Ibrahim et al., Endocrinol Diabetes Res 2019, 5:1
DOI: 10.4172/2470-7570.1000135

Endocrinology & Diabetes Research

Research Article A SciTechnol Journal

Association of Apelin Genetic Variants with Type Two Diabetes Mellitus in Egyptian Population

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Abstract

Background and Aim: Apelin, the newly identified adipokine and the endogenous ligand for the APJ receptor is related to obesity and insulin resistance. The aim of the study was to investigate the association of 2 single-nucleotide polymorphisms (SNPs) in the apelin gene (APLN) with susceptibility to Type Two Diabetes Mellitus (T2DM) in the Egyptian population.

Methods: Two SNPs on APLN were genotyped in 145 diabetic patients and 135 nondiabetic individuals, aged 40-60 years. Real-time polymerase chain reaction (RT-PCR) was used to analyze the 2 SNPs in both the diabetic and the healthy subjects. The association of the 2 SNPs (rs2281068 and rs3115759) in APLN and T2DM risk was investigated. Allele and genotype frequencies between patients and control groups were compared using the Chi-square (χ^2) test.

Results: In the apelin gene; GT/TT genotype of apelin risk genotypes of the rs2281068 variants was found to be significantly related with the risk of T2DM with the power (OR : 9.623, CI : 35.52 - 16.77) (P ≤ 0.001). while on the contrary, the GA/AA genotype of the rs3115759 variants was not at increased risk for T2DM (OR :1.25, CI : 0.785-2.09) (P=0.3408).

Conclusions: Both association and functional studies suggested that SNP rs2281068 in APLN is associated with the risk of T2DM in the Egyptian population.

Keywords

Apelin; Single Nucleotide Polymorphism; Type Two Diabetes Mellitus

Introduction

Diabetes is a fast-growing health problem in Egypt with a significant impact on morbidity, mortality, and health care resources. Currently, the prevalence of type two diabetes (T2DM) in Egypt is around 15.6% of all adults aged 20 to 79. This sharp rise could be attributed to either an increased pattern of the traditional risk factors for T2D such as obesity and physical inactivity or other risk factors unique to Egypt. It has been estimated that approximately 8.615 million Egyptians will be affected by diabetes by the year 2030 [1].

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Received: July 07, 2018 **Accepted:** December 23, 2018 **Published:** January 11, 2019

The adipose tissue is no longer considered just an energy storage site; it actively plays a role in the maintenance of energy homeostasis by secreting hormones called adipokines. Adipokines have been recognized to be involved in body fat distribution, insulin sensitivity and contribute to the etiology of T2DM, obesity, and metabolic syndrome [2].

Apelin, the endogenous ligand for the APJ receptor (an orphan G-protein-coupled receptor), is a bioactive peptide that is expressed in a wide variety of tissues [3]. The apelin gene (APLN) in humans is located on chromosome Xq25–26.1, a region close to a susceptibility locus for obesity that has been identified by linkage mapped in Finnish [4]. We therefore hypothesized that genetic variants at the APLN gene region might be associated with T2DM and related metabolic disorders.

During the recent years, there is a mounting evidence that apelin has pleiotropic effects on lipid and glucose metabolisms and therefore is correlated with metabolic disorders including diabetes [5]. Recent studies support an intimate relation between apelin, obesity, and insulin resistance. Although plenty of studies showed a positive correlation between plasma apelin concentrations and BMI in human subjects [6], the association between APLN polymorphisms and obesity phenotypes as well as T2DM has not been investigated in the egyptian population.

To date, only few studies have reported the associations of common genetic variants of APLN with T2DM [7]. However, genetic findings need replication. Thus, in this study we sought to investigate the relationship between APLN tagging SNPs and T2DM in the egyptian population. BMI was used as measurements of obesity. BMI represents the adipocity of the whole body [8].

Subjects and Methods

Study design

A total of 280 participants (146 male and 134 female) were enrolled in the study: Subjects were selected according to our defined inclusion criteria, which was: age 40-60 years. 145 patients with T2DM and 135 age and sex matched nondiabetic healthy control subjects. 145 T2DM obese patients without complications were recruited from admitted patients of the internal medicine department of Kasr Al Ainy University Hospitals. Participants were classified as having T2DM if they had one or more components of the American Diabetes Association criteria: FPG ≥ 126 mg/dl (7.0 mmol/l) or 2-h plasma glucose ≥ 200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water or in a patient with classic symptoms of hyperglycemia or hyperglycemia crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l). 135 Non diabetic subjects were randomly recruited from the local population Cairo city to act as control. They had normal fasting blood glucose levels, were not suffering any health problems, and had a negative history for T2DM and CVD. All participants gave their informed consent prior to participation, and the study was conducted in accordance with the approval of the Ethics Committee of the Faculty of Pharmacy, cairo

