Original Article

Association of MicroRNA-146a rs2910164 Gene Polymorphism with Metabolic Syndrome

(miRNA-146a / rs2910164 / metabolic syndrome)

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Abstract. Alteration in microRNA-146a (miRNA-146a) expression is an important event in the pathogenesis of many human diseases. MiRNA-146a rs2910164 is a functional polymorphism that showed association with several diseases. Metabolic syndrome is an aggregation of multiple risk factors including impaired glucose tolerance, increased high-density lipoprotein, abdominal obesity, and high blood pressure. The aim of this study was to assess the relation of miRNA-146a rs2910164 with metabolic syndrome and its component traits in Egyptian women from the Suez Canal area. The study included 100 healthy female subjects and 100 metabolic syndrome patients. The component traits of metabolic syndrome were determined and the genotypes of the polymorphisms were assessed using the polymerase chain reaction-restriction fragment length polymorphism technique using the restriction enzyme *Hpy*188I. The rare C allele had a significantly higher frequency in metabolic syndrome patients (P = 0.013). The heterozygote GC and the rare CC genotypes showed a significant increase in body mass index, waist circumference, triglycerides, total cholesterol, low-density lipoprotein, systolic and diastolic blood pressure. The GC genotype was associated with higher fasting blood glucose, fasting serum insulin and insulin resistance. The carriers of CC genotype had significantly lower HDL compared with the GG genotype carriers. In conclusion, The C allele of miRNA-146a rs2910164 showed positive association with increased susceptibility to metabolic syndrome and its phenotypes in the study population.

Introduction

MicroRNAs (miRNAs) belong to a class of small non-coding regulatory RNA that act through binding to the 3′-UTR of target mRNA leading to transcriptional repression or degradation of the target mRNA at the post-transcriptional level (Ambros, 2004). It has also been reported that miRNAs can increase expression of the target genes (Cordes et al., 2009). MiRNAs have emerged to play important roles in many physiological and pathophysiological processes such as embryonic development, organogenesis, tumorigenesis and other human diseases such as arrhythmia, ischaemic heart disease, cardiac hypertrophy, viral hepatitis and diabetes (Li et al., 2010).

MiRNA-146a is one of the inflammation-related miRNAs that plays an important role in the immune system (Perry et al., 2008). Its expression in inflammatory cells is induced by lipopolysaccharide and is nuclear factor-κB-dependent. MiRNA-146a causes negative feedback regulation of the innate immune response via targeting the pro-inflammatory adapter proteins TNF receptor-associated factor 6 and interleukin-1 receptor-associated kinase 1 (Taganov et al., 2006; Iyer et al., 2012).

MiRNA-146α is involved in the pathogenesis of autoimmune diseases and several types of cancer (Li et al., 2010). In addition, miRNA-146a may also be involved in the pathological processes of inflammatory human degenerative diseases, such as prion disease (Lukiw et al., 2011; Saba et al., 2012), Alzheimer's disease (Sethi and Lukiw, 2009; Cui et al., 2010) and epilepsy (Aronica

Received October 13, 2014. Accepted November 11, 2014.

This work is funded by the authors.

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Abbreviations: ANOVA – analysis of variance, BMI – body mass index, CAD – coronary artery diseases, FBG – fasting blood glucose, HDL-C – high-density lipoprotein cholesterol, HOMA-IR – homeostasis model assessment of insulin resistance, LDL-C – low-density lipoprotein cholesterol, miRNA – micro RNA, PCR-RFLP – polymerase chain reaction-restriction fragment length polymorphism, QUICKI – quantitative insulin sensitivity check index, SD – standard deviation, SNP – single-nucleotide polymorphism, TC – total cholesterol, TG – triglycerides, TNF-α – tumour necrosis factor α.

et al., 2000). The level of miRNA-146a is regulated by a single-nucleotide polymorphism (SNP). This G/C SNP (rs2910164) is located within the seed sequence of pre-miRNA-146a, which is the miRNA-146a precursor (Kogo et al., 2011). MiRNA-146a rs2910164 SNP showed association with increased risk of several types of cancer (Xu et al., 2011; Yue et al., 2011; Hezova et al., 2012; Min et al., 2012; Ma et al., 2013).

Metabolic syndrome is a combination of several traits, including elevated plasma triglyceride (TG), reduced high-density lipoprotein cholesterol (HDL-C), elevated blood pressure, raised plasma glucose, and abdominal obesity. Metabolic syndrome is considered as a risk factor for coronary artery diseases (CAD), diabetes and fatty liver, and several cancers (Alberti et al., 2009; Grundy et al., 2014).

