseases [41–45]. Both arginine and cysteine are polar aliphatic essential amino acids but they are different in size, solubility, charge, and function. Arginine is positive charged hydrophilic large amino acid while cysteine is neutral hydrophobic small amino acid [46]. Arginine plays an important role in stabilization of protein, because it is responsible for salt bridges formation by making bonds with negative charged particles, to form stable hydrogen bonds that stabilize protein [47]. On the other side, cysteine usually participates in the formation of disulfide bonds and not hydrogen bonds [48]. Hence, the mutation of arginine to cysteine can probably affect the protein function due to the great difference between arginine and cysteine in function and structure.

β-catenin, the central mediator of the wnt/ β-catenin signaling pathway, can translocate from the cytoplasm to the nucleus, where it functions as a transcriptional co-activator and modulates hundreds of pathogenic genes. Of note, β-catenin can be detected in human serum due to its accumulation in the cytoplasm and/or nucleus. Recent studies reported β-catenin correlation with several diseases. However, there was controversy about its increase or decrease with the diseases progression. The elevation of β-catenin serum level was reported in HBV-related liver diseases, hepatocellular carcinoma, Colorectal Cancer and Adenoma [26,49,50]. While, other studies reported its decrease in osteoporosis and type 2 diabetes [51–53].

Regarding serum β-catenin level, our results showed relatively decrease in the obese group (16.84±34.6) than non-obese group (20.56±38.5) but with no significant power. In addition, the diagnostic accuracy of serum β- catenin is not perfect (AUC= 0.567, $p=0.261$) with sensitivity of 77.1% and specificity of 16.7%.

The slightly decrease in serum β-catenin may be explained by (C>T) ***Arg113Cys*** missense mutation of wnt10a which probably led to destabilization of β catenin. This, in turn, resulted in decrease its level in the serum due to less accumulation in the cytoplasm and/or nucleus. The combining between both (C>T) ***Arg113Cys*** missense mutation and down-regulation in the serum β-catenin level probably caused impaired wnt/ β-catenin signaling, and hence, prevent suppression of adipogenesis.

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Table 1: Anthropometric parameters and sex distribution in obese and non-obese groups

		obese (n=48)	Non-obese (n=48)	U	*P*
Age		31.62±8.4	27.5±4.94	2 .166	0.07
		23-50	18-45		
Weight Kg		86.52±9.6	60.08±4.64	17.155	<0.001**
		70-113	49-70		
Hight cm		162.56±7.7	164.1±5.55	1.890	0.061
		150-189	152-177		
BMI kg/cm2		33.14±3.26	22.12±1.15	22.061	<0.001**
		26.5-42.7	20-24.8		
Waist circumference		113.22±20.6	82.08±5.13	10.160	<0.001**
		80-162	74-91		
Waist to hip ratio		0.86±0.02	0.79±0.05	8.631	<0.001***
		0.8-0.97	0.5-0.85		
sex	Female n(%)	35 (72.9%)	22 (45.8%)	7.29	0.007*
	Male n(%)	13 (27.1%)	26 (54.2%)		
		OR (CI 95%) = 3.18 (1.35-7.47)			
		P = 0.002**			

mann-whitney test is used

BMI=body mass index. OR= odds ratio. CI= confidence interval.

All data are represented as mean ± S.

* $P < 0.05$ is significant, **$P < 0.01$ is very high significant, ***$P < 0.001$ is very high significant, and $P > 0.05$ is non-significant

Table 2: Concentration of FBS, lipid profile parameters and serum β-catenin in both obese and non-obese groups

	Obese (n=48)	Non-obese (n=48)	U	*P*
FBS	143.43±51.3	83.06±8.22	8.043	< 0.001 ***
	77-300	70-98		
Total-Ch	212.19±24.3	176.1±18.58	8.170	< 0.001 ***
	163-266	146-205		
TG	143.18±45.6	65.54±22.3	7.825	< 0.001 ***
	62.5-325	35-140		
HDL	49.34±13.2	68.5±7.11	8.833	< 0.001 ***
	35-58	60-88		
LDL	134.27±24.2	93.36±11.9	10.495	< 0.001 ***
	54-180	75-125		
β-catenin (pg/ml)	16.84±34.6	20.56±38.5	0.460	0.646

Association between *β-catenin level and obesity risk*

β-catenin (pg/ml)	Crude OR (95% CI)	*P*	Adjusted OR(95% CI)[a]	*P*
<5	1	-	1	-
6-15	3.9 (0.87-0.987)	0.768	4.7 (0.16-217.2)	0.986
15<	7.51 (0.15-0.33)	0.543	7.75 (0.23-203.2)	0.657

mann-whitney test is used.

