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Original Article

Study Protocol for the Interactions between Dietary Patterns and *ARL15* and *ADIPOQ* Genes Polymorphisms on Cardiometabolic Risk Factors

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Abstract

Background: Cardiovascular diseases (CVDs) are recognized as one of the leading causes of death worldwide. Studies have shown the impact of genetic predisposition and dietary factors on developing these diseases. Dietary patterns and genetic factors such as polymorphisms related to the level of adiponectin may also interact with each other and produce variances in the effects of these factors on different individuals. The purpose of this study is to investigate the interactions between food intake patterns and polymorphisms on *ADIPOQ* and *ARL15* genes in relation to cardiometabolic risk factors. **Methods:** This cross-sectional study is conducted on 380 adults (20 to 70 years old) living in Yazd, Iran. Individuals were selected from the participants in Yazd Health Study (YaHS) and its sub-study called Taghziyeh Mardom-e Yazd (TAMYZ) after reviewing the inclusion and exclusion criteria. YaHS is a population-based cohort study which has been conducted on 9962 adults living in Yazd since 2014. In the present study, rotated principle component analysis (PCA) with Varimax rotation is used to identify the major dietary patterns. The polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) method is used in order to identify rs1501299 and rs6450176 variants (on *ADIPOQ* and *ARL15* genes, respectively). General linear models (GLM) as well as regression models are used to investigate the interactions between the studied genotypes and the extracted dietary patterns. **Conclusions:** The results of this study can help to personalize dietary recommendations for the prevention of CVDs according to the genetic predisposition of individuals.

Keywords: *Adiponectin, ADIPOQ, ARL15, cardiovascular diseases, diet, gene--environment interaction, genetic variation, heart diseases, nutrigenomics, polymorphism, rs1501299, rs6450176*

Introduction

Cardiovascular diseases (CVDs) are known as one of the most important causes of death worldwide, so that in the world, about 17.3 million deaths are recorded per year due to these diseases, which accounted for 31.5% of all deaths due to various factors in 2013.[1] Coronary artery disease (CAD) is the most common type of CVDs and its main feature is the presence of sclerotic lesions in the coronary arteries of the heart. These lesions progressively narrow the arteries and reduce the blood flow received via the heart muscle. This disorder in the blood flow to the heart, depending on the extent of the disorder, causes complications during activity or rest and can eventually lead to heart muscle failure, cardiorespiratory problems and myocardial infarction (MI) and eventually death.[2] Various risk factors have been identified for CVDs including: aging, male gender, genetics, menopause, type 2 diabetes mellitus (T2DM), sedentary lifestyle, unhealthy dietary patterns, smoking, dyslipidemia, chronic inflammation, obesity, overweight and hypertension.[3-7]

So far, many studies have examined the relationship between dietary patterns and risk factors of CVDs. Most studies believe that Western dietary patterns, rich in red and processed meats, saturated fats, refined grains, and high-fat dairy products are directly related to the risk of developing these diseases, and on the other hand, healthy dietary patterns, including higher intake of fruits, vegetables, fish, whole grains, low-fat dairy, and nuts are inversely related to the chance of developing CVDs and their risk factors.[8-10] For example, a study by Mirmiran *et al*.[11] (2016) showed that in Tehran, high adherence to a Western dietary pattern, including red and processed meats, and sweetened beverages are related to the risk of CVDs. The study of Esmaillzadeh *et al*.[12] (2008) also showed that in Iranian women, adherence to the

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healthy dietary pattern was inversely associated with the coronary heart diseases risk factors such as hypertension and hyperlipidemia.

Adiponectin is a protein secreted by adipose tissue and has anti-inflammatory properties and increases insulin sensitivity.[13] As a result, decreased levels of this protein are associated with several diseases such as obesity, metabolic syndrome, T2DM, and CVDs.[14,15] Adiponectin levels are highly influenced by genetic factors. Genome-wide association studies (GWAS) has identified a number of genetic regions associated with the levels of this protein, including single nucleotide polymorphisms (SNPs) on the Adiponectin (*ADIPOQ*) and ADP-ribosylation factor-like 15*(ARL15)* genes.[16,17] Numerous nutritional studies have also revealed a link between food intake patterns and adiponectin levels.[18,19] Studies showed that following healthy dietary patterns including high intake of whole grain, low fat dairy, dietary fibers, fruits, vegetables, nuts and legumes and low in saturated fats, red and processed meats are related to the elevated levels of adiponectin and unhealthy dietary patterns such as western food intake pattern can decline the levels of this adipokine.[20]