The prevalence of metabolic syndrome increases in women, particularly those in the childbearing age (Ramos and Olden, 2008). Genetic factors, as well as environmental factors, are thought to play a role in the development of metabolic syndrome (Joy et al., 2008). Metabolic syndrome was found to be associated with many gene polymorphisms, such as oestrogen receptor α (Ghattas et al., 2013), tumour necrosis factor α (TNF-α) (Gupta et al., 2012), angiotensin-converting enzyme (Xi et al., 2012), and cholesteryl ester transfer protein (Povel et al., 2011).

This study aimed to analyse the relation of miRNA-146a rs2910164 SNP with metabolic syndrome and its component traits in Egyptian women from Suez Canal area. To the best of our knowledge, this is the first study to investigate the relation of this polymorphism with metabolic syndrome.

Material and Methods

Study population

A cross-sectional study was conducted in 200 Egyptian female subjects of the same ethnic group, divided into 100 healthy subjects and 100 metabolic syndrome patients. Patients were selected from the Outpatient Clinic of the Ismailia General Hospital and Suez Canal University Hospital. Metabolic syndrome was diagnosed according to the definition of the Third Report of the National Cholesterol Education Program's Adult Treatment Panel (ATPIII), i.e. the presence of any three or more of the following factors: fasting blood glucose (FBG) ≥ 100 mg/dl or known diabetes, serum TG ≥ 150 mg/dl, HDL-C < 50 mg/dl in women, blood pressure ≥ 13085/ mmHg or treated hypertension, and waist circumference ≥ 88 cm in women (Alberti et al., 2009).

The study included no smokers. We excluded all patients with heart disease, diabetes mellitus type I, any type of cancer, renal failure or chronic liver disease. Pregnant or lactating women were also excluded.

The present study was conducted according to the principles of the Declaration of Helsinki, and all the participants provided written informed consent. The study protocol was approved by the Faculty of Pharmacy, Suez Canal University Research Ethics Committee.

Body mass index (BMI), waist circumference, and systolic and diastolic blood pressure were determined for all the subjects.

Laboratory measurements

Peripheral blood was drawn after a 12 h fast, where a portion was collected in EDTA anticoagulant tubes for DNA extraction and the remaining portion was used for separation of serum and assessment of the following:

Glucose homeostasis traits: FBG was measured by the enzymatic colorimetric method (Biodiagnostic, Giza, Egypt) and fasting serum insulin was measured by enzyme-linked immune sorbent assay (ELISA) (Monobind Inc., Lake Forest, CA). FBG and serum insulin values were used for calculation of the homeostasis model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985) and the quantitative insulin sensitivity check index (QUICKI) (Katz et al., 2000).

Lipid profile: TG, total cholesterol (TC) and HDL-C were measured by enzymatic colorimetric methods (Biodiagnostic). Low-density lipoprotein cholesterol (LDL-C) was calculated (Friedewald et al., 1972).

Genomic DNA extraction and genotyping

Genomic DNA was isolated from 300 µl of whole blood collected in EDTA anticoagulated tubes using the Wizard genomic DNA purification kit (Promega, Madison, WI). The pre-miRNA-146a G/C (rs2910164) polymorphism was genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique (Zeng et al., 2010). Two primers, 5'-CATGGGTTGTGTCAGTGTTAGA-3' and 5'-CCAAGAGTCTCGTATAACAGCA-3', were used for amplification of a 372 bp long DNA fragment. The PCR reaction was conducted in a total volume of 25 µl containing 1 µl genomic DNA (~100 ng/µl), 1 µl of each primer (10 pmol/µl), 12.5 µl Go Taq® Green Master Mix (2×) (Promega) and 9.5 µl DNase-free water. Thermal cycling was performed in an Eppendorf Mastercycler® machine (Eppendorf, Hamburg, Germany). The PCR cycle was composed of 8 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 53 °C, and 30 s at 72 °C, with a final elongation at 72 °C for 10 min.

The PCR product was digested with *Hpy*188I (New England Biolabs, Inc., Beverly, MA), 5 units for 90 min at 37 °C, followed by electrophoresis in 2% agarose gel. The GG genotype displayed two bands at 211 and 161 bp, the CC genotype yielded three bands at 211, 134 and 27 bp, while the GC genotype produced four bands at 211, 161, 134, and 27 bp.

