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Evaluation of Apelin Genetic Variant rs 2235306 in Prediabetic and Type 2 Diabetic Individuals

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Abstract: Background: Apelin is a new peptide that may contribute to the pathogenesis of insulin resistance and type 2 diabetes .The study aimed to investigate the relation of APLN rs2235306 in prediabetic and diabetic patients. **Methods:** 200 patients were selected and divided them into 4 groups representing the 4 key stages of diabetes,control group, impaired fasting, early diabetic and old diabetic. SNP rs2235306 was genotyped by real-time PCR technique.Comparison of allele and genotype distribution was done by mann-whitney test. comparison with different clinical features was done by ANOVA and student's t-test. **Results:** rs2235306 is associated with post prandial insulin in male normal individuals (6.5±1.3) p= 0.046 and female normal individuals(7.7±1.7) p=0.041.It is also associated with insulin resistance in female diabetic patients (4.6±2.6) p=0.045. Poor glycemic control in diabetic females is associated with rs2235306 (10.1±2.2) p=0.03. **Conclusion:** apelin genetic variant rs2235306 and insulin secretion may be closely related taking in consideration the degree of glycemic control.

Key words: prediabetes, apelin ,diabetes mellitus type2, polymorphism, single nucleotide

INTRODUCTION

Type 2 diabetes is a metabolic disorder characterized by 2 major defects: decreased secretion of insulin by the pancreas and resistance to the action of insulin in various tissues especially adipose tissue which results in impaired glucose uptake. (Lawrence, J.M., *et al*., 2008)

Prediabetes is described as a grey area between normal blood glucose and diabetic levels (Nathan, D.M., *et al*., 2005). It is also referred as impaired glucose tolerance and always associated with insulin resistance and usually presented in overweight people or with positive family history (Barr, E.L., *et al*., 2007)

Apelin is a new peptide that was was identified by professor M.Fujino 1998 (Samreen Riaz, *et al*., 2009). It is an adipocyte secreted factor regulated by insulin and is increased in adipose tissue and plasma with obesity (Dray, C., *et al*., 2.10). In pancreas, it inhibits the insulin secretion induced by glucose (Wang, G., *et al*., 2009). This may suggest that apelin may contribute to the pathogenesis of insulin resistance and type 2 diabetes (Wang, G., *et al*., 2004)

The apelin gene APLN is located on chromosome xq 25-q26, a region close to the susceptibility locus for obesity (Zhang rong, *et al*., 2009). we therefore hypothesized that genetic variants at the APLN gene region might be associated with type 2 diabetes at its different stages.

The aim of this study was to evaluate the association between the genetic variant rs2235306 on APLN in prediabetic and type 2 diabetic patients with their related clinical features.

Methods:

Subjects:

The study was performed on 200 unrelated subjects (98 males,102 females) divided into four groups representing the four key stages of diabetes.

Group1: control group: included 50 normal individuals , age between 40-60 years with normal glucose regulation. Fasting plasma glucose < 100mg/dl and 2-hour plasma glucose <140 mg/dl confirmed by a standard 75 gm oral glucose tolerance test (OGTT) with negative family history of diabetes.

Group 2 : Included 50 patients with impaired glucose tolerance with fasting plasma glucose 100-125 mg/dl or2-hour plasma glucose 140-199 mg/dl according to the ADA criteria with positive family history and BMI > 25.

Group 3: Included 50 type 2 diabetic patients with early onset (6 months- 1year) duration defined by 1999 WHO criteria for diabetes.

Group 4 :Included 50 type 2 diabetic patients with old onset (6-10 years) duration.

All the cases and controls were recruited from the outpatient clinic at the national research centre of Egypt. Awritten informed consent was obtained from each participant.

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Clinical Studies:

All subjects underwent detailed clinical investigation.Anthropometric measurements of height and weight were collected. BMI was calculated as weight in (KG) divided by height in (m^2). Fundus, ECG, neurological examination was done to exclude diabetic complications.Fasting and post prandial plasma glucose was measured during OGTT by obtaining samples at 0 and120 minutes by using standerdised enzymatic procedures (Bio Merieux) however, fasting and post prandial insulin was measured by chemiluminescence assay (Immulite 1000). HBA1C was measured by ion exchange chromatography (Stanbio Laboratory).

