The Relationship Between Genetic Variants Associated with Premature Menopause and Lipid Profile in Women Recruited from MASHAD Cohort Study

Mohammad Reza Mirinezhad

Mashhad University of Medical Sciences

Hamideh Ghazizadeh

International UNESCO Center for Health-Related Basic Sciences and Human Nutrition, Mashhad University of Medical Sciences, Mashhad, Iran

Maliheh Aghsizadeh

Birjand University of Medical Sciences

Mohammad Zamiri Bidary

Mashhad University of Medical Sciences

Alireza Naghipour

Mashhad University of Medical Sciences

Elahe Hasanzadeh

Mashhad University of Medical Sciences

Mahdiyeh Yaghooti-Khorasani

Mashhad University of Medical Sciences

Ali Ebrahimi Dabagh

Varastegan Institute for Medical Sciences, Mashhad, Iran

Mohammad Reza Shadmand Foumani Moghadam

Varastegan Institute for Medical Sciences, Mashhad, Iran

Nazanin Sheikh Andalibi

Mashhad University of Medical Sciences

Zeynab Naseri Far

Mashhad University of Medical Sciences

Habibollah Esmaily

Mashhad University of Medical Sciences

Gordon A Ferns

Brighton and Sussex Medical School

Tayebeh Hamzehloei

Mashhad University of Medical Sciences

Alireza Pasdar

Division of Applied Medicine, Medical School, University of Aberdeen
Majid Ghayour-Mobarhan (✉ ghayourm@mums.ac.ir)
Mashhad University of Medical Sciences

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Abstract

Background and aim: Premature menopause (PM) is defined by the occurrence of the menopause before the age of 40 years. It is often associated with cardiovascular disease (CVD). The purpose of this study was to explore the relationship between PM-associated genotypes cardio metabolic disorder risk factors.

Methods: One hundred seventeen women with PM and one hundred eighty-three healthy women without PM were recruited in this study. DNA was extracted and analyzed using ASO-PCR or Tetra ARMS-PCR. Lipid profiles were also assessed.

Results: Multivariate logistic regression analysis showed that individuals with GG vs. TT genotype of the rs1046089 SNP were more likely to have high serum LDL risk ($p = 0.03$) compared to the control group. There was also a significant association between low serum HDL risk and rs2303369 and rs4806660 SNP genotypes in the PM group. In the PM group, the percentage of those with a high total cholesterol was lower in those with a CC genotype compared to those with a TT genotype ($p = 0.03$).

Conclusion: Some SNPs reported to be associated with PM appear to be independently associated with dyslipidemia. These results may be helpful to identify subjects with PM who may be susceptible to CVD.

Introduction

Menopause is defined as occurring when menstruation stops for 12 consecutive months due to loss of ovarian follicular activity; this usually occurs around the age of 45-55 years¹. Menopause that happens before the age of 40 years is called premature menopause (PM) or premature ovarian insufficiency (POI), which may be natural or due to or reproductive surgeries²⁻⁴. This condition is accompanied by amenorrhea, estrogen deficiency and an increase of gonadotrophin levels⁵. It has been reported that about 3.6% of women develop PM⁶. Various factors such as smoking⁷, certain medications⁸, infections and genetic and autoimmune disorders have been associated with PM⁹. Iatrogenic causes such as radiotherapy, chemotherapy, pelvic surgery are also associated with PM^{5,9-14}. About 60-90% of PM cases are idiopathic¹⁴. Several studies have also found that PM increases the risk of hypertension¹⁵, cardiovascular disease³, osteoporosis¹⁶ cerebral infarction¹⁷, all-cause mortality¹⁸ type 2 diabetes mellitus¹⁵, and other negative health consequences¹⁶.

Genetic factors also play a significant role in PM¹⁹. The heritability of menopausal age is estimated about 30-85%²⁰. About 20-25% of PM cases are due to genetic causes¹⁴. Genome-wide association studies (GWAS) have identified a polymorphism (rs16991615) of minichromosome maintenance 8 homologous recombination repair factor (MCM8) gene involved in the age of natural menopause²¹. Also, rs1046089 and rs4806660, located on Proline Rich Coiled-Coil 2A (PRRC2A) and transmembrane (TMEM) gene, respectively are associated with the age at menopause^{22,23}. GWAS identified several other variants that are associated with PM²⁴.

Deleterious changes in risk factors for cardiometabolic disorders often occur around the age of menopause²⁵⁻²⁸. Estrogen is involved in dilating blood vessels and helping blood flow²⁵. Various studies have also shown that estrogen therapy in postmenopausal women reduces serum total cholesterol and low density lipoprotein (LDL) cholesterol concentrations, and increases serum high-density lipoprotein (HDL) cholesterol and triglyceride concentrations^{29,30}. Moreover, lack of ovarian function in the menopause is involved in the activation of the renin-angiotensin system, leads to immunodeficiency, inflammation and endothelial dysfunction^{25,31}. These are associated with obesity, diabetes and high blood pressure^{25,31}. Several studies have shown that age at menopause is associated with cardiovascular disease^{18,32-34}. A Japanese study found women with early menopause had a higher risk for hypercholesterolemia³⁵, and another study showed that early menopause is associated with hypertension³⁶. Sarnowski et al. founded that genetic variants associated with early menopause are also associated with increased cardiovascular disease risk³⁷. There appeared to be a need to evaluate the relationship between PM-related variants with lipid profile and susceptibility to cardiometabolic disease risk factors. Few studies have been done on this subject. We aimed to explore the associations between PM-related variants with lipid profile and susceptibility to cardiometabolic disease risk factors in Mashhad stroke and heart atherosclerotic disorders (MASHAD) cohort study population.