Statistical analysis

Student's *t*-test was used to compare the characteristics of the metabolic syndrome patients and the control group. The χ^2 test was used to analyse the genotype and allele frequencies and to assess the compatibility of the genotype frequencies with Hardy-Weinberg equilibri-

Table 1. General and biochemical characteristics of the study population

Variables	Control (N = 100)	Metabolic syndrome patients (N = 100)	P
Age (years)	39.98 ± 12.13	38.76 ± 9.72	0.433
BMI (kg/m2)	23.10 ± 1.68	32.31 ± 5.55*	0.001
Waist circumference (cm)	76.77 ± 8.50	107.12 ± 10.99*	0.001
Systolic blood pressure (mmHg)	109.15 ± 9.02	139.85 ± 16.85*	0.001
Diastolic blood pressure (mmHg)	72.40 ± 6.98	89.75 ± 8.36*	0.001
Glucose homeostasis traits			
FBG (mg/dl)	84.26 ± 10.67	157.02 ± 55.10*	0.001
Fasting serum insulin (μIU/ml)	8.50 ± 1.11	15.80 ± 5.58*	0.001
HOMA-IR	1.80 ± 0.45	6.91 ± 4.89*	0.001
QUICKI	0.351 ± 0.016	0.301 ± 0.026*	0.001
Lipid profile			
TG (mg/dl)	117.54 ± 22.65	202.00 ± 62.41*	0.001
TC (mg/dl)	166.85 ± 27.93	229.55 ± 37.22*	0.001
HDL-C (mg/dl)	59.06 ± 10.56	42.73 ± 5.66*	0.001
LDL-C (mg/dl)	84.37 ± 29.24	142.06 ± 35.11*	0.001

Data are presented as mean ± SD. Comparisons were performed by unpaired Student's *t*-test.
* Significantly different from normal control at $P < 0.05$

um. In order to determine the relative risks, odds ratios and 95% confidence intervals were used. Associations of genotypes with the metabolic syndrome component traits were analysed by the one-way analysis of variance (ANOVA) test followed by Tukey's test for multiple comparisons. Analysis was performed using SPSS, version 17.0. Data are presented as means ± standard deviations (SD). A value of $P < 0.05$ was considered statistically significant.

Results

The clinical and biochemical parameters of the metabolic syndrome patients and the control subjects are summarized in Table 1. Patients had higher BMI, waist circumference, systolic and diastolic blood pressure than the control group. Patients also showed significantly increased FBG, fasting serum insulin levels, and insulin resistance, represented by higher HOMA-IR and lower QUICKI values. Concerning the lipid profiles, the serum values of TG, TC, and LDL-C were higher in the metabolic syndrome patients, with lower serum HDL-C than the control group. Both groups were age matched.

As shown in Table 2; the minor C allele of the miRNA-146a rs2910164 polymorphism had a significantly higher frequency in metabolic syndrome patients than in the control subjects (OR = 0.599, 95% CI = 0.3980.900–, P = 0.013). The frequencies of GC (OR = 0.480, 95% CI = 0.2600.887–, P = 0.018) and CC genotypes (OR = 0.399, 95% CI = 0.1640.971–, P = 0.040) were significantly higher than the frequency of the GG genotype in metabolic syndrome patients. The genotype distribution was compatible with Hardy-Weinberg equilibrium in the whole study sample ($\chi^2 = 0.07$, P = 0.791), as well as in subjects with metabolic syndrome ($\chi^2 = 0.92$, P = 0.338) and control subjects ($\chi^2 = 0.12$, P = 0.729).

We also investigated the relation of miRNA-146a rs2910164 genotypes with different clinical parameters in the study population. As illustrated in Table 3; in comparison with the reference GG genotype; the heterozygote GC genotype showed a significant increase in

Table 2. Allele frequencies and genotype distribution of miRNA-146a rs2910164 polymorphism in the control group and metabolic syndrome patients

	Control N = 100	Metabolic syndrome N = 100	P	OR (95% CI)
G allele	136	112		
C allele	64	88	0.013*a	0.599 (0.398–0.900)
Genotype				
GG	47	29		
GC	42	54	0.018*b	0.480 (0.260–0.887)
CC	11	17	0.040*c	0.399 (0.164–0.971)

Comparisons were performed with the χ^2 test; (CI) = confidence interval; OR = odds ratio;
a G vs. C; b GG vs. GC; c GG vs. CC; * indicates significant difference at $P < 0.05$.