FBS=fasting blood glucose, Total-Ch= total cholesterol, TG=triglycride, LDL= low density lipoprotein, and HDL= high density lipoprotein

a OR was adjusted for age

*** $P < 0.001$ is very high significant

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Table 3: Correlation between study parameters

		BMI	FBS	Total-Ch	TG	HDL	LDL	β-catenin
Age	**r**	0.225	0.213	0.227	0.233	-0.199	0.235	-0.152
	p	0.124	0.145	0.121	0.110	0.175	0.107	0.303
Weight Kg	**r**	0.575**	0.040	0.482**	0.088	-0.154	0.537**	0.126
	p	<0.001	0.785	0.001	0.552	0.295	<0.001	0.393
Height cm	**r**	-0.351*	-0.443**	-0.171	-0.231	-0.093	-0.003	0.018
	p	0.015	0.002	0.246	0.114	0.529	0.984	0.904
BMI	**r**	1	0.487***	0.713**	0.336*	-0.084	0.606***	0.133
	p	-	<0.001	<0.001	0.019	0.570	<0.001	0.369
FBS	**r**	0.487***	1	0.415**	0.244	0.174	0.211	-0.006
	p	<0.001	-	0.003	0.095	0.236	0.150	0.965
Total-Ch	**r**	0.713***	0.415**	1	0.628***	-0.198	0.805**	0.132
	p	<0.001	0.003	-	<0.001	0.177	<0.001	0.371
TG	**r**	0.336*	0.244	0.628***	1	-0.274	0.287*	0.037
	p	0.019	0.095	<0.001	-	0.059	0.048	0.801
HDL	**r**	-0.084	0.174	-0.198	-0.274	1	-0.60***	0.016
	p	0.570	0.236	0.177	0.059	-	<0.001	0.912
LDL	**r**	0.606***	0.211	0.805***	0.287*	-0.601***	1	0.104
	p	<0.001	0.150	<0.001	0.048	<0.001	-	0.482
Waist circumference	**r**	0.440**	0.264	0.597***	0.235	-0.153	0.575***	0.073
	p	0.002	0.069	<0.001	0.108	0.300	<0.001	0.624
Waist to hip ratio	**r**	0.282	0.301*	0.297*	0.100	0.119	0.207	-0.149
	p	0.052	0.038	0.040	0.499	0.421	0.158	0.313
β-catenin	**r**	0.133	-0.006	0.132	0.037	0.016	0.104	1
	p	0.369	0.965	0.371	0.801	0.912	0.482	-

* $P < 0.05$ is significant, **$P < 0.01$ is very high significant, ***$P < 0.001$ is very high significant, and $P > 0.05$ is non-significant

BMI= body mass index, FBS=fasting blood glucose, Total-Ch= total cholesterol, TG=triglyceride, LDL= low density lipoprotein, and HDL= high density lipoprotein

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Table 4: Distribution of genotypes and alleles in the Wnt10a SNPs (rs121908119- rs141074983)

			Group		X^2	P
			Obese	**Non-obese**		
Wnt10a C>A (rs121908119)						
Gene	**CC**	**n**	22	32	4.35	0.114
		%	45.8%	66.7%		
	CA	**n**	25	15		
		%	52.1%	31.2%		
	AA	**n**	1	1		
		%	2.1%	2.1%		
Allele	**C**	**n**	69	79	2.94	0.084
		%	71.9%	82.3%		
	A	**n**	27	17		
		%	28.1%	17.7%		
HWE			X^2= 18.17 P= <0.001	X^2= 15.78 P=<0.001		
Wnt10a C>T (rs141074983)						
Gene	**CC**	**n**	37	46	7.21	0.027*
		%	77.1%	95.8%		
	CT	**n**	6	1		
		%	12.5%	2.1%		
	TT	**n**	5	1		
		%	10.4%	2.1%		
Allele	**C**	**n**	80	93	9.87	0.001**
		%	83.3%	96.8		
	T	**n**	16	3		
		%	16.7%	3.2%		

Genotype comparing was done by chi square independence test

P < 0.05 is significant

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Table 5: genotype distribution under different genetic models

