PPARα polymorphisms association with total cholesterol and LDL-C levels in a Mexican population

A.I. ORTEGA-MELÉNDEZ[1,2], S. MONTERO-MOLINA[1], R.F. JIMÉNEZ-ORTEGA[3], E. RAMÍREZ-LÓPEZ[4], E. CAMPOS-GÓNGORA[4], R. VELÁZQUEZ-CRUZ[5], Z. JIMÉNEZ-SALAS[4], I. BALDERAS-RENTERÍA[1]

[1]Universidad Autónoma de Nuevo León, Facultad de Ciencias Químicas, Laboratorio de Farmacología Molecular y Modelos Biológicos, San Nicolás de los Garza, Nuevo León, México
[2]Universidad ETAC, Campus Coacalco, Estado de México, México
[3]Universidad Privada del Estado de México, Licenciatura en Nutrición, Texcoco, Estado de México, México
[4]Universidad Autónoma de Nuevo León, Centro de Investigación en Nutrición y Salud Pública (CINSP), Monterrey, Nuevo León, México
[5]Instituto Nacional de Medicina Genómica (INMEGEN), Laboratorio de Genómica del Metabolismo Óseo, Ciudad de México, México.

Abstract. – **OBJECTIVE:** Peroxisome proliferator-activated receptor alpha (PPARα) is a nuclear transcription factor with a role in gene expression changes associated to lipid metabolism. PPARα polymorphic variants have been previously correlated to serum lipid profile but in Mexico, there is no previous report about that association. For this reason, the aim of this study was to investigate the relationship between PPARα polymorphic variants and lipids level in serum in a Mexican population.

PATIENTS AND METHODS: Two-hundred and forty women from the Northeast region of Mexico were included in the study. Anthropometric characteristics and serum lipid profile (such as triglycerides, total cholesterol, LDL cholesterol and HDL cholesterol) were evaluated. Genomic DNA extraction and purification were made from blood samples. Real-time PCR and TaqMan probes were used for genotyping of rs1800206 and rs4253778 single nucleotide polymorphisms (SNPs).

RESULTS: Linear regression analysis (adjusted by age and body mass index (BMI)) showed a significant statistical association of rs4253778 with total cholesterol (*p*=0.034) and LDL cholesterol (*p*=0.037). Any significant association was found between rs1800206 and lipid levels.

CONCLUSIONS: These results suggested that rs4253778 (C allele) is associated with high levels of total cholesterol and LDL in a Mexican women population.

Key Words:
Cardiovascular disease (CVD), Peroxisome-proliferator activated receptor alpha (PPARα), Single nucleotide polymorphism (SNP).

Introduction

Cardiovascular diseases (CVD) are the leading cause of morbidity and mortality worldwide. Although rates of age-adjusted mortality have declined in developed countries, in low and middle-income countries have increased significantly[1]. In Mexico, one of the main cardiovascular risk factors is blood cholesterol, which can lead to acute myocardial infarction as well as for silent myocardial ischemia. It was reported that 34% of population had blood cholesterol levels between 200-240 mg/dL and 16.4 % had levels above 240 mg/dL[2], especially in women and men over 60 years[3].

Cardiovascular disease is a multifactorial disorder that is been thought to result from an interaction between genetic background and environmental factors such as diet, smoking and physical activity. It is usually associated with conventional risk factors, including hypertension, diabetes mellitus and dyslipidemia[4]. It is well-known that high LDL-cholesterol (LDL-c) and triglycerides (TG) levels and low HDL-cholesterol (HDL-c) levels are risk predictable factors for cardiovascular events[5]. Epidemiological data have indicated that dyslipidemias are the most prevalent risk factor for cardiovascular diseases in Mexico, coupled with a continuous increase in prevalence rate during the last decades[6]. The exact cause of dyslipidemia is not known.

Genetics play an important role on lipid homeostasis. The peroxisome proliferator-activated

receptor alpha (PPARα), which is a ligand-activated transcriptional factor that belongs to the family of nuclear receptor and play a critical physiological role as lipid sensor and regulator of lipid metabolism. It is mainly expressed in tissues with extensive fatty acid catabolism and its activation leads to changes in the transcription expression of genes involved in fatty acid beta-oxidation and is a major regulator of energy homeostasis[7].