Table 3. The relationship between miRNA-146a rs2910164 genotypes and different clinical parameters in the study population

Variables	Carriers of GG (N =76)	Carriers of GC (N = 96)	P (GC vs GG)	Carriers of CC (N = 28)	P (CC vs GG)
BMI (kg/m^2)	26.00 ± 6.22	28.58 ± 5.71*	0.017	29.35 ± 6.65*	0.035
Waist circumference (cm)	85.54 ± 18.19	94.95 ± 17.35*	0.002	99.04 ± 15.34*	0.002
Systolic blood pressure (mmHg)	114.87 ± 16.45	129.06 ± 21.17*	0.001	135.00 ± 17.53*	0.001
Diastolic blood pressure (mmHg)	75.07 ± 9.75	83.85 ± 11.34*	0.001	87.86 ± 9.95*	0.001
Glucose homeostasis traits					
FBG (mg/dl)	106.37 ± 43.87	128.15 ± 56.34*	0.022	133.64 ± 62.36	0.054
Fasting serum insulin (μIU/ml)	10.69 ± 4.39	12.96 ± 5.71*	0.017	13.63 ± 6.28	0.063
HOMA-IR	3.27 ± 3.25	4.92 ± 4.59*	0.033	5.34 ± 5.28	0.073
QUICKI	0.335 ± 0.029	0.321 ± 0.034*	0.012	0.320 ± 0.036	0.086
Lipid profile					
TG (mg/dl)	140.76 ± 45.21	169.04 ± 74.52*	0.009	179.57 ± 49.14*	0.013
TC (mg/dl)	178.64 ± 35.75	206.67 ± 48.35*	0.001	222.25 ± 38.91*	0.001
HDL-C (mg/dl)	53.71 ± 12.59	49.65 ± 11.63	0.060	47.54 ± 8.03*	0.044
LDL-C (mg/dl)	95.49 ± 35.53	121.99 ± 46.93*	0.001	131.25 ± 32.67*	0.001

Data are presented as mean ± SD. Comparisons were performed by one way ANOVA test followed by the Tukey's test for multiple comparison; * indicates significant difference from carriers of GG at $P < 0.05$.

FBG (P = 0.022), fasting serum insulin (P = 0.017), HOMA-IR (P = 0.033), QUICKI (P = 0.012), BMI (P = 0.017), waist circumference (P = 0.002), TG (P = 0.009), TC (P = 0.001), LDL (P = 0.001), systolic and diastolic blood pressure (P = 0.001).

Table 3 also shows that the rare CC genotype was also associated with higher BMI (P = 0.035), waist circumference (P = 0.002), TG (P = 0.013), TC (P = 0.001), LDL (P = 0.001), systolic and diastolic blood pressure (P = 0.001). The carriers of CC genotype had significantly lower HDL compared with the GG genotype carriers (P = 0.044).

Discussion

In this study, we aimed to investigate the association of miRNA-146a rs2910164 SNP with the susceptibility to metabolic syndrome in the study population. Our results revealed that the C allele was more frequent in patients with metabolic syndrome compared with the G allele. The C allele was reported to increase the expression level of miRNA-146a (Shen et al., 2008; Kogo et al., 2011; Xiong et al., 2014). The role of miRNA-146a in the pathogenesis of inflammation and other degenerative aspects may explain its possible contribution to the course of metabolic syndrome. The association of metabolic syndrome with inflammation is well documented (Eckel et al., 2005). Xiong et al. (2014) stated that the GC and CC genotypes of miRNA-146a rs2910164 polymorphism are associated with increased risk of CAD, whose risk in turn increases with the presence of increasing numbers of metabolic syndrome criteria (Haffner, 2006).

Our results show that the carriers of GC genotype had significantly higher FBG and increased insulin resistance compared to the carriers of the GG genotype. These results counteract the findings of Ciccacci et al. (2013), who reported no significant association of miRNA-146a rs2910164 SNP with type 2 diabetes mellitus. However, Rong et al. (2013) found that circulating miRNA-146a levels were elevated in new diabetic patients compared with controls. Similar results were reported by Kong et al. (2011).

We also found that the carriers of GC and CC genotypes had significantly higher BMI, waist circumference, TC, TG, and LDL. HDL levels were significantly lower in carriers of the homozygote CC genotype compared to the reference GG genotype. Chartoumpekis et al. (2012) showed up-regulation of miRNA-146a in adipose tissue during the development of obesity using the C57BLJ6 mice fed a high-fat diet as a model. MiRNA-146a is potentially involved in the differentiation of adipocytes by targeting C/EBP β (Tanaka et al., 1997) and apoE, respectively (Huang et al., 2009).

Increased expression of miRNA-146a in human senescent endothelial cells can be linked to the elevated expression of miRNA-146a in atherosclerotic plaques (Olivieri et al., 2013; Raitoharju et al., 2013). These previously reported results can explain the association of the miRNA-146a rs2910164 C allele with increased blood pressure in the current study.

In conclusion, this study aimed to examine the relation of miRNA-146a rs2910164 SNP with metabolic syndrome in Egyptian women. The rare C allele showed positive association with increased susceptibility to metabolic syndrome and its phenotypes in the study population. Our findings are limited by the relatively small sample size. Further studies on larger scales and from different ethnicities are required to support these results.

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