Model	Genotype		Group: Obese	Group: Non-obese	X^2	P	OR (CI 95%)	P
Co dominant model	CC	n	37	46	7.21	0.027*	1	-
		%	77.1%	95.8%				
	CT	n	6	1			CT 7.41 (0.8-171.3)	0.067
		%	12.5%	2.1%				
	TT	n	5	1			TT 6.2 (0.56-146.9)	0.069
		%	10.4%	2.1%				
Dominance model	CC	n	37	46	4.77	0.02*	1	
		%	77.1%	95.8%				
	CT+TT	n	11	2			6.8 (1.3-47.6)	0.007*
		%	22.9%	4.2%				
Recessive model	TT	n	5	1	2.84	0.09	1	
		%	10.4%	2.1%				
	CC+CT	n	43	47			0.18 (0.01-1.73)	0.09
		%	89.6%	97.9%				
Homozygote (TT vs. CC)	TT	n	5	1	3.37	0.06	6.22 (0.65-146.3)	0.066
		%	10.4%	2.1%				
	CC	n	37	46			1	
		%	77.1%	95.8%				
Heterozygote (CT vs. CC)	CT	n	6	1	4.3	0.03*	6.22 (0.65-146.3)	0.066
		%	12.5%	2.1%				
	CC	n	37	46			1	
		%	77.1%	95.8%				
Allele	C	n	80	93	9.87	0.001**	T 7.4 (0.8-171.8)	0.057
		%	83.3 %	96.8				
	T	n	16	3				
		%	16.7%	3.2%				

* $P < 0.05$ is significant, **$P < 0.01$ is very high significant, ***$P < 0.001$ is very high significant, and $P > 0.05$ is non-significant

Table 6: Association between β catenin and different genotypes of Wnt10a SNPS in obese group

		Mean ± SD	**Kruskal Wallis**	**P**
β catenin (pg/ml)	**CA**	15.49 ± 8.24	0.35	0.7
	CC	21.53 ± 9.26		
	AA	6.72 ± 4.27		
β catenin (pg/ml)	**CT**	4.83 ± 3.60	0.25	0.75
	CC	19.31 ± 11.23		
	TT	4.98 ± 2.1		

$P < 0.05$ is significant

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Table 7: Multivariate logistic regression for independent predictors for obesity

	Wald	*P*	OR	CI 95%	
				Lower	Upper
Age	**14.468**	**0.06**	**1.375**	**1.167**	**1.621**
Sex	**2.075**	**0.150**	**2.284**	**0.742**	**7.029**
genotype TT	**2.321**	**0.087**	**1.270**	**0.001**	**35.922**
genotype CT	**2.070**	**0.791**	**0.057**	**0.021**	**10.052**
Allele T	**6.654**	**0.009***	**5.621**	**1.235**	**18.542**
Fasting blood glucose	**1.1214**	**0.124**	**2.245**	**0.254**	**4.184**
Total-cholesterol	**2.987**	**0.0654**	**2.039**	**0.874**	**28.541**
TG	**2.885**	**0.0745**	**2.582**	**0.254**	**5.214**
HDL	**2.654**	**0.0985**	**0.888**	**0.021**	**4.321**
LDL	**3.124**	**0.0521**	**23.984**	**0.987**	**28.654**
Waist-circumference	**2.232**	**0.0952**	**4.094**	**0.854**	**17.5421**
Waist/hip ratio	**1.254**	**0.2145**	**1.65**	**0.632**	**12.3214**