Common variants of the PPARα have been described. The rs1800206 and rs4253778 SNPs has been associated with variation in lipid serum levels in European and Asian populations[8-11]. The rs1800206:C>G or L162V is a leucine-to-valine change in codon 162, represented by a C to G substitution at the DNA-binding domain, encodes for a more active PPARα depending on the concentration of the ligand. The L162V polymorphism has been associated with increased levels of triglycerides, total cholesterol, LDL cholesterol and ApoA1 and ApoB[12]. On the other hand, the intron 7 polymorphism (rs4253778: G>C) consist in a G to C substitution. The 7C allele is associated with a significantly earlier age at diagnosis of increased total cholesterol and LDL cholesterol[12,13]. However, there are only a few studies for Mexican population, for this reason, the aim of this study was to perform an analysis of association between rs1800206 and rs4253778 polymorphisms and serum lipid profile (LDL cholesterol, HDL cholesterol, total cholesterol and triglycerides) in women from a Northeastern Mexican population.

Patients and Methods

Participants and Study Design

Eligible participants were women between 18 and 50 years old and born in Northeast Mexico, whose parents and grandparents identified themselves as Mexican mestizos. Two hundred and forty unrelated women were recruited in the Faculty of Public Health and Nutrition (FASPyN), Monterrey, Nuevo León, Mexico. Pregnant women, athletes and women with history of diseases that could alter the lipid profile (such as dyslipidemia, diabetes and CVD) were excluded from the study. Data related to demographic characteristics and information regarding smoking status, menopausal status, estrogen use, medical history and use of medication were obtained from self-administered questionnaires. FASPyN Research Ethics Committee approved this study (Registration Number 15-FaSPyN-SA-01) and all participants signed the informed consent form.

Anthropometric and Biochemical Evaluations

Anthropometric parameters, such as body weight, height, waist and hip circumference were routinely measured in this study. Furthermore, total cholesterol, LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), glucose and triglycerides were measured from non-fasting venous samples using enzymatic methods implemented in the Advia 2400 Chemistry System (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA). LDL-C and HDL-C were measured using direct assays.

Genomic DNA Extraction

Blood samples were obtained and stored at 4°C until use. Genomic DNA was extracted from the peripheral blood of all participants using a commercial isolation kit (QIAGEN system Inc., Valencia, CA, USA), according to the manufacturer's instructions.

Single Nucleotide Polymorphism Selection and Genotyping

Single nucleotide polymorphisms (SNPs) were identified through a research in dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). We selected two SNPs of the PPARα gene on the basis of the following criteria: (1) validation status (validated in Western Eurasian, Latin American and Asian ancestry); (2) degree of heterozygosity, i.e., minor allele frequencies (MAF) $\geq$ 0.05, and (3) previous evidence of association with lipid measurements[14].

Genotyping of SNPs was performed using commercial predesigned TaqMan Probes (Applied Biosystems, Foster City, CA, USA) in QuantStudio 6 Real-Time PCR System. The sequence of the probe for rs1800206 was: CCAGTATTGTCGATTTCACAAGTGCC/GTTTCTGTCGGGATGTCACACAACGG and ACACTTGAAGCTTGATATCTAGTTTG/CGATTCAAAAGCTTCATTTCCCATAT for rs4253778. Genotyping quality control was performed with PLINK software[15]. Genotyping success rate was expected to be > 95 %, minor allele frequency $\geq$ 0.05 % and $p \geq$ 0.001 for Hardy-Weinberg equilibrium.

Statistical Analysis

All data from the study population are shown as mean $\pm$ SD (standard deviation) for quantitative variables and absolute and relative frequencies for qualitative variables. Hardy-Weinberg equilibrium was tested for each SNP using the standard x^2 test. Linear regression analysis was used

for testing association between serum lipid profile and genotype using an additive genetic model adjusted by age and BMI. All statistical analysis were performed with Statistical Package for Social Sciences software (SPSS 20.0; SPSS Inc., Chicago, IL, USA) and PLINK. p-values < 0.05 were considered statistically significant. Linkage disequilibrium (LD) and haplotype frequencies were estimated using Haploview 4.2[16].

Statistical power was calculated with Quanto 1.1 software, for a significance level of 0.05 and MAF of 5% in 240 unrelated young women between 18-50 years of age with a minimal power of 80% to detect differences in lipid profile, under an additive model. The significance threshold after multiple test correction for each gene was estimated using the Bonferroni correction. Multiple logistic regression models for risk alleles were performed to identify the associations of the genotypes with lipid profile (triglycerides, cholesterol, LDL cholesterol and HDL cholesterol), adjusting for confounding variables such as age or height.