University, Egypt. A detailed medical history and drug treatment(s) were collected for all subjects. The following exclusion criteria were used for all study participants: Patients suffering from T_1DM, insulin treatment, thyroid, hepatic, acute infectious diseases, acute or chronic inflammatory disease, autoimmune disease, any hematologic disorder (assessed by complete blood count for every participant), and cancer were excluded from this study. Both the nondiabetic control group and the diabetic group were selected to have matching BMI and had a similar distribution of sex and age. Body mass index (BMI) of all Subjects was calculated as weight (kg)/height (m^2) and subjects with a BMI equal or more than 30 kg/m^2 were considered as obese subjects. The characteristics and biochemical data of patients and healthy controls are summarized in Table 1.

Biochemical assay

Participants were weighed in a gown and without shoes. Participants were weighed in a gown and without shoes. Blood pressure was measured with an automated monitor two times over a 5-minute rest period, and the average of the two blood pressures was used in study analyses. About 10 mL of fasting venous blood was obtained from all participants. Aliquots of blood were collected on EDTA for estimation of plasma glucose and extraction of DNA. The remaining portion of the blood samples were collected in serum separation tubes for determination of insulin, total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triacylglycerol (TAG) using standard laboratory methods. Serum apelin levels were estimated using enzyme-linked immunoassay kits (Phoenix Pharmaceuticals Inc., Burlingame, CA, USA.) [9]. The homeostasis model assessment of insulin resistance index (HOMA-IR) was calculated from fasting insulin and glucose levels as described [10].

Genotyping of polymorphisms

Genomic DNA was extracted from whole blood using DNA extraction kit and stored at -80°C in aliquots until required. This was done using TIANamp Genomic DNA kit (Beijing, China) according to the manufacturer's instructions. The concentration of the extracted DNA was determined by using Qubit 2.0 Fluorometer. The genotype of apelin SNPs (rs 2281068 C/T and rs 315759 G/A) were performed by using Applied Biosystems TaqMan technology with the ABI 7500 Real-Time PCR System platform (Applied Biosystems). The primers for the rs3115758 polymorphism were ggAggACATATTTTATATgTAACAAT and gAgAATgTTgAgCATACACTCTA. For the rs3115759 polymorphism, the primers were AgATgTTTAAATgTCgAATTATTg and AATgTgACTgCTTCTgCAT, Data were analyzed with the platform's System SDS software (version 1.2.3; Applied Biosystems).

Table 1: Clinical and Hemodynamic Characteristics of Participants.

Variables	Control	T2DM
Sex	135 (70 M/65 F)	145 (76 M/69 F)
Age (years)	48.51 ± 0.66	49.38 ± 0.55
BMI (Kg/m²)	30.51 ± 0.26	31.26 ± 0.32
D.M Duration (years)	--------	4.8 ± 0.14
SBP (mm Hg)	121.2 ± 8.67	119.9 ± 7.54
DBP (mm Hg)	79.5 ± 6.41	81.32 ± 5.32
FBG (mg/dL)	90.05 ± 1.09	210.3 ± 4.92[a]
Serum Insulin (µIU/mL)	6.61 ± 0.28	16.55 ± 0.48[a]
HOMA-I.R index	1.68 ± 0.06	8.42 ±0.39[a]
Serum TAG (mg/dL)	118.6 ± 2.3	173.9 ± 4.05[a]
Serum TC (mg/dL)	163.3 ± 1.70	226.7 ± 3.09[a]
Serum HDL-C (mg/dL)	49.53 ± 0.57	34.71 ± 0.92[a]
Serum LDL-C (mg/dL)	88.37 ± 1.81	156.6 ± 3.4[a]
Serum Apelin (ng/mL)	1.2 ± 0.09	3.9 ± 0.21[a]

Abbreviations: T2DM: Type 2 Diabetes Mellitus; BMI: Body Mass Index; FBG: Fasting Blood Glucose; TAG: Triacylglycerol; TC: Total Cholesterol; HDL-C: High-Density Lipoprotein; LDL-C: Low-Density Lipoprotein Cholesterol.