$P < 0.05$ is significant

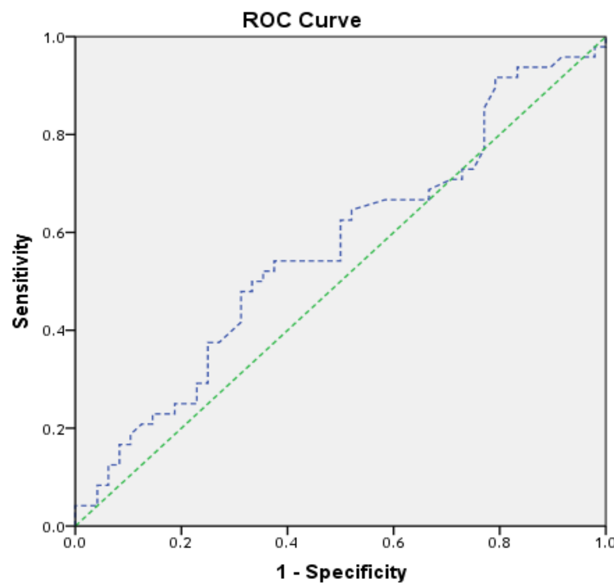

Fig 1: receiver operating curve for β catenin

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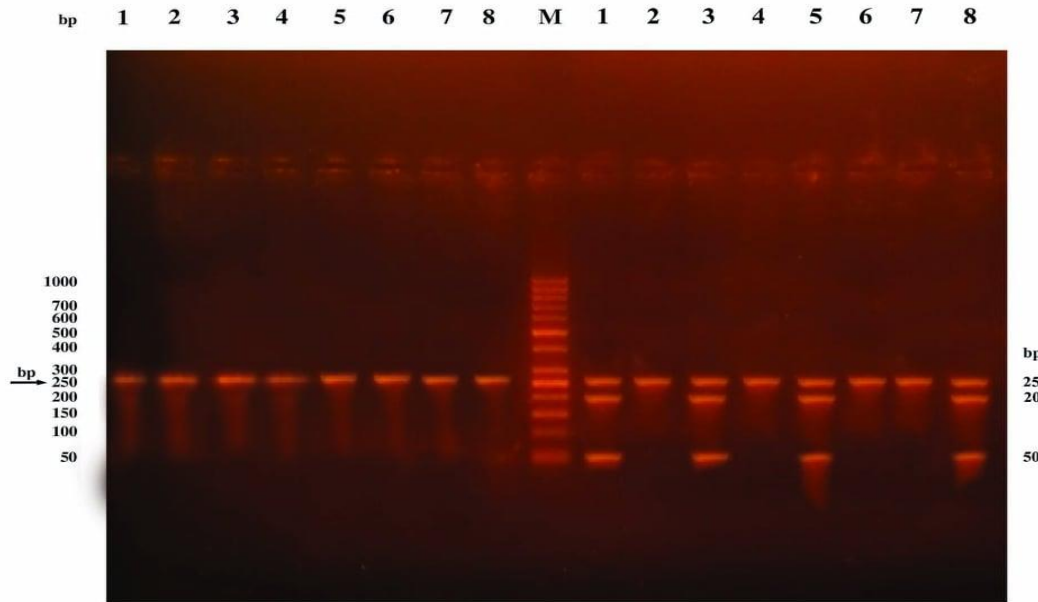

Fig 2: Agarose gel photo for Wnt10a C>A (rs121908119) polymorphism

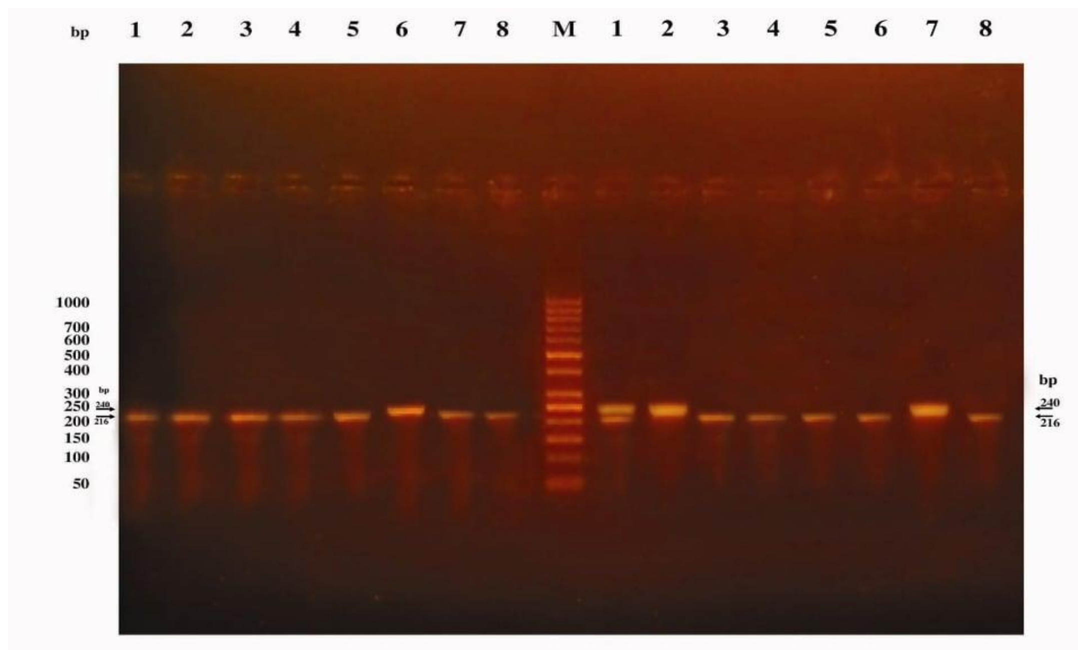

Fig 3: Agarose gel photo for Wnt10a C>T (rs141074983) polymorphism

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