Results

Anthropometric and Biochemical Evaluations

Results were expressed in mean ± SD. For anthropometric characteristics, the mean age was 29.56 ± 10.53 years old, weight 64.17 ± 14.01, BMI 25.33 ± 5.39 kg/m^2, glucose 87.55 ± 25.61 mg/dL and systolic and diastolic arterial pressure values were normal. Biochemical evaluation results are shown in Table I and mean values were within the normal range.

Genotyping

Genotyping was successfully performed using the extracted DNA. Data from 14 samples in which

Table I. Anthropometric and biochemical evaluations of the study population.

Variables	Mean ± SD
Age (years)	29.56 ± 10.53
Systolic Pressure (mmHg)	106.31 ± 11.67
Diastolic Pressure (mmHg)	69.03 ± 10.96
Height (cm)	159.15 ± 5.75
Weight (Kg)	64.17 ± 14.01
BMI (Kg/m^2)	25.33 ± 5.39
Waist circumference (cm)	85.86 ± 13.23
Hip circumference (cm)	100.87 ± 10.37
Blood glucose (mg/dL)	87.55 ± 25.61
Triglycerides (mg/dL)	97.10 ± 45.32
Total cholesterol (mg/dL)	156.70 ± 38.26
HDL cholesterol (mg/dL)	47.98 ± 11.79
LDL cholesterol (mg/dL)	89.29 ± 37.65

N = 240. BMI = Body mass index. HDL = High density lipoprotein. LDL = Low density lipoprotein. SD = Standard deviation.

DNA was not genotyped successfully were excluded. Both SNPs analyzed in this study showed a minor allele frequency (MAF) > 5 % in Western Eurasian ancestry population according to dbSNP database. Allele and genotype frequencies of both SNPs are shown in Table II. A MAF of 4.65 % and 13.77 % were reported for rs1800206 and rs4253778, respectively. Hardy-Weinberg equilibrium showed a value of p=1.0 for rs1800206 and p=0.153 for rs4253778. The distributions of allele and genotype frequencies of both SNPs did not differ from Hardy-Weinberg equilibrium. Therefore, association results obtained in this study can be considered as reliable.

PPARα Polymorphisms and Serum Lipid Profile

Both PPARα SNPs were analyzed in searching of allelic association, adjusted by age and BMI. To know if there is a relationship between the SNPs

Table II. Allele and genotype frequencies and p values of Hardy-Weinberg equilibrium for analyzed SNPs.

SNP	Genotype	n	%	H-W equilibrium
rs1800206	C/C	205	90.70	p=1.000
	C/G	21	90.30	
	G/G	0	0	
Allele frequency	C	431	95.35	
	G	21	4.65	
rs4253778	C/C	7	3.11	p=0.153
	C/G	48	21.33	
	G/G	170	75.56	
Allele frequency	C	62	13.77	
	G	388	86.23	

Table III. rs1800206 polymorphism association with anthropometric and biochemical measurements.

Variable	CC (n=205) Mean ± SD	CG (n=21) Mean ± SD	β	Confidence interval	*p*	*p* adjust
Weight (Kg)	64.31 ± 14.20	61.19 ± 10.17	- 3.122	-9.335 ; 1.265	0.225	0.200
BMI (Kg/m²)	25.40 ± 5.50	24.01 ± 3.81	- 1.384	-3.788 ; 1.020	0.259	0.127
Blood glucose (mg/dL)	87.18 ± 25.85	89.94 ± 28.76	2.678	-9.183 ; 14.593	0.658	0.775
Triglycerides (mg/dL)	97.31 ± 43.64	89.76 ± 47.13	- 8.346	-29.114 ; 12.421	0.431	0.325
Total cholesterol (mg/dL)	156.70 ± 38.73	160.87 ± 42.51	9.863	-10.639 ; 30.366	0.346	0.681
HDL cholesterol (mg/dL)	47.89 ± 12.00	49.52 ± 11.64	1.635	-3.842 ; 7.112	0.558	0.479
LDL cholesterol (mg/dL)	89.34 ± 37.54	93.38 ± 40.06	4.040	-13.238 ; 21.317	0.647	0.680

SD = Standard deviation.

and some of the anthropometric, biochemical and body composition variables in our study population, a linear regression analysis was performed with the rs1800206 and rs4253778 SNPs of the PPARα gene, adjusting for age for the codominant model. Results from statistical analysis showed that rs1800206 was not associated to any variable (Table III). For rs4253778 SNP, a significant statistical association was found with total cholesterol (β= -13.55, *p*=0.034) and LDL (β=-10.56, *p*=0.037). No association was found with HDL cholesterol and triglycerides (Table IV). Linkage disequilibrium (LD) did not reveal a significant relation between PPARα SNPs. A significant LD value (r² = 0.53) was observed.