Data are given as mean ± SEM

[a]Significant difference from control group at $p < 0.05$

Statistical analysis

The distribution of the alleles of apelin SNPs (rs 2281068 C/T and rs 315759 G/A) was tested for Hardy–Weinberg equilibrium ($p>0.05$). Proportions of genotypes of alleles were compared by X^2 analysis, Odd ratios (ORs) and 95% confidence intervals (CI). Descriptive statistics were computed for all variables. The results were expressed as means ± SEM. To determine the statistical significance of laboratory findings, multiple comparisons were achieved using analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Differences in serum apelin concentrations between individuals with different genotypes were tested by Student's t-test. The significance level was set at 0.05 or less. All statistical analyses were performed with SPSS, version 17.0 (SPSS Inc.).

Results

Clinical and biochemical characteristics of the study subjects

A total of 280 subjects were included in this study are given in Table 1 with their clinical characteristics and all conventional risk factors for obesity complications, including BMI, hypertension (defined as a systolic blood pressure (BP) #140 mmHg, a diastolic BP #90 mmHg, or both), FBG, insulin, HDL, TAG, and TC ($p<0.05$). As shown in Table 1, there was no significant difference in the age, BMI, or sex distribution between the different study groups. Type 2 diabetic patients (T_2DM) showed significantly higher levels of FBG, insulin, and HOMA-IR compared with healthy individuals. Among the lipid profiles, the levels of TAG, TC, and LDL-C were significantly higher in T2DM patients than controls. Whereas levels of HDL-C were lower in T2DM patients than controls. In the present study the serum apelin level in T2DM patients showed significant increase to 3.9 ± 0.21 ng/mL than the healthy control group 1.2 ± 0.09 ng/mL ($p \leq 0.05$).

Genotypes and allele distribution of apelin gene rs 3115759 G/A

The observed genotype distributions of Apelin rs 3115759 G/A SNP agreed with those expected from Hardy–Weinberg equilibrium in all study groups. Results presented in Table 2 indicated that there is no significant differences in the frequencies of apelin rs3115759 GA+AA genotype and A allele in T2DM patients compared to the controls (53.2% *versus* 47.5%) X^2= 0.907, P = 0.3408 and (51.4% *versus* 43.8%) X^2=3.302, P = 0.0692 respectively.

Association between apelin gene rs 3115759 G/A polymorphisms with Type 2 diabetes mellitus

As indicated in Table 2 Subjects with the GA/AA genotype and A allele were not at increased risk for T2DM (OR = 1.25, CI = 0.785-2.09, P = 0.3408) and (OR = 1.36, CI = 0.97-1.89, P = 0.0692) respectively compared with those having the GG genotype and G allele.

The relation between apelin gene rs 3115759 G/A polymorphism and serum apelin levels

As illustrated in Table 3 it was revealed that serum level of apelin in patients with GA/AA genotype in the T2DM was higher than those with the GG genotype but with no significant difference (4.13 ± 0.25 ng/mL and 3.63 ± 0.12 ng/mL, respectively) ($p \leq 0.05$). Likewise the serum apelin level of GA/AA genotype carriers was higher but not significantly difference from that of the GG genotype in the healthy control (1.09 ± 0.08 ng/mL and 0.95 ± 0.06 ng/mL, respectively) ($p \leq 0.05$).

Genotypes and allele distribution of apelin rs 2281068 C/T

Results presented in Table 4 revealed significantly higher frequencies of rs 2281068 CT/TT genotype and T allele in T2DM patients compared to controls (75.3% *versus* 19.3%) $X^2 = 71.64$, $P \leq 0.0001$ and (70.8% *versus* 22.3%) $X^2 = 131.8$, $p \leq 0.0001$ respectively. The observed genotype distributions of apelin rs 2281068 C/T SNP agreed with those expected from Hardy–Weinberg equilibrium in all study groups.

Association between apelin gene rs 2281068 C/T polymorphisms with Type 2 diabetes mellitus

Carriers of GT/TT genotype and T allele of apelin rs 2281068 were significantly more likely associated with T2DM (OR = 9.623, CI = 35.52 - 16.77, $p \leq 0.001$) and (OR = 8.44, CI = 5.75-12.37, $p \leq 0.001$) respectively as shown in Table 4.

The relation between apelin rs 2281068 C/T gene polymorphism and serum apelin level

CT/TT genotype carriers (n = 101) of T2DM patients exhibited significantly higher serum apelin values (4.83 ± 0.25 ng/mL) than *CC* (n = 44) (3.21 ± 0.18 ng/mL). Similarly, controls with CT/TT genotype (n = 26) showed increased levels of serum apelin (1.31 ± 0.04 ng/mL) compared with *CC* (n = 109) (0.92 ± 0.07 ng/mL) as shown in Table 5.