The adiponectin gene (*ADIPOQ*) is located on the large arm of chromosome No. 3 and encodes the adiponectin protein. The relation of SNPs on this gene with adiponectin levels, risk of overweight and obesity, CVD, T2DM, and metabolic syndrome has been studied in various studies. One of these SNPs is rs1501299 (+276G>T) which is located in the intron 2 region of *ADIPOQ* gene.[21] Studies have shown that this SNP can affect the incidence of CVDs and its risk factors. For example, a meta-analysis study showed that genotypes of rs1501299 are associated with the risk of coronary artery disease in diabetic patients.[22] Another study by Luis DA *et al*.[23] also showed a significant relationship between rs1501299 and insulin resistance, serum adiponectin level and metabolic syndrome. Several studies have also shown a significant interaction between theses variants and blood levels of saturated fatty acids, or intake of various fats on the level of adiponectin.[24-26]

The *ARL15* gene is located on the large arm of chromosome No. 5 and encodes the ADP-ribosylation factor-like 15 protein. Studies in this field have shown the association between SNPs of this gene and adiponectin levels. Some studies have also indicated the association between these SNPs and blood lipid levels and T2DM.[16,27] Rs6450176 is located in the intronic region of *ARL15* gene. Association between this SNP and CVD risk factors has been shown in previous studies. For example, Sun *et al*.[27] reported a significant relationship between rs6450176 genotypes and serum lipid levels in Chines population.

Little is known about the relationship between *ARL15* and *ADIPOQ* gene polymorphisms and adiponectin levels and consequently risk factors of CVDs, and according to the lack of a study investigating the interactions between SNPs of these genes and dietary intake patterns on risk factors of CVDs in Iran, this study is designed to investigate the interactions between rs6450176 (G> A) and rs1501299 (G> C) SNPs and dietary patterns, on risk factors of CVDs in an adult population living in Yazd, Iran. The present protocol study also has been written to summarize the most important steps of performing this research and to discuss about the limitations in reaching the goals of the study.

Materials and Methods

Participants

This cross-sectional study is conducted on 380 healthy adults (20 to 70 years old) living in Yazd. Sample size has been calculated using QUANTO software. Samples were selected from the participants in the recruitment phase of Yazd Health Study (YaHS) after reviewing the inclusion and exclusion criteria. YaHS is a population-based cohort study on 9962 adults living in Yazd, which has been conducted since 2014. The purpose of YaHS and its subset study, Taghziyeh Mardom-e Yazd (TAMYZ), was to investigate the changing incidence of non-communicable diseases and also the relationship between non-communicable diseases and related risk factors in the population of Yazd city-Iran. Nutritional data such as food intake frequency have been collected in TAMYZ study. A bio-bank called Zist Bank-e-Yazd (ZIBA) has been established since 2015 to preserve the study's biological samples, including blood, serum and urine samples (www.yahs-ziba.com). Lots of data such as medical history, socioeconomic status, physical activity, anthropometric measurements including weight, height, body mass index (BMI), waist circumference and body fat percentage, mental health status, nutritional information, blood pressure and biochemical parameters including fasting blood glucose (FBS) and lipid profile have been measured during YaHS and TAMYZ. Details of YaHS and TAMYZ methodology and early results were published previously.[28]

To select the individuals, YaHS study participants were first selected based on inclusion criteria including having a whole blood sample in the ZIBA biobank and having nutritional and biochemical data in the database. Then people with any of the exclusion criteria, including under-reporting or over-reporting of energy intake (who their energy intake will be outside the range of 800--4200 kcal/day),[29] having inflammatory or metabolic diseases, cancers, liver, kidney or thyroid diseases, acute or chronic infections, receiving corticosteroid medicines and pregnant and breastfeeding women were excluded from the study, then 380 of the remaining individuals were randomly selected to participate in the study.

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Assessment of dietary intake and major dietary patterns identification,

Dietary intake of participants in the YaHS and TAMYZ study was obtained using the food frequency questionnaire (FFQ). In fact, the questionnaire used in this study is the modified form of the questionnaire used in the Tehran Lipid and Glucose Study, in which 10 items of frequently used traditional food of Yazd including some traditional sweets and breads have been added (178 items) and its shape has also changed to 4-choice questions.[30] Validity and reproducibility of the used FFQ have been confirmed previously.[31]

Principle component analysis (PCA) with varimax rotation will be used to identify major dietary patterns. This method is a statistical method to reduce data and extract dietary patterns based on correlation between food groups.[32,33] The food groups will be selected based on previous studies and then the food items will be categorized into these food groups that already have been selected by literature review. In the next step, the created food groups will be adjusted based on the energy intake of individuals by residual regression method. Eventually, the large number of food groups will be reduced to a small number of dietary patterns by factor analysis based on criteria such as scree plot and eigenvalue greater than 1.5. The Kaiser--Meyar--Olkin and Barlett's test *P* values will be assessed to measure sampling adequacy and the relationship between variables, respectively.[34]