Discussion

Cardiovascular diseases are a severe public health problem that generate high socioeconomic costs and is the leading cause of death globally. Association studies and genome-wide analysis using polymorphic markers across the genome have provided a useful method for identifying CVD-related genes, as well as helping to identify quantitatively genetic loci that influence variables involved in CVD pathogenesis[17].

In this study, we analyzed the association of two polymorphisms of PPARα gene (rs1800206 and rs4253778) with profiles of serum lipids, LDL, HDL, total cholesterol and triglycerides in women 18 to 50 years old from the northeast region of Mexico. We found that SNP rs1800206 did not present a significant association with any of the variables and genotype frequencies obtained were similar to previous reports[18]. Gu et al[9] analyzed the association of two PPARα variants (rs1800206 and rs4253778) and a statistical significance was reported with dyslipidemia under a codominant and additive model (*p*<0.001) in an Asian population composed of 820 individuals. They reported that CG genotype appeared to have a higher risk for dyslipidemia. Previously, Costa-Urrutia et al[19] reported an association between allele G of PPARα rs1800206 and central obesity risk in a Mexican-mestizo population. However, Binia et al[20] reported a study for rs1800206 in a Mexican population as well and they did not find association with total cholesterol, LDL cholesterol or HDL cholesterol, which is consistent with our results. Additionally, it is possible that a study with a larger population could show results similar to other previous reports, where a statistically significant association was found between the

Table IV. rs4253778 polymorphism association with anthropometric and biochemical measurements.

Variable	CC (n=7) Mean ± SD	CG (n=48) Mean ± SD	GG (n=170) Mean ± SD	β	Confidence interval	*p*	*p* adjust
Weight (Kg)	63.43 ± 11.81	66.55 ± 15.88	63.29 ± 13.38	- 1.958	-5.492 ; 1.586	0.279	0.454
BMI (Kg/m²)	24.83 ± 3.87	26.19 ± 5.64	24.99 ± 5.35	- 0.672	-2.041 ; 0.697	0.336	0.568
Blood glucose (mg/dL)	86.09 ± 22.79	90.53 ± 39.33	86.70 ± 21.23	- 3.623	-10.299 ; 7.983	0.282	0.706
Triglycerides (mg/dL)	85.90 ± 28.45	98.13 ± 52.48	96.75 ± 41.95	- 0.924	-12.834 ; 10.986	0.897	0.524
Total cholesterol (mg/dL)	186.54 ± 46.06	161.92 ± 39.91	154.49 ± 37.40	- 13.551	-25.177 ; -1.926	0.022	**0.034***
HDL cholesterol (mg/dL)	56.22 ± 9.52	46.44 ± 10.74	48.11 ± 12.30	- 0.685	-3.744 ; 2.374	0.661	0.511
LDL cholesterol (mg/dL)	113.13 ± 54.28	95.85 ± 38.66	87.02 ± 36.43	- 10.563	-20.117 ; -1.009	0.030	**0.037***

SD = Standard deviation, * *p* < 0.05.

SNP rs1800206 with changes in triglycerides, total cholesterol, LDL-cholesterol, and HDL-cholesterol in Western Eurasian ancestry, Indian, and African American populations[13,21-24].

On the other hand, rs4253778 presented a significant statistical association with total cholesterol (p=0.030) and LDL-cholesterol (p=0.037). These data were also consistent with those reported by Gu et al[9] where they observed an association with an increase in total cholesterol, LDL-cholesterol, progression of atherosclerosis and an increase in the risk of myocardial infarction. Although it seems that the rs4253778 polymorphism has an influence on alterations in metabolism, the results obtained in this work did not show an association with a modified lipid profile.