Methods

117 women who had PM were included in the case group. Healthy women (n = 183) were recruited into the control group. All of participants were recruited as part of the MASHAD study. The MASHAD study is a cohort study from 2010-2020 that were included 9704 participants aged 35-65 years who will be follow-up exams every three years until 2020³⁸. The inclusion criteria were as follows: the diagnostic criterion was based on the definition of PM: 1) Women who go through menopause before the age of 40 years; 2) 12 continuous months have passed since the last bleeding; and 3) serum FSH > 40 IU/L. Exclusion criteria were: women over 40 years old, with a history of diseases and surgeries affecting menstruation (oophorectomy, hysterectomy), history of genetic abnormalities and syndromes associated with an early menopause is a part of their manifestation, history of using drugs affecting menstruation. Blood samples were collected into vacutainer® plain tubes, and were taken after 14 hours fast. Blood samples were centrifuged at 4° C in 5000 rpm for 15 minutes and the serum part was used for lipid profile measurement. Body Mass Index (BMI) was measured using standard method³⁸. Kidney, liver, and thyroid activity were normal in all participants.

DNA extraction and quality controls

Participants' DNA was extracted from 200 µl blood or buffy coat samples using a DNA extraction kit (Pars Tous, Mashhad, Iran). Qualitative and quantitative quality control was performed by agarose gel electrophoresis (Pars Tous, Mashhad, Iran) and Nano drop 2000 (Thermo Fisher Scientific, USA) in 280 and 260 nanometer wavelengths, respectively.

Allele-specific oligonucleotide polymerase chain reaction (ASO-PCR)

The ASO-PCR reaction volume was 15 µl which included: 1.5 µl water, 2 µl genomic DNA, 1 µl of each primer, and 7.5 µl master mix (Pars Tous, Mashhad, Iran). First, to carry out PCR, we performed one cycle of denaturation for 7 minutes at 95°C. After that, 35 cycles include the following: 95°C for 30 sec, annealing for 30 s at 60°C, 72°C for 30 sec, and eventually one cycle of 7 min was done for final extension.

Tetra amplification refractory mutation system PCR (ARMS-PCR)

Tetra ARMS was carried out by the same method and the same composition of 15 µl reaction volume that performed in ASO-PCR. Primers were designed with Primer1 software³⁹.

Lipid profile measurements and dyslipidemia diagnosis

Total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were measured from serum which was taken from participants 12 hours fasting using standard method^{40, 41}. The NCEP ATPIII criteria⁴² were used to diagnose dyslipidemia. 1) If serum cholesterol levels ≥ 200 mg/dl (5.2 mmol/l) is considered hypercholesterolemia. 2) If HDL cholesterol levels < 40 mg/dl (< 1.04 mmol/l) for men and < 50 mg/dl (< 1.3 mmol/l) for women is considered low HDL cholesterol. 3) If LDL cholesterol levels ≥ 130 mg/dl (≥ 3.4 mmol/l) is considered high LDL cholesterol. 4) If serum triglycerides ≥ 150 mg/dl is considered Hypertriglyceridemia.

Physical activity level assessment

The equations of James and Schofield for energy requirements, were used to assess physical activity of all participants. Questions regarding the physical activity level were based on the mentioned equations which were selected from World Health Organization MONICA project questionnaires⁴³. The level of physical activity was calculated by total energy expenditure (TTE) and basal metabolism rate (BMR) during a whole day and night⁴⁴.

Ethics

All steps of the study were approved by the Mashhad University of Medical Sciences (MUMS) Ethics Committee. Informed consents were obtained from all subjects and the procedure and possible risks were explained completely, pursuant to the Declaration of Helsinki⁴⁵.

Statistical analysis

All analysis tests were performed by Statistical Package for Social Sciences (SPSS) (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). The values in this study have been reported frequently with percentage or mean and standard deviation. Single Nucleotide

Polymorphisms' (SNPs') genotypes between PM cases and healthy controls were compared by Chi-square test. Assessment of normal distribution in quantitative data was performed by Kolmogorov-Smirnov test. Man-Whitney test was used for comparing quantity of normally distributed values between the subgroups. Besides, we used multivariate logistic regression to prevent confounder's factors from affecting our results. P-value < 0.05 was statistically significant.

Results

The clinical characteristics of the population have been summarized in Table 1. In our cross-sectional analysis, participants had a mean age of 55 years averagely. Differences in lipid profile factors serum level between different genotypes of PM-related polymorphisms were examined (Table 2). Furthermore, Table 3 and 4 show the results of multivariate logistic regression analysis before and after adjustment for age and physical activity level.

In the PM cases, serum total cholesterol was significantly different between various rs16991615, rs244715, rs4806660, and rs10183486 SNP genotypes; however, this association was not observed in the healthy controls. Interestingly, three of four of the investigated factors including serum total cholesterol, triglyceride, and HDL were substantially associated with different genotypes of rs4806660 SNP in participants with PM and this association was not detected in controls group.

These results for rs1046089 showed individuals with GG genotype were more likely to have low serum LDL (OR= 5.48, CI= 1.14-26.34, p = 0.02) risk than individuals with the AA genotype in control group using a multivariate logistic regression test. Also, the results demonstrate that there was a significant association with high TG risk in CC variant vs. TT in rs10183486 in PM group (OR= 4.63, CI= 1.17-18.29, p = 0.02). Furthermore, these results suggest that individuals carried the recessive homozygous genotype (CC) of rs2303369 SNP compared to individuals with dominant homozygous genotype (TT) had an increased risk of a high serum LDL and low serum HDL and the risk of high TC was decreased in control group

The risk of low HDL was increased in individuals carrying rs2303369 variant (CT) compared to non-carriers (TT) in both studied groups, (OR, 6.6; 95% CI, 1.88-19.53, P=0.003 in PM group) and (OR= 4.21, CI= 1.17-15.18, p = 0.02 in control group).