DNA extraction and genotyping

After selecting the samples from the participants of the YaHS study, the whole blood samples of the individuals available in the ZIBA Biobank will be used to extract DNA. In order to extract the DNA, the DNA Extraction Mini Kit *(spin column; Favorgen; Taiwan; Catalogue number: FABGK001)* will be used according to the manufacturer's instructions. The genotypes of rs6450176 and rs1501299 SNPs will then be determined by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP). The extracted DNA residue will be stored in ZIBA Biobank for future researches. The location of the SNPs in the sequence for rs6450176 and rs1501299, respectively, are as follows: CTACTAAGAGGGGAG[G/A] CAGATCCAGGACAC and TAAACTATATGAAG[G/T] CATTCATTATTAACTAA.

For PCR, pre-designed primers in the studies of Sun *et al*. and Kaur *et al*. will be used [Table 1].[27,35] In the next step, PCR products for rs6450176 and rs1501299 SNPs will digested by ALW26I *(Thermo Fisher Scientific; US; Catalogue number: ER0031)* andMva1269I *(Thermo Fisher Scientific; US; Catalogue number: ER0962)* restriction enzymes, respectively, at 37°C overnight and then will be visualized on agarose gel 2%. For each of the SNPs, three conditions may occur after the digest operation, depending on whether they are digested or undigested by the enzymes. The digestion will result in fragments of 321bp and 183bp for G allele of rs1501299 and 287bp and 234bp for G allele of rs6450176. The results of digestion process will be interpreted according to Table 1.

Data analysis method

Statistical analyses will be performed using SPSS software (version 20.0 for Windows; SPSS, Chicago, Illinois). As mentioned previously, the PCA method will be used to determine dietary patterns. General linear model (GLM) and regression model will be used to investigate the interaction of genotypes and extracted food patterns. We will use ANOVA, independent *t*-test and GLM to compare the means of the quantitative variables between the genotypes. Also, Chi-square test will be used to compare the values of qualitative variables between the groups. Confounders such as physical activity, age, gender, socioeconomic status and level of education will be adjusted in the statistical models. For all tests, *P* value less than 0.05 will be considered as significant.

Discussion

Environmental factors such as dietary patterns and physical activity are important and influential factors associated to CVDs and their related risk factors. On the other hand, genetic variations are also important factor influencing the incidence of diseases and also affect the response of different people to environmental changes. This is why similar dietary patterns have different effects on people health. Therefore, it seems that when studying the relationship between dietary factors and diseases, genetic differences should also be considered.[36] The present study will investigate the interactions between dietary patterns of individuals and some genetic factors in the population of Yazd-Iran.

Table 1: Details of single nucleotide polymorphisms (SNPs) that will be genotyped

SNP rs number	Gene	Chromosome	primers	Enzyme	Restriction fragments
rs6450176	*ARL15*	5	TCGTGTTGGCCCATTTTAGG	ALW26I (BsmAI)	AA: 521 bp
			CCCTTATGACACCTCCCCAA		GG: 287 and 234 bp
					AG: 521,287 and 234 bp
rs1501299	*ADIPOQ*	3	GTCTCTCCATGGCTGACAGT	Mva1269I (BsmI)	TT: 504 bp
			GGTGAAGATGGGAAAGGGGA		GG: 321 and 183 bp
					TG: 504, 321 and 183 bp

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This study has some limitations, including the fact that due to the cross-sectional nature of the study, the causality cannot be deduced. Only two SNPs related to adiponectin levels will be examined, and other existing SNPs as well as gene-gene interactions will not be examined. The use of FFQ also has limitations such as measurement error. The strengths of this study include the following: In this study, many confounders such as physical activity, age, gender, socio-economic status and level of education will be examined and will be adjusted in statistical analysis. Factor analysis will be used to examine people's dietary patterns, which will examine the entire diet of individuals instead of evaluating single foods. The samples of this study also were selected from the samples of a large and population-based study that its vast amount of data has been carefully collected by trained and careful individuals.

To the best of our knowledge, this research is the first study to investigate the possible interactions between dietary patterns and polymorphisms related to adiponectin levels on cardiometabolic risk factors in Iran and the results can help personalization of dietary recommendations for the prevention of CVDs with regard to the genetic predisposition of individuals.

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Conflicts of interest

There are no conflicts of interest.

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