Interestingly, according to what was reported by Mazzotti et al[25] and Chen et al[26], they observed divergences in the association results of the two SNPs analyzed between two different populations in South America (São Paulo and Ciudad de Cuiaba). These studies showed that in the population of São Paulo the C allele of rs4253778 was associated with high HDL-cholesterol levels, low levels of triglycerides and low levels of very low-density lipoproteins. However, the population of Ciudad de Cuiaba was found associated with dyslipidemia. These results are controversial and could be explained by the differential stratification of the two populations studied.

One of the most important SNPs of PPARα gene is rs4253778 and HaploReg database has predicted this SNP as a variation that alters the binding sites of members of the IRF family of transcription factors (encoded by 9 genes in humans IRF1-IRF9)[27]. It is possible that this polymorphism is in linkage disequilibrium with a functional variant and some region of the PPARα gene that may result in its expression[28]. On the other hand, the G allele of SNP rs4253778 is associated with an increased fatty acid oxidation and an increase in the development of slow-twitch muscle fibres, which use oxygen more efficiently in continuous muscle activity, characteristic of endurance athletes[29].

In addition, it is essential to mention that Mexican population is a mixture with a complex genetic structure that includes a European component, a Native American component and a small portion of African genes[30,31]. Spurious association signals produced by differences in ancestral background can be a confusing factor in genetic association studies[32,33]. Unfortunately, in this work we did not have data on ancestry markers for our study population. In this way, spurious associations caused by population stratification could be avoided[34].

Limitations

The present study has some limitations: first, the SNPs of the PPARα gene were selected based on previous reports from Europe, South America, and Asia, therefore, other SNPs could contribute to the variation of serum lipid profile, in Mexican population. Second, the lack of association between SNP rs1800206 and serum lipid profile may be due to the small sample size. Although our sample size is the minimum necessary to identify the effect of the PPARα gene variants with the variation of lipid profiles, it is possible that an increase in our population significantly improves the data obtained. Finally, our study does not have ancestry markers that help to avoid spurious results due to differences in the ancestral background.

It is important to keep looking for some indicators that help researchers to predict the risk for cardiovascular events in order to exert preventive actions to diminish the prevalence of diseases associated to dyslipidemia. Although additional studies are needed, these results could be helpful to identify genetic risk factors in Mexican population. Our results suggest the association between the rs4253778 polymorphism of PPARα and total cholesterol and LDL cholesterol in women from Northeast Mexico.

Conclusions

In the present study, we carried out a report of the association of the rs1800206 and rs4253778 polymorphisms with serum lipids, LDL, HDL, total cholesterol and triglycerides in women from the Northeast of Mexico. Our results suggested an association with lipid profiles in blood serum that are similar to data reported from other geographical regions, supporting the hypothesis that these polymorphisms play an important role in the variation of serum lipid levels, in this population.

would also like to extend our gratitude to the volunteers who participated in this study.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Authors' Contributions

Alejandra I. Ortega-Meléndez was responsible for data acquisition, perform the experiments and drafting of manuscript.
Sonia Montero-Molina and Rogelio F. Jiménez-Ortega collaborated in study design, performed analysis and interpretation of data, as well as drafting of manuscript.
Erik Ramírez-López carried out anthropometric parameters determination and contributed to interpretation of data and critical revision of manuscript.
Eduardo Campos-Góngora and Rafael Velázquez-Cruz participated in study design and critical revision of the manuscript.
Zacarías Jiménez-Salas and Isaías Balderas-Rentería were responsible for the study conception and design, drafting and revision of the manuscript for important intellectual content of the article.
All authors read and approved the submitted article.

References

1) Anand S, Bradshaw C, Prabhakaran D. Prevention and management of CVD in LMICs: why do ethnicity, culture and context matter? BMC Med 2020; 18: 7.

2) Escobedo-de la Peña J, de Jesús-Pérez R, Schargrodsky H, Champagne B. Prevalence of dyslipidemias in Mexico City and its relation to other cardiovascular risk factors. Results from the CARMELA study. Gac Med Mex, 2014; 150: 128-136.

3) Shamah-Levy T, Vielma-Orozco E, Heredia-Hernández O, Romero-Martínez M, Mojica-Cuevas J, Cuevas-Nasu J, Santaella-Castell JA, Rivera-Dommarco J. Encuesta Nacional de Salud y Nutrición 2018-2019. Resultados Nacionales, Instituto Nacional de Salud Pública. 2020; ISBN 978-607-511-205-3.