Discussion

Our findings suggest that serum levels of several parameters in the fasted serum lipid profile consisting of total cholesterol, LDL, and HDL, but not serum triglycerides, were associated with PM. Further analyses indicated that genotypes of polymorphisms, which were previously reported to be related with the incidence of PM, are substantially associated with the level of lipid profile factors in PM cases. Moreover, we found that some genotypes in specific polymorphisms including rs4806660, rs10183486, and rs2303369 SNPs were significantly related to abnormalities regarding total cholesterol, LDL and HDL level in the cases' serum.

Initially our results found significant difference between PM cases and control participants for serum HDL, LDL, and total cholesterol levels, and these factors were significantly higher in the PM group. Gulhan and his coworkers have also found that among the 4 lipid factors, only total cholesterol and LDL were substantially different between cases diagnosed with premature ovarian failure (POF) and control subjects. POF group in this study had higher levels of total cholesterol and LDL ⁴⁶. Gulhan *et al.* work had included only women with previous history of successful childbirth and without any hormone therapy within the last 6 months, this inconsistency in HDL serum level had happened. However, this difference might have occurred due to their use of a small sample size as it was as one third as our study or due to the role of age as a confounding factor and also the role of some genetic variants related to PM. Interestingly, a recent study on 3 Dutch university medical centers did not report any significant difference in lipid profile between previously POI-diagnosed participants and population-based controls ⁴⁷. In their study, secondary amenorrhea (cessation of menstruation for at least 3 consecutive months) was one of the criteria for including POI cases; While, this period was too short compared to our criteria (12 consecutive months) and this important thing might have affected their results. Moreover, they have not indicated whether their participants had any previous history of surgeries, diseases, or taking medications related to female reproductive tract or not; Thus, this factor might have not been considered in their study which cause this disagreement.

Knauff *et al.* have reported changes in lipid profile of cases with POF compared to the controls is potentially related to the rate of ovarian function ⁴⁸. Another study which included cases who enter menopause by surgical ovariectomy, clarified that this intervention on female's reproductive tract, has caused impaired lipid metabolism 6 months post-surgery and substantial increase in all four lipid indicators (Total Cholesterol, Triglyceride, LDL, and HDL) ⁴⁹. Moreover it has been demonstrated that POI cases had significant lower level of Free Androgen Index (FAI) than people with regular menstrual cycles and there, it has been suggested that higher FAI was associated with high serum triglyceride and LDL in POI cases ⁵⁰. Overall, these results suggest that impairment in sexual hormones level, as a result of decreased females' reproductive system activity, is related to weaken lipid metabolism.

Our study has for the first time investigated the association between PM-related polymorphisms' genotypes and lipid profile status. In our analysis, we observed that genotypes of 2 different SNPs including rs4806660 (TMEM150B) and rs10183486 (TLK1) are substantially associated with abnormalities of lipid profile. Regarding the first one, low level of HDL was observed by 5.26 times higher in PM cases with TC genotype rather than CC participants. This association was not found in the controls. It is possible that these results are due to hormonal disorders that occur in postmenopausal individuals and consequently the lipid profile in individuals is impaired. Furthermore, for the second mentioned SNP, hypercholesterolemia was found over 450 percent higher in cases who carried CC than those with TT genotype. Previous studies have reported that both rs4806660 ⁵¹ and rs10183486 ⁵² polymorphisms are associated with the incidence of PM. While it has been proved that TLK1 gene encodes nuclear serine-threonine kinases ⁵² in which actively transfers signals from receptors of estrogen

within the cell membrane to the nucleus⁵³, the exact function of TMEM150B gene product, called transmembrane protein 150B, has not yet been discussed.

Several SNPs, have been found to be associated with PM, a condition in which ovaries stop releasing sexual hormones, especially estrogen, making females infertile as early as before the age of 40. This estrogen hormone deficiency affects metabolism of lipids and thus, PM cases might face some lipid profile abnormalities that could increase the risk of cardiovascular disorders in participants of our study.

Our study was mainly limited by its design as a cross-sectional study, and changes in the level of lipid profile factors were not prospectively assessed. Data regarding pattern of the target population diet for the consumption of oils and meats, as the most common lipids, were not recorded in this study. We suggest future studies consider these limitations to achieve more reliable results. Moreover, several PM-related SNPs were not investigated in our study that might be associated with the cases' metabolic status and it is highly recommended to include these polymorphisms in the analysis.

In conclusion, SNPs which are previously found to be attributed to PM, could cause impairment in cases' lipid profile status through hormonal abnormalities. Present study clarifies that TC, CC and, CT genotypes of rs4806660, rs10183486, and rs2303369 SNPs, respectively increase the rate of dyslipidemia by approximately 5 times compared to their reference genotype (rs4806660: CC; rs10183486: TT; rs2303369: TT) in PM cases. But, GG and CC genotypes of rs1046089 and rs2303369 SNPs, respectively increase the rate of dyslipidemia about 6 times compared to their reference genotype in healthy population.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all subjects using protocols approved by the Ethics Committee of the Mashhad University of Medical Sciences. All participants were able to read and understand and were willing to provide written, informed consent.

Consent to publish

Not applicable

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare no conflict of interests.