Genomic DNA was extracted from peripheral blood leukocytes with a commercial kit (Nucleospin DNA Blood, Macherey-Nagel (MN), and Germany).

Snp Selection And Genotyping:

Tagging SNP of APLN was selected from release 2 phase II data of the hap map project (htt:// hapmap.ncbi.nlm.nih.gov/) by using the tagger pairwise method of haploview software. The genotyping of SNP rs2235306 was done. By using Taqman technology with (SLAN ADVANSURE, real-time PCR system, KOREA). The PCR program was denaturation 95c for 15 minutes, amplification (40 cycles) 95c for 15 seconds,58 c for60 sec. Farward primer GCATCTGACCCCAGAGATA and reverse primer AAGGAAGGAACAGAGCCG.

Statistical Analysis:

APLN is located on x chromosome that is why statistical analysis were conducted in a sex specific manner. One way analysis of variance (ANOVA) and student's t test were used to compare mean values of the different clinical features across different genotypes.Mann-whitney test was used to compare genotype frequencies between male and female subjects in the different groups. All statistical analysis was performed by SAS (version8.0). For all statistical tests, P values less than 0.05 were considered statistically significant.

Results:

Stratifying by gender failed to detect any evidence of association between the genotyped SNP rs2235306 alleles with impaired glucose tolerance patients (prediabetes) and type 2 diabetes mellitus. (Table 1-3)

In the quantitative trait analysis, we analysed the association between the SNP rs22353306 and clinical features related to glucose metabolism in the 4 groups representing the stages of diabetic progression. It was clear that the T allele carriers of SNP rs2235306 in males and CC allele carriers in females have a higher post prandial plasma insulin than the other genotypes in the control group (6.5±1.3) and (7.7±1.7) respectively. (table 4). In addition, SNP rs 225306 was found to be associated with insulin resistance in female diabetic patients with CC alleles (4.6±2.6), compared to the CT alleles who have higher HBA1C level compared to other genotypes (10.1±2.2) (Table 5).

Table 1: Association analysis between APLN SNP rs2235306 in control subjects and impaired fasting group (group2)after stratifying by gender.

	MALE			FEMALE			
GROUPS	T	C	*P Value*	TT	CT	CC	*P Value*
Controls	12(24.0%)	13(26.0%)	0.81	8(16.0%)	7(14.0%)	9(18.0%)	0.49
Group(2)	13(26.0%)	12(24.0%)		8(16.0%)	10(20.0%)	7(14.0%)	

Table 2: Association analysis between APLN SNP rs2235306 in control subjects and Type 2 diabetes after stratifying by gender.

	MALE			FEMALE			
GROUPS	T	C	*P Value*	TT	CT	CC	*P Value*
Controls	12(24.0%)	13(26.0%)	0.9	8(16.0%)	7(14.0%)	9(18.0%)	1.0
Group(4)	14(28.0%)	11(22.0%)		8(16.0%)	8(16.0%)	9(18.0%)	

Table 3: Association analysis between APLN SNP rs2235306 in impaired fasting group (Group2) and Type 2 diabetes (group4)after stratifying by gender.

	MALE			FEMALE			
GROUPS	T	C	*P Value*	TT	CT	CC	*P Value*
Group(2)	13(26.0%)	12(26.0%)	0.74	8(16.0%)	10(20.0%)	7(14.0%)	0.49
Group(4)	14(28.0%)	11(22.0%)		8(16.0%)	8(16.0%)	9(18.0%)	

Table 4: Association between rs 2235306 and clinical features in the normal glucose regulation subjects (Group 1)

Variables	MALE			FEMALE			
	T(12)	C(13)	*P value*	TT(8)	CT(8)	CC(9)	*P value*
BMI(kg/m2)	20.5±2.8	20.66±1.96	0.89	19.2±1.58	20.2±2.9	19.6±6.8	0.91
Fasting plasma glucose(mg/dl)	87.6±12.6	82.5± 6.5	0.22	87.1± 6.08	87.6±5.7	88.2±9.3	0.92
2-hour plasma glucose(mg/dl)	92.7±10.4	90.4± 5.9	0.53	94.1± 9.5	95.3±1.0	94.2±7.9	0.93
HBA1C (%)	4.9±0.56	5.0± 0.41	0.71	4.8 ± 0.4	4.8±0.51	4.8±0.28	0.82