Table 2: Differences in allele distribution and genotype frequency of apelin gene single nucleotide polymorphism (SNP) rs 3115759 G/A between control and TDM groups.

Groups	*n*	Genotype frequency		OR	95% CI	Allele frequency		OR	95% CI
		GG	GA + AA			G	A		
Control	135	71 (52.5%)	64 (47.5%)			152 (56.2%)	118 (43.8%)		
T2DM	145	68 (46.8%)	77 (53.2%)	1.25	0.785-2.09	141 (48.6 %)	149 (51.4%)	1.36	0.97-1.89
		X^2=0.9073, P= 0.3408				X^2=3.302, P=0.0692			

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
Results are expressed as number and in parentheses percent. p<0.05 is considered statistically significant after adjusting for age, sex, and BMI. Chi squared (X2) test was performed to compare categorical data.

Table 3: Genotypes of apelin rs 3115759 G/A gene polymorphism and serum apelin in study groups.

Groups	Genotype	n	Apelin (ng/ml)
Control	GG	96	0.95 ± 0.06
	GA + AA	39	1.09 ± 0.08
T2DM	GG	48	3.63 ± 0.12
	GA + AA	97	4.13 ± 0.25

Data are given as mean ± SEM
[a]Significant difference from GG genotype at $p \leq 0.05$

Table 4: Differences in allele distribution and genotype frequency of apelin gene single nucleotide polymorphism (SNP) rs 2281068 C/T between control and TDM groups.

Groups	*n*	Genotype frequency		OR	95% CI	Allele frequency		OR	95% CI
		CC	CT + TT			C	T		
Control	135	109 (80.7%)	26 (19.3%)			210 (77.7%)	60 (22.3%)		
T2DM	145	44 (30.3%)	101 (75.3%)	9.623	5.52 - 16.77	85 (29.2%)	205 (70.8%)	8.44	5.75-12.37
		X^2=71.64, P=0.0001				X^2=131.8, P=0.0001			

Abbreviations: OR: Odds Ratio; 95% CI: 95% Confidence Interval.

Results are expressed as number and in parentheses percent. $p < 0.05$ is considered statistically significant after adjusting for age, sex, and BMI. Chi squared (X^2) test was performed to compare categorical data.

Table 5: Genotypes of apelin rs 2281068 C/T gene polymorphism and serum apelin in study groups.

Groups	Genotype	n	Apelin (ng/ml)
Control	CC	109	0.92 ± 0.07
	CT + TT	26	1.31 ± 0.04[a]
T2DM	CC	44	3.21 ± 0.18
	CT + TT	101	4.83 ± 0.25[a]

Data are given as mean ± SEM
[a]Significant difference from CC genotype at $p \leq 0.05$

Discussion

This is the first study to investigate the relationship between the genetic variants (rs2281068 and rs3115759) in the APLN and T2DM in the Egyptian population in the literature. Common variants in adipokine genes (e.g. ADIPOQ, RBP4) were reported to affect the susceptibility of T2DM and related metabolic disorder [11].

In 1998, Tatemoto et al. [12] isolated a 36-amino acid peptide from bovine stomach extracts and named it apelin, apelin was the endogenous ligand of G-protein-coupled receptor termed APJ. Recently, apelin was identified in mouse adipocytes and human subcutaneous adipose tissue and recognized as an adipokine which is up-regulated in obese subjects with hyperinsulinemia [13].

Previous studies that addressed the relation between apelin and obesity yielded conflicting results. Elevated plasma apelin concentrations were reported in subjects with morbid obesity and T2DM [14]. Likely, in our study, this was supported by the positive correlation found between plasma apelin concentrations and BMI in our obese diabetic subjects.

In addition, Soriguer et al. [15] showed that elevated plasma apelin concentrations were only evident in obese people with glucose intolerance or diabetes but not in obese people with normal glucose. Interestingly, an increase of insulin concentrations can be essential to upregulate the APLN gene. All these suggest that apelin is involved in the pathogenesis of insulin resistance and T2DM.

The current study genotyped two tagging SNPs (rs2281068 and rs3115759) of APLN region in an Egyptian population with and without diabetes. Our analysis showed that the SNP rs2281068 was highly associated with T2DM prevalence. On the contrary, our second SNP rs3115759 showed no association with T2DM in the Egyptian population.