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Authors' Contributions

We declare that We contributed significantly towards the research study i.e., (a) conception (M.R M and Z N-F), design (M A and A E-D) and/or analysis and interpretation of data (H Gh, N S-A, M Z-B, A N and M.R S-F-M) and to (b) drafting the article (E H and M Y-Kh) or revising it critically for important intellectual content (H E and G.A F) and on (c) final approval of the version (T H, A P and M G-M) to be published.

Conflict of interest

The authors have no conflict of interest to disclose.

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Tables

Table 1
Demographic features and characteristics of the study population

Characteristics	PM cases, N = 117	Controls, N = 183	P-value
BMI (kg/m ²)	28.78 ± 5.06	29.34 ± 4.22	0.323
FBG (mg/dl)	88.74 ± 20.06	88.96 ± 31.43	0.948
PAL	1.78 ± 0.25	1.70 ± 0.23	0.008
Non smoker	86 (74.8%)	146 (79.8%)	NA
Ex-smoker	10 (8.7%)	9 (4.9%)	0.390
Current smoker	19 (16.5%)	28 (15.3%)	NA
TC (mg/dl)	207.8 ± 35.8	188.8 ± 33.1	< 0.001
TG (mg/dl)	111.0 (82.0-162.5)	117.0 (80.0-159.0)	0.684
LDL-C (mg/dl)	128.39 ± 33.96	110.47 ± 33.42	< 0.001
HDL-C (mg/dl)	47.45 ± 9.33	45.06 ± 11.34	0.040

Data are shown as Mean ± SD; Student t-test and Chi-square test were used; PM: Premature Menopause; SD: Standard Deviation; BMI: Body Mass Index; FBG: Fasting Blood Glucose; PAL: Physical Activity Level; TC: Total Cholesterol; TG: Triglyceride; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

Table 2

Association of genotypes related to PM with lipid profile in our target population divided into PM cases and healthy controls

Polymorphisms	PM cases (N = 117)				Controls (N = 183)			
rs16991615	AA	GA	GG	P	AA	GA	GG	P
Serum Total Cholesterol (mg/dl)	222.3 ± 41.4 ^a	195.8 ± 35.3 ^b	209.4 ± 29.5 ^{ab}	0.01	190.2 ± 33.8	191.2 ± 33.5	186.4 ± 32.7	0.64
Triglyceride (mg/dl)	108.0 (78.0-181.0)	112.0 (82.0-147.0)	107.0 (89.0-156.0)	0.96	98.5 (65.8-121.3)	129.0 (87.0-159.0)	108.0 (79.5-162.3)	0.14
HDL (mg/dl)	48.6 ± 7.7	45.9 ± 8.9	48.0 ± 10.5	0.44	45.0 (40.8-53.0)	44.0 (35.0-53.0)	43.0 (35.6-54.3)	0.58
LDL (mg/dl)	136.1 ± 37.8	120.3 ± 29.9	130.6 ± 34.1	0.15	116.7 ± 35.2	112.7 ± 34.0	107.1 ± 32.5	0.39
rs244715	GG	AG	AA	P	GG	AG	AA	P
Serum Total Cholesterol (mg/dl)	213.3 ± 44.4 ^{ab}	216.7 ± 35.5 ^a	198.8 ± 32.3 ^b	0.04	185.8 ± 25.8	182.9 ± 29.2	192.1 ± 35.0	0.21
Triglyceride (mg/dl)	140.5 (77.5-202.7)	112.0 (85.0-179.0)	108.0 (82.7-151.5)	0.75	107.0 (84.0-231.3)	122.0 (85.5-163.5)	113.0 (77.0-149.5)	0.55
HDL (mg/dl)	46.3 ± 8.6	47.8 ± 8.2	47.4 ± 10.5	0.88	43.5 (33.8-49.3)	42.0 (36.3-55.7)	44.0 (35.6-52.9)	0.93
LDL (mg/dl)	126.8 (111.9-158.8)	134.4 (115.9-146.5)	124.1 (93.1-142.6)	0.18	96.1 ± 36.4 ^{ab}	97.2 ± 34.3 ^a	118.2 ± 30.5 ^b	< 0.001
rs451417	AA	CA	CC	P	AA	CA	CC	P
Serum Total Cholesterol (mg/dl)	201.0 (191.0-236.0)	207.5 (178.0-231.7)	208.5 (181.2-223.2)	0.71	194.5 (174.0-211.5)	181.5 (164.5-201.5)	192.0 (162.0-217.0)	0.31
Triglyceride (mg/dl)	120.0 (82.0-185.0)	111.5 (78.3-153.0)	107.5 (84.5-151.5)	0.51	106.0 (88.3-144.0)	124.0 (77.3-166.5)	115.0 (80.0-154.0)	0.94
HDL (mg/dl)	47.5 ± 9.1	46.2 ± 9.0	48.4 ± 9.8	0.56	45.5 (39.3-54.4)	43.5 (34.3-54.5)	42.8 (36.6-52.1)	0.78