HOMA-insulin resistance	0.84±0.36	0.75±0.43	0.57	0.96 ± 0.7	0.71±0.6	0.61±0.35	0.42
Fasting insulin(mU /ml)	2.01±2.1	1.75±1.17	0.72	3.8± 3.3	2.8±2.7	2.7±1.4	0.63
2-hour insulin(mU/ml)	6.5±1.3	5.67±1.16	0.046	5.6 ± 2.4	5.2±2.9	7.7±1.7	0.041

Table 5: Association between rs2235306 and clinical features in the diabetic group (Group 4)

Variables	MALE			FEMALE			
	T(14)	C(11)	*P value*	TT(8)	CT(8)	CC(9)	*P value*
BMI(kg/m2)	26.6±8.4	28.0±4.2	0.61	21.8±2.7	23.6±5.6	26.7±4.5	0.095
Fasting plasma glucose(mg/dl)	198.6±60.4	177.0±46	0.33	19.1±6.7	20.3±7.8	18.7±4.6	0.8
2-hour plasma glucose(mg/dl)	264.7±56.6	257.0±62	0.75	25.8±9.7	24.3±12.6	26.1±6.6	0.92
HBA1C (%)	9.25±1.8	9.08±2.0	0.82	7.8±1.7	10.1±2.2	7.8±1.8	0.03
HOMA-insulin resistance	6.08±2.6	5.65±2.03	0.65	1.85±0.9	2.8±2.5	4.6±2.6	0.045
Fasting insulin(mU /ml)	11.9±4.6	18.8±9.2	0.071	4.4±2.4	6.7±6.1	9.5±4.6	0.13
2-hour insulin(mU/ml)	16.9±5.5	18.8±8.8	0.51	18.1±9.4	21.9±6.6	27.1±12.4	0.19

Discussion:

Insulin resistance is widely recognized as a fundamental defect that precedes the development of type 2 diabetes. Adipose tissue factors called adipokines, have been shown to play an important role in the link between obesity and insulin resistance (Hu, C., *et al*., 2009).

Apelin, an adipocyte-secreted factor upregulated by insulin, is increased in plasma with obesity. It was recently identified as a new player in the control of glucose homeostasis (Erdem, G., *et al*., 2008). There are controversies in the literature regarding the regulation of apelin in subjects with altered glucose metabolism (Li, L., *et al*., 2006). In patients with newly diagnosed type 2 diabetes plasma apelin levels were decreased (Ren, F., *et al*., 2009), however increased plasma levels of apelin were observed in individuals with glucose intolerance and type2 diabetes (Soriguer, F., *et al*., 2009).

Common variants of the APLN gene were reported to affect the susceptibility of type 2 diabetes and related metabolic disorders (Zhang rong, *et al*., 2009; Rayalam, S., *et al*., 2008).

In our study we genotyped APLN variant rs2235306 and studied its association with different clinical features at 4 key stages of diabetes. It was found that male T allele and female CC allele carriers had a higher post prandial plasma insulin in normal individuals. Fasting and post prandial plasma glucose were not associated with rs2235306. On the contrary, Zhang *et al* 2009., reported an association with fasting plasma glucose level in normal male subjects(Zhang rong, *et al*., 2009).

No significant association was reported between obese impaired fasting individuals with rs2235306, however obese type 2 diabetic females with CC allele carriers were found to have positive association. Liao *et al* 2011,reported a significant association of another variant of APLN rs 3115757 with BMI as a measurment of obesity.

Surprisingly, poor glycemic control in female CT allele carriers was associated with rs 2235306 which may predict the relation between the degree of hyperglycemia and the different apelin variants together with apelin serum levels.

Thus, this study may suggest that apelin variants and insulin secretion may be closely related taking in consideration the degree of glycemic control.

We recommend that studies including another APLN gene variants are needed to give a global idea about the relation of apelin variants with insulin resistance on the other hand determination of circulating apelin levels in our samples is highly recommended. Besides, studies in other populations are needed to confirm our findings.

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