Zhang et al. [16] selected 3 single-nucleotide polymorphisms (SNPs) that could capture all common variants in the APLN gene region and genotyped them in 1892 type 2 diabetic patients and 1808 normal glucose regulation controls. None of the SNPs or haplotypes showed evidence of an association with type 2 diabetes; however, rs2235306 was nominally associated with fasting plasma glucose levels in the male subjects with normal glucose regulation (p=0.04) It suggests that APLN genetic variants may contribute to clinical features related to glucose metabolism in the Chinese population. In contrast to this study, the relationship of variations in the APLN with T2DM was invastigated in our study. The apelin gene variant rs2281068 AA risk genotype was significantly associated with an increased risk of T2DM (OR = 8.44, CI = 5.75-12.37, $p \leq 0.001$). The GA and AA genotypes of the rs2281068 variant, taken together, showed significant differences between diabetic patients and controls ($p \leq 0.001$). The GA + AA genotype combination was associated with a significant increase in the risk of T2DM (OR = 9.623, CI = 35.52 - 16.77, $p \leq 0.001$). Recently, Zhang and his colleagues [17] have assessed the associations of polymorphisms within APLN with hypertension in diabetic patients in a Chinese Han population and reported a negative result. Another association was next validated within APLN and T2DM in a general population by Zheng et al. [18] who analyzed 2 SNPs (rs2281068 and rs3115759) in APLN and genotyped them in 500 diabetic patients and 1468 nondiabetic individuals in a chinese population. The analysis showed that the SNP rs2281068 in APLN was significantly related to diabetes mellitus. In our study, as in Zheng 's study, SNP rs2281068 that contributed to the development of T2DM was analyzed and was associated significantly with T2DM.

We further genotyped another SNP in APLN (rs3115759), but genotyping did show any association with T2DM. Unlike this study, Pakizeh et al. [19] evaluated the possible relationship between both polymorphisms (rs3115758 and rs3115759) and another metabolic syndrome as cardiovascular diseases in Turkish population, both SNPs were associated significantly with cardiovascular diseases. In addition, the heterozygous (GA) and homozygous mutant (AA) genotypes of rs3115759 of the apelin gene were significantly higher in hypertensive patients as compared to normotensive patients.

The strength of the current study resides in that we thought to measure the circulating apelin concentration in our sample, precluding the analysis for the association between APLN genetic variants and circulating apelin levels. Our results revealed that the CT/TT carriers in rs2281068 was significantly associated with high apelin levels togather with hyperinsulinemia in diabetic patients . No significant difference was observed between any SNP and other variables. all this evidence shows that several mechanisms may account for the APLN effect on metabolic disorders. firstly, apelin is involved in a negative feedback mechanism between adipocyte and insulin [20]. Secondly, hyperinsulinemia upregulates the expression of APLN whereas elevated apelin concentrations enhance the glucose use and insulin sensitivity in muscle cells [21].

Another study conducted by Liao et al. [22], four tagging APLN SNPs (rs3115757, rs2235310, rs3761581and rs2235307) were genotyped in 1627 Chinese subjects. They treated primary adipocytes with high glucose plus insulin because of a close relation between insulin resistance and obesity. The minor homozygote CC of the rs3115757 SNP was associated with a high BMI in women. Accordingly, genetic association and functional studies suggest that genetic variants in APLN may influence apelin expression and are associated with the susceptibility of obesity phenotypes. In our study, it is notable that the rate of obesity presented by BMI and WC in the diabetic group was higher too when compared with the non diabetic group. Thus, the relationship between apelin and T2DM indirectly suggests that there may be other risk factors associated.

Together with our data, it is likely that APLN polymorphisms may have affected prevalence of T2DM in response to hyerinsulinemia. Thus, understanding the contribution of such an adipokine and its genetic polymorphisms in obesity-associated disorders appear to be of major importance. However, studies in other populations are needed to confirm our finding.

Conclusion

Interestingly, to our knowledge, this is the first study to demonstrate an association between common SNPs (rs2281068 and rs3115759) in the apelin gene and T2DM in a Egyptian population. Obesity-associated diseases, such as T2DM, have variable gene-to-gene and gene-to-environment interactions in different populations. Genetic variants in the APLN should be studied prospectively in a wider population to fully understand the mechanism underlying the association of APLN polymorphisms in the etiopathology of T2DM in Egyptian population.

Conflicts of Interest

There are no conflicts of interest.

Author Contributions

All authors contributed to the design of the study, data collection and analysis, data interpretation and manuscript writing.

Acknowledgements

We would like to thank all those who participated in the study. We are also grateful to the health nurses and laboratory technicians for their contribution.

Funding

This research was supported financially by Cairo University (Project No:3245).

Ethical Approval

The study was approved by the Committee on Medical Ethics of Cairo University. The study was carried out in accordance with the regulations and recommendations of the Declaration of Helsinki. (REC number: GH2008H).

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