Polymorphisms	PM cases (N = 117)				Controls (N = 183)			
LDL (mg/dl)	132.3 ± 38.3	122.0 ± 29.8	130.7 ± 33.9	0.39	117.4 ± 28.9	104.7 ± 34.1	113.9 ± 33.3	0.14
rs1046089	AA	GA	GG	P	AA	GA	GG	P
Serum Total Cholesterol (mg/dl)	196.0 (177.0-225.0)	207.0 (184.0-232.0)	209.0 (186.5-224.5)	0.76	177.1 ± 23.4	192.5 ± 33.1	188.0 ± 35.0	0.13
Triglyceride (mg/dl)	112.0 (79.0-214.0)	112.0 (82.0-153.0)	109.0 (84.0-154.5)	0.87	112.0 (80.0-155.0)	118.0 (84.0-162.5)	117.0 (76.0-159.0)	0.55
HDL (mg/dl)	50.4 ± 7.7	47.4 ± 9.4	46.6 ± 9.7	0.49	52.0 (40.0–58.0) ^a	45.0 (34.0–55.0) ^{ab}	43.0 (35.1–50.0) ^b	0.03
LDL (mg/dl)	122.5 ± 40.1	127.2 ± 32.8	132.2 ± 34.7	0.65	75.5 (58.6–111.0) ^a	108.0 (87.1–137.0) ^b	122.5 (101.1-138.6) ^{bc}	< 0.001
rs7246479	AA	TA	TT	P	AA	TA	TT	P
Serum Total Cholesterol (mg/dl)	201.5 (196.3-233.5)	200.5 (176.0-225.5)	213.0 (200.0-234.0)	0.12	178.6 ± 21.3	187.2 ± 31.8	191.3 ± 35.1	0.48
Triglyceride (mg/dl)	101.5 (73.3–167.0)	108.0 (81.3-150.7)	118.0 (94.0-186.0)	0.29	145.0 (93.5-164.3)	118.5 (84.7-152.3)	107.0 (77.0-161.0)	0.63
HDL (mg/dl)	48.7 ± 7.1	47.6 ± 9.2	46.8 ± 10.4	0.83	53.5 (41.5–57.7)	44.0 (35.0-52.5)	42.8 (36.0-53.1)	0.26
LDL (mg/dl)	137.9 ± 31.2	123.8 ± 30.7	133.6 ± 39.8	0.23	82.6 ± 29.4 ^a	107.8 ± 33.9 ^{ab}	115.5 ± 32.0 ^b	0.02
rs4806660	CC	TC	TT	P	CC	TC	TT	P
Serum Total Cholesterol (mg/dl)	200.6 ± 34.8 ^{ab}	217.0 ± 34.3 ^a	199.8 ± 35.9 ^b	0.04	161.4 ± 25.1	189.1 ± 33.2	190.6 ± 32.9	0.08
Triglyceride (mg/dl)	93.5 (73.3–168.0) ^{ab}	122.0 (97.5-183.5) ^a	100.0 (73.0-140.0) ^b	0.02	133.0 (100.0-167.0)	111.5 (80.7-160.5)	117.0 (76.7–156.0)	0.66

Polymorphisms	PM cases (N = 117)				Controls (N = 183)			
HDL (mg/dl)	53.5 ± 12.3 ^a	44.9 ± 8.0 ^b	48.7 ± 9.0 ^{ab}	0.01	46.0 (40.0–56.0)	43.7 (35.5–54.3)	43.2 (35.7–52.5)	0.82
LDL (mg/dl)	112.2 ± 32.9	134.7 ± 34.9	125.6 ± 32.1	0.08	73.1 ± 26.1 ^a	104.3 ± 33.0 ^b	118.6 ± 31.4 ^c	< 0.001
rs10183486	TT	CT	CC	P	TT	CT	CC	P
Serum Total Cholesterol (mg/dl)	197.7 ± 27.7 ^{ab}	199.3 ± 34.6 ^a	220.1 ± 35.9 ^b	0.01	193.9 ± 35.3	192.4 ± 36.5	185.2 ± 29.5	0.32
Triglyceride (mg/dl)	97.5 (74.7–119.5)	107.5 (80.7–150.7)	122.0 (94.0–185.0)	0.12	143.0 (91.0–180.5)	111.0 (77.5–144.0)	120.0 (82.5–164.5)	0.23
HDL (mg/dl)	47.2 ± 6.4	47.7 ± 11.2	47.2 ± 7.5	0.95	35.5 (31.4–39.3) ^a	44.0 (39.0–54.5) ^b	43.5 (34.0–53.3) ^{bc}	0.02
LDL (mg/dl)	118.9 ± 25.1 ^{ab}	119.5 ± 31.1 ^a	140.9 ± 35.5 ^b	0.003	120.5 ± 29.3	114.4 ± 34.3	106.1 ± 32.7	0.17
rs2303369	TT	CT	CC	P	TT	CT	CC	P
Serum Total Cholesterol (mg/dl)	206.5 ± 29.7	213.6 ± 36.8	200.8 ± 36.5	0.23	181.9 ± 19.6	192.6 ± 32.8	185.9 ± 35.3	0.19
Triglyceride (mg/dl)	98.0 (69.0–129.0)	117.0 (94.5–182.0)	106.0 (77.5–152.0)	0.05	106.0 (75.0–150.0)	120.0 (84.5–162.3)	117.0 (76.0–154.5)	0.36
HDL (mg/dl)	52.0 ± 10.1 ^a	44.6 ± 8.5 ^b	48.9 ± 9.1 ^{ab}	0.01	56.5 (49.8–60.8) ^a	44.5 (34.0–55.0) ^b	42.8 (35.3–48.7) ^{bc}	0.002
LDL (mg/dl)	117.6 (100.9–139.6)	134.4 (116.3–149.0)	123.6 (103.1–147.8)	0.14	85.8 ± 27.6 ^a	109.7 ± 32.2 ^b	117.1 ± 33.2 ^{bc}	0.001

PM: Premature Menopause; HDL: High-Density Lipoprotein; LDL: Low Density Lipoprotein; One-way analysis of variance (ANOVA) and Kruskal-Wallis tests were used; Anomalous letters indicate a significant difference; Significant P value <0.05

Table 3

Investigation of the relationship between the risk of polymorphisms related to premature menopause and dyslipidemia factors in the study population in a crude model