4) Hirashiki A, Yamada Y, Murase Y, Suzuki Y, Kataoka H, Morimoto Y, Tajika T, Murohara T, Yokota M. Association of gene polymorphisms with coronary artery disease in low- or high-risk subjects defined by conventional risk factors. J Am Coll Cardiol 2003; 42: 1429-1437.

5) Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000; 86: 943-949.

6) Hernández-Alcaraz C, Aguilar-Salinas CA, Mendoza-Herrera K, Pedroza-Tobías A, Villalpando S, Shamah-Levy T, Rivera-Dommarco J, Hernández-Ávila M, Barquera S. Dyslipidemia prevalence, awareness, treatment and control in Mexico: results of the Ensanut 2012. Salud Publica Mex 2020; 62: 137-146.

7) Fruchart JC. Peroxisome proliferator-activated receptor-alpha (PPARalpha): at the crossroads of obesity, diabetes and cardiovascular disease. Atherosclerosis 2009; 205: 1-8.

8) Lacquemant C, Lepretre F, Pineda-Torra I, Manraj M, Charpentier G, Ruiz J, Staels B, Froguel P. Mutation screening of the PPARalpha gene in type 2 diabetes associated with coronary heart disease. Diabetes Metab 2000; 26: 393-401.

9) Gu SJ, Guo ZR, Zhou ZY, Hu XS, Wu M. PPAR α and PPAR γ polymorphisms as risk factors for dyslipidemia in a Chinese Han population. Lipids Health Dis 2014; 26: 13-23.

10) Vohl MC, Lepage P, Gaudet D, Brewer CG, Bétard C, Perron P, Houde G, Cellier C, Faith JM, Després JP, Morgan K, Hudson TJ. Molecular scanning of the human PPARa gene: association of the L162V mutation with hyperapobetalipoproteinemia. J Lipid Res 2000; 41: 945-952.

11) Yong EL, Li J, Liu MH. Single gene contributions: genetic variants of peroxisome proliferator-activated receptor (isoforms alpha, beta/delta and gamma) and mechanisms of dyslipidemias. Curr Opin Lipidol 2008; 19: 106-112.

12) Contreras AV, Torres N, Tovar AR. PPAR-α as a key nutritional and environmental sensor for metabolic adaptation. Adv Nutr 2013; 4: 439-452.

13) Purushothaman S, Ajitkumar VK, Renuka Nair, RR. Association of PPARα intron 7 polymorphism with coronary artery disease: A cross-sectional study. ISRN Cardiol 2011; 816025.

14) Frazier-Wood AC, Ordovas JM, Straka RJ, Hixson JE, Borecki IB, Tiwari HK, Arnett DK. The PPAR alpha gene is associated with triglyceride, low-density cholesterol and inflammation marker response to fenofibrate intervention: the GOLDN study. Pharmacogenomics J 2013; 13: 312-317.

15) Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ, Sham PC. PLINK: A tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–575.

16) Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263-265.

17) Cymet T. Genetics of cardiovascular disease. Compr Ther 2010; 36: 18–19.

18) A global reference for human genetic variation, The 1000 Genomes Project Consortium, Nature 2015; 526: 68-74.

19) Costa-Urrutia P, Abud C, Franco-Trecu V, Colistro V, Rodríguez-Arellano ME, Vázquez-Pérez J, Granados J, Seelaender M. Genetic obesity risk and attenuation effect of physical fitness in Mexican-Mestizo Population: a case-control study. Ann Hum Genet 2017; 81: 106-116.

20) Martínez-Conde D, Hernández-Morán B, Ramírez-Solano M, Pérez-Ortega C, Rodríguez-Carmona Y, Castan I, Rubio-Aliaga I, Vadillo-Ortega F, Márquez-Velasco R, Bojalil R, López-Alvarenga JC, Valet P, Kussmann M, Silva-Zolezzi I, Tejero ME. Improvement of cardiometabolic markers after fish oil intervention in young Mexican adults and the role of PPARα L162v and PPARɡ2 P12A. J Nutr Biochem 2017; 43: 98-106.

21) Flavell DM, Pineda-Torra I, Jamshidi Y, Evans D, Diamond JR, Elkeles RS, Bujac SR, Miller G, Talmud PJ, Staels B, Humphries SE. Variation in the PPARalpha gene is associated with altered function in vitro and plasma lipid concentrations in Type II diabetic subjects. Diabetologia 2000; 43: 673-680.