Polymorphisms Lipid profile abnormalities	PM cases		Controls					
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs16991615	GA/AA		GG/AA		GA/AA		GG/AA	
High LDL (mg/dl)	0.65 (0.24– 1.74)	0.39	1.15 (0.45– 2.97)	0.77	0.65 (0.23– 1.84)	0.42	0.46 (0.16– 1.29)	0.14
High Triglyceride (mg/dl)	0.51 (0.17– 1.50)	0.22	0.88 (0.33– 2.35)	0.79	1.93 (0.51– 7.32)	0.33	2.41 (0.65– 9.03)	0.19
Low HDL (mg/dl)	1.55 (0.55– 4.31)	0.40	1.01 (0.39– 2.66)	0.98	1.08 (0.36– 3.21)	0.89	0.89 (0.30– 2.60)	0.83
High Total Cholesterol (mg/dl)	0.36 (0.13– 1.02)	0.05	0.90 (0.32– 2.51)	0.84	0.69 (0.24– 1.94)	0.48	0.64 (0.23– 1.78)	0.39
rs244715	AG/GG		AA/GG		AG/GG		AA/GG	
High LDL (mg/dl)	2.06 (0.57– 7.47)	0.27	1.04 (0.29– 3.69)	0.95	1.22 (0.13– 11.48)	0.86	3.17 (0.36– 28.01)	0.30
High Triglyceride (mg/dl)	0.42 (0.12– 1.55)	0.19	0.39 (0.11– 1.38)	0.14	1.13 (0.19– 6.66)	0.89	0.67 (0.12– 3.83)	0.65
Low HDL (mg/dl)	0.54 (0.13– 2.25)	0.39	0.52 (0.13– 2.16)	0.37	0.41 (0.05– 3.75)	0.43	0.37 (0.04– 3.24)	0.37
High Total Cholesterol (mg/dl)	1.87 (0.50– 6.95)	0.35	0.77 (0.22– 2.73)	0.69	0.71 (0.12– 4.26)	0.71	1.36 (0.24– 7.74)	0.73
rs451417	CA/AA		CC/AA		CA/AA		CC/AA	
High LDL (mg/dl)	1.24 (0.47– 3.26)	0.67	1.80 (0.72– 4.52)	0.21	0.54 (0.18– 1.55)	0.25	1.19 (0.43– 3.29)	0.74
High Triglyceride (mg/dl)	0.69 (0.25– 1.92)	0.48	0.56 (0.21– 1.49)	0.24	1.96 (0.59– 6.48)	0.27	1.52 (0.46– 5.02)	0.49

Polymorphisms Lipid profile abnormalities	PM cases		Controls					
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Low HDL (mg/dl)	1.26 (0.46– 3.44)	0.65	0.98 (0.39– 2.50)	0.97	0.83 (0.29– 2.31)	0.72	1.34 (0.48– 3.74)	0.58
High Total Cholesterol (mg/dl)	0.88 (0.33– 2.36)	0.81	1.08 (0.42– 2.75)	0.88	0.57 (0.21– 1.60)	0.29	1.06 (0.39– 2.85)	0.91
rs1046089	GA/AA		GG/AA		GA/AA		GG/AA	
High LDL (mg/dl)	2.59 (0.63– 10.63)	0.19	3.50 (0.80– 15.34)	0.10	4.82 (1.06– 21.99)	0.04	6.84 (1.49– 31.47)	0.01
High Triglyceride (mg/dl)	0.46 (0.13– 1.70)	0.24	0.58 (0.15– 2.27)	0.43	1.05 (0.39– 2.84)	0.93	0.78 (0.28– 2.19)	0.63
Low HDL (mg/dl)	2.05 (0.57– 7.44)	0.28	2.50 (0.63– 9.86)	0.19	1.85 (0.74– 4.67)	0.19	3.47 (1.30– 9.26)	0.01
High Total Cholesterol (mg/dl)	1.92 (0.53– 6.96)	0.32	2.22 (0.57– 8.68)	0.25	2.33 (0.79– 6.86)	0.12	1.96 (0.65– 5.90)	0.23
rs7246479	TA/AA		TT/AA		TA/AA		TT/AA	
High LDL (mg/dl)	0.46 (0.13– 1.62)	0.23	1.37 (0.36– 5.25)	0.65	2.87 (0.34– 24.54)	0.34	3.93 (0.46– 33.39)	0.21
High Triglyceride (mg/dl)	1.04 (0.25– 4.30)	0.96	2.25 (0.52– 9.77)	0.28	0.34 (0.08– 1.49)	0.15	0.44 (0.10– 1.87)	0.26
Low HDL (mg/dl)	1.17 (0.34– 4.09)	0.80	1.37 (0.36– 5.25)	0.65	5.60 (1.06– 29.47)	0.04	7.27 (1.38– 38.39)	0.02
High Total Cholesterol (mg/dl)	0.53 (0.15– 1.94)	0.34	1.69 (0.40– 7.10)	0.48	1.53 (0.29– 8.04)	0.62	1.86 (0.35– 9.72)	0.47
rs4806660	TC/CC		TT/CC		TC/CC		TT/CC	
High LDL(mg/dl)	2.73 (0.73– 10.21)	0.14	1.63 (0.43– 6.14)	0.47	NA	NA	NA	NA

Polymorphisms Lipid profile abnormalities	PM cases		Controls					
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
High Triglyceride (mg/dl)	1.88 (0.45–7.77)	0.39	0.97 (0.23–4.19)	0.97	0.62 (0.13–2.97)	0.55	0.46 (0.09–2.19)	0.33
Low HDL(mg/dl)	6.00 (1.55–23.25)	0.01	2.67 (0.71–10.05)	0.15	0.77 (0.14–4.23)	0.77	0.78 (0.14–4.22)	0.77
High Total Cholesterol(mg/dl)	2.14 (0.58–7.93)	0.25	0.63 (0.18–2.27)	0.48	NA	NA	NA	NA
rs10183486	CT/TT		CC/TT		CT/TT		CC/TT	
High LDL (mg/dl)	0.82 (0.23–2.94)	0.77	2.71 (0.74–9.92)	0.13	1.95 (0.38–10.02)	0.42	1.43 (0.28–7.34)	0.67
High Triglyceride (mg/dl)	1.75 (0.34–8.98)	0.50	3.39 (0.67–17.25)	0.14	0.33 (0.08–1.37)	0.13	0.66 (0.17–2.61)	0.55
Low HDL (mg/dl)	0.73 (0.19–2.72)	0.64	0.97 (0.25–3.71)	0.96	0.25 (0.03–2.10)	0.20	0.22 (0.03–1.81)	0.16
High Total Cholesterol (mg/dl)	1.51 (0.43–5.35)	0.53	4.58 (1.21–17.35)	0.03	0.82 (0.20–3.27)	0.77	0.57 (0.14–2.27)	0.42
rs2303369	CT/TT		CC/TT		CT/TT		CC/TT	
High LDL (mg/dl)	2.42 (0.82–7.12)	0.11	1.34 (0.44–4.10)	0.61	6.77 (0.85–53.84)	0.07	9.06 (1.14–72.26)	0.04
High Triglyceride (mg/dl)	3.23 (0.84–12.50)	0.09	2.21 (0.54–8.99)	0.27	1.43 (0.42–4.81)	0.57	1.05 (0.30–3.64)	0.94
Low HDL (mg/dl)	5.86 (1.89–18.18)	0.002	2.19 (0.72–6.70)	0.17	5.18 (1.55–17.38)	0.01	10.59 (3.02–37.08)	< 0.001
High Total Cholesterol (mg/dl)	0.76 (0.23–2.44)	0.64	0.31 (0.09–1.01)	0.05	2.89 (0.77–10.86)	0.12	2.21 (0.58–8.45)	0.25

Multivariate logistic regression models were performed; PM: Premature Menopause; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; High LDL >130 and Normal LDL <130; High Triglyceride >150

and Normal Triglyceride <150; Low HDL <40 (Male) or <50 (Female) and Normal HDL >40 (Male) or >50 (Female), High Total Cholesterol >200 and Normal Total Cholesterol <200; Normal groups of all lipid profile measures were considered as references; The common genotypes of each studied variants were considered as references; Significant P-value <0.05.

Table 4

Investigation of the relationship between the risk of polymorphisms related to premature menopause and dyslipidemia factors in the study population in an adjusted model for age and physical activity level

Polymorphisms Lipid profile abnormalities	PM cases				Control			
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
rs16991615	GA/AA		GG/AA		GA/AA		GG/AA	
High LDL (mg/dl)	0.63 (0.23– 1.73)	0.37	1.21 (0.46– 3.18)	0.69	0.65 (0.21– 2.05)	0.47	0.37 (0.12– 1.17)	0.09
High Triglyceride (mg/dl)	0.45 (0.15– 1.37)	0.16	0.85 (0.31– 2.35)	0.76	1.46 (0.36– 5.81)	0.59	1.79 (0.46– 7.01)	0.39
Low HDL (mg/dl)	1.46 (0.52– 4.13)	0.46	0.97 (0.36– 2.59)	0.96	0.75 (0.23– 2.44)	0.64	0.62 (0.19– 1.97)	0.42
High Total Cholesterol (mg/dl)	0.35 (0.12– 0.99)	0.05	0.92 (0.32– 2.59)	0.87	0.68 (0.22– 2.11)	0.51	0.55 (0.18– 1.68)	0.29
rs244715	AG/GG		AA/GG		AG/GG		AA/GG	
High LDL (mg/dl)	2.12 (0.57– 7.83)	0.25	1.02 (0.28– 3.70)	0.96	1.18 (0.12– 11.38)	0.88	3.17 (0.35– 28.53)	0.30
High Triglyceride (mg/dl)	0.39 (0.10– 1.48)	0.17	0.37 (0.10– 1.37)	0.13	1.51 (0.24– 9.55)	0.65	0.76 (0.12– 4.65)	0.77
Low HDL (mg/dl)	0.51 (0.12– 2.18)	0.36	0.51 (0.12– 2.17)	0.36	0.51 (0.05– 4.81)	0.55	0.40 (0.04– 3.69)	0.42
High Total Cholesterol (mg/dl)	1.87 (0.50– 7.02)	0.35	0.76 (0.21– 2.71)	0.67	0.68 (0.11– 4.20)	0.68	1.34 (0.23– 7.68)	0.74
rs451417	CA/AA		CC/AA		CA/AA		CC/AA	
High LDL (mg/dl)	1.26 (0.46– 3.41)	0.64	1.81 (0.71– 4.61)	0.21	0.51 (0.17– 1.52)	0.23	0.98 (0.34– 2.79)	0.97
High Triglyceride (mg/dl)	0.56 (0.19– 1.62)	0.56	0.52 (0.19– 1.42)	0.20	1.83 (0.54– 6.19)	0.32	1.32 (0.39– 4.49)	0.65