22) Shin MJ, Kanaya AM, Krauss RM. Polymorphisms in the peroxisome proliferator activated receptor α gene are associated with levels of apolipoprotein CIII and triglyceride in African-Americans but not in Caucasian. Atherosclerosis 2008; 198: 313-319.

23) Flavell DM, Jamshidi Y, Hawe E, Pineda-Torra I, Taskinen MR, Frick MH. Peroxisome proliferator-activated receptor α gene variants influence progression of coronary atherosclerosis and risk of coronary artery disease. Circulation 2002; 105: 1440-1445.

24) Zak I, Balcerzyk A, Sarecka B, Niemiec P, Ciemniewski Z, Dyląg S. Contemporaneous carrier-state of two or three "proatherosclerotic" variants of APOE, ICAM1, PPARA and PAI-1 genes differentiate CAD patients from healthy individuals. Clin Chim Acta 2005; 362: 110-188.

25) Mazzotti DR, Singulane CC, Ota VK, Rodrigues TP, Furuya TK, De Souza FJ. PPARα polymorphisms as risk factors for dyslipidemia in a Brazilian population. Mol Genet Metab 2011; 102: 189-193.

26) Chen ES, Mazzotti DR, Furuya TK, Cendoroglo MS, Ramos LR, Araujo LQ. Association of PPARα gene polymorphisms and lipid serum levels in a Brazilian elderly population. Exp Mol Pathol 2010; 88: 197-201.

27) Paun A, Pitha PM. The IRF family, revisited. Biochimie 2007; 89: 744-753.

28) Lopez-Leon S, Tuvblad C, Forero DA. Sports genetics: The PPARA gene and athletes' high ability in endurance sports. A systematic review and meta-analysis. Biol Sport 2016; 33: 3-6.

29) Tural E, Kara N, Agaoglu SA, Elbistan M, Tasmektepligil MY, Imamoglu O. PPAR-α and PPARGC1A gene variants have strong effects on aerobic performance of Turkish elite endurance athletes. Mol Biol Rep 2014; 41: 5799-5804.

30) Moreno-Estrada A, Gignoux CR, Fernández-López JC, Zakharia F, Sikora M, Contreras AV, Acuña-Alonzo V, Sandoval K, Eng C, Romero-Hidalgo S, Ortiz-Tello P, Robles V, Kenny EE, Nuño-Arana I, Barquera-Lozano R, Macín-Pérez G, Granados-Arriola J, Huntsman S, Galanter JM, Via M, Ford JG, Chapela R, Rodriguez-Cintron W, Rodríguez-Santana JR, Romieu I, Sienra-Monge JJ, del Rio Navarro B, London SJ, Ruiz-Linares A, Garcia-Herrera R, Estrada K, Hidalgo-Miranda A, Jimenez-Sanchez G, Carnevale A, Soberón X, Canizales-Quinteros S, Rangel-Villalobos H, Silva-Zolezzi I, Gonzalez Burchard E, Bustamante CD. Human genetics. The Genetics of Mexico Recapitulates Native American Substructure and Affects Biomedical Traits. Science 2014; 344: 1280-1285.

31) Price AL, Patterson N, Yu F, Cox DR, Waliszewska A, McDonald GJ, Tandon A, Schirmer C, Neubauer J, Bedoya G, Duque C, Villegas A, Bortolini MC, Salzano FM, Gallo C, Mazzotti G, Tello-Ruiz M, Riba L, Aguilar-Salinas CA, Canizales-Quinteros S, Menjivar M, Klitz W, Henderson B, Haiman CA, Winkler C, Tusie-Luna T, Ruiz-Linares A, Reich D. A Genomewide admixture map for Latino populations. Am J Hum Genet 2007; 80: 1024-1036.

32) Cardon LR, Palmer, LJ. Population stratification and spurious allelic association. Lancet 2003; 361: 598-604.

33) Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. Nat Genet 2004; 36: 512-517.

34) Velázquez-Cruz R, García-Ortiz H, Castillejos-López M, Quiterio M, Valdés-Flores M, Orozco L. WNT3A gene polymorphisms are associated with bone mineral density variation in postmenopausal mestizo women of an urban Mexican population: findings of a pathway-based high-density single nucleotide screening. Age (Dordr) 2014; 36: 1483-1492.