Polymorphisms Lipid profile abnormalities	PM cases		Control					
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Low HDL (mg/dl)	1.11 (0.40– 3.11)	0.83	0.94 (0.36– 2.44)	0.91	0.73 (0.25– 2.11)	0.56	1.24 (0.42– 3.63)	0.68
High Total Cholesterol (mg/dl)	0.83 (0.30– 2.28)	0.72	1.07 (0.41– 2.76)	0.88	0.55 (0.19– 1.56)	0.26	0.89 (0.32– 2.48)	0.83
rs1046089	GA/AA		GG/AA		GA/AA		GG/AA	
High LDL (mg/dl)	2.53 (0.61– 10.52)	0.20	3.65 (0.82– 16.24)	0.08	3.37 (0.70– 16.27)	0.12	5.48 (1.14– 26.34)	0.03
High Triglyceride (mg/dl)	0.45 (0.11– 1.71)	0.24	0.60 (0.14– 2.47)	0.48	0.65 (0.21– 2.02)	0.46	0.48 (0.15– 1.54)	0.22
Low HDL (mg/dl)	2.12 (0.57– 7.83)	0.25	2.69 (0.67– 10.80)	0.16	1.41 (0.51– 3.88)	0.50	2.63 (0.93– 7.47)	0.06
High Total Cholesterol (mg/dl)	1.91 (0.52– 7.01)	0.32	2.28 (0.57– 9.04)	0.23	1.63 (0.52– 5.14)	0.39	1.49 (0.47– 4.75)	0.49
rs7246479	TA/AA		TT/AA		TA/AA		TT/AA	
High LDL (mg/dl)	0.45 (0.12– 1.63)	0.23	1.47 (0.37– 5.81)	0.57	1.68 (0.18– 15.66)	0.64	2.09 (0.22– 19.70)	0.52
High Triglyceride (mg/dl)	0.95 (0.22– 4.04)	0.95	2.31 (0.51– 10.33)	0.27	0.18 (0.03– 1.02)	0.05	0.18 (0.03– 1.08)	0.06
Low HDL (mg/dl)	1.09 (0.30– 3.88)	0.89	1.32 (0.33– 5.18)	0.68	4.37 (0.76– 24.92)	0.09	5.33 (0.90– 31.30)	0.06
High Total Cholesterol (mg/dl)	0.52 (0.14– 1.94)	0.33	1.77 (0.41– 7.54)	0.43	0.83 (0.14– 4.98)	0.84	0.93 (0.15– 5.68)	0.94
rs4806660	TC/CC		TT/CC		TC/CC		TT/CC	
High LDL (mg/dl)	2.67 (0.68– 10.48)	0.15	1.58 (0.39– 6.33)	0.51	-	-	-	-

Polymorphisms Lipid profile abnormalities	PM cases		Control					
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
High Triglyceride (mg/dl)	1.53 (0.35–6.61)	0.56	0.70 (0.15–3.21)	0.64	0.51 (0.10–2.63)	0.42	0.36 (0.07–1.85)	0.22
Low HDL (mg/dl)	5.26 (1.32–20.94)	0.01	2.19 (0.56–8.62)	0.25	0.63 (0.10–3.88)	0.61	0.60 (0.10–3.68)	0.58
High Total Cholesterol (mg/dl)	2.14 (0.56–8.22)	0.26	0.59 (0.15–2.25)	0.44	NA	NA	NA	NA
rs10183486	CT/TT		CC/TT		CT/TT		CC/TT	
High LDL (mg/dl)	0.70 (0.18–2.61)	0.59	2.41 (0.64–9.10)	0.19	2.01 (0.38–10.55)	0.40	1.29 (0.24–6.78)	0.76
High Triglyceride (mg/dl)	1.87 (0.33–10.40)	0.47	3.86 (0.70–21.30)	0.12	0.35 (0.08–1.52)	0.16	0.68 (0.16–2.83)	0.60
Low HDL (mg/dl)	0.74 (0.19–2.91)	0.67	1.02 (0.25–4.09)	0.97	0.27 (0.03–2.31)	0.23	0.22 (0.02–1.89)	0.16
High Total Cholesterol (mg/dl)	1.50 (0.40–5.58)	0.54	4.63 (1.17–18.29)	0.02	0.82 (0.20–3.35)	0.78	0.51 (0.12–2.08)	0.34
rs2303369	CT/TT		CC/TT		CT/TT		CC/TT	
High LDL (mg/dl)	2.18 (0.72–6.55)	0.16	1.22 (0.39–3.84)	0.72	4.40 (0.52–36.78)	0.17	6.94 (0.83–57.53)	0.07
High Triglyceride (mg/dl)	3.01 (0.75–11.97)	0.11	1.84 (0.43–7.74)	0.40	0.97 (0.25–3.72)	0.96	0.73 (0.18–2.81)	0.64
Low HDL (mg/dl)	6.06 (1.88–19.53)	0.003	2.03 (0.64–6.41)	0.22	4.21 (1.17–15.18)	0.02	8.55 (2.33–31.25)	0.001
High Total Cholesterol (mg/dl)	0.68 (0.20–2.26)	0.53	0.26 (0.07–0.90)	0.03	1.92 (0.47–7.75)	0.35	1.62 (0.40–6.57)	0.49

Multivariate logistic regression models were performed; PM: Premature Menopause; LDL: Low-Density Lipoprotein; HDL: High-Density Lipoprotein; High LDL >130 and Normal LDL <130; High Triglyceride >150

and Normal Triglyceride <150; Low HDL <40 (Male) or <50 (Female) and Normal HDL >40 (Male) or >50 (Female), High Total Cholesterol >200 and Normal Total Cholesterol <200; Normal groups of all lipid profile measures were considered as references; The common genotypes of each studied variants were considered as references; Adjusted with Age & Physical Activity Level; Significant P-value <0.05.