





Rationale, design, and preliminary results of the Iran-premature coronary artery disease study (I-PAD): A multi-center case-control study of different Iranian ethnicities

Ehsan Zarepur⁽¹⁾ , Noushin Mohammadifard⁽²⁾, Marjan Mansourian⁽³⁾, Hamidreza Roohafza⁽²⁾, Masoumeh Sadeghi⁽⁴⁾, Alireza Khosravi⁽⁵⁾, Fatemeh Nouri⁽⁶⁾, Nahid Azdaki⁽⁷⁾, Nahid Salehi⁽⁸⁾, Masoud Lofizadeh⁽⁹⁾, Nizal Sarrafzadegan^(10,11) 

Original Article

Abstract

BACKGROUND: Premature coronary artery disease (CAD) is still prevalent worldwide and may differ in various ethnicities. Due to the presence of different ethnicities in Iran, the Iran-premature coronary artery disease (I-PAD) study aimed to determine the frequency of premature CAD and related risk factors based on each ethnicity.

METHODS: In this multi-center case-control study, 4000 patients with premature CAD from ten different ethnicities who lived in different cities of Iran and underwent coronary angiography were enrolled (women aged ≤ 70 and men ≤ 60 years). Patients with CAD defined as obstruction equal or above 75% in at least a single coronary artery or left main $\geq 50\%$ were included in the case group, while patients with normal coronary arteries were included in the control group. Lifestyle behaviors, cardiometabolic risk factors, anthropometric measurements, and other variables were collected. Serum, whole blood, buffy coat, plasma, urine, stool, and saliva samples were stored.

RESULTS: The number of patients enrolled until April 2020 was 2071. The mean age of patients was 53.51 ± 7.52 and 934 (45.09%) of patients were women. To date, about 39.6% of the patients were normal. Also, about 26.0% were with one-vessel disease (1VD), 15.0% with two-vessel disease (2VD), and 15.2% with three-vessel disease (3VD). More than 30000 patients' biosamples from across the country have been stored.

CONCLUSION: Knowing the frequency of premature CAD according to different ethnicities with major differences in their lifestyle behaviors and risk factors can assist health decision-makers. In addition, I-PAD biosamples will be an invaluable source.

Keywords: Coronary Artery Disease; Ethnic Groups; Risk Factors; Biological Specimen Banks; Iran

Date of submission: 17 June 2020, *Date of acceptance:* 08 Sep. 2020

Introduction

Cardiovascular disease (CVD) is one of the most important causes of disability that accounts for up

to 30% of mortality worldwide. The prevalence of CVD in Iran is also high, while some studies report it more than 30%.¹⁻³

- 1- Resident, Interventional Cardiology Research Center, Cardiovascular Research Institute AND Department of Cardiology, Isfahan University of Medical Sciences, Isfahan, Iran
 - 2- Assistant Professor, Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 3- Associate Professor, Pediatric Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 4- Professor, Cardiac Rehabilitation Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 5- Professor, Hypertension Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 6- Heart Failure Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 7- Associate Professor, Cardiovascular Diseases Research Center AND Razi Clinical Research Development Unit, Birjand University of Medical Sciences, Birjand, Iran
 - 8- Associate Professor, Cardiovascular Research Center, Health Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran
 - 9- Associate Professor, Social Determinants of Health Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran
 - 10- Professor, Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
 - 11- Professor, Faculty of Medicine, School of Population and Public Health, University of British Columbia, Vancouver, Canada
- Address for correspondence: Nizal Sarrafzadegan; Professor, Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran; Professor, Faculty of Medicine, School of Population and Public Health, University of British Columbia, Vancouver, Canada; Email: sarrafzadegan@gmail.com

How to cite this article: Zarepur E, Mohammadifard N, Mansourian M, Roohafza H, Sadeghi M, Khosravi A, et al. **Rationale, design, and preliminary results of the Iran-premature coronary artery disease study (I-PAD): A multi-center case-control study of different Iranian ethnicities.** ARYA Atheroscler 2020; 16(6): 295-300.

Premature coronary artery disease (CAD) in Iran is highly frequent which justifies the reason for further studies and better understanding of its risk factors and determinants.²⁻⁵

Behavioral and cardiometabolic risk factors like hypertension (HTN), diabetes, dyslipidemia, smoking, unhealthy diet, and low physical activity are important risk factors that have been reported in numerous studies previously. However, studies have shown that some of these factors, such as smoking, family history, unhealthy nutrition, and blood lipids play a stronger role in premature CAD.⁵⁻⁸

The prevalence of CAD differs in various populations which may be connected to their ethnicities.⁹⁻¹² Iran is one of the countries with multiple ethnicities, including Fars (Persian), Azari (Azeri, Turk, or Tork), Kurd (Kord), Arab, Lor (Lur), Gilak, Balouch (Balouchi or Balooch), Turkman (Torkman or Turkmen), Qashqaei (Qashghai), and Bakhtiari. These ten ethnicities consist more than 95 percent of Iran's population. Fars is considered to be the largest ethnic group in Iran, estimated to comprise between 50% and 70% of the population, followed by Azari, as the second largest ethnic group (15% to 24%), Kurd from 7% to 10%, Gilak from 3% to 6%, Arab from 2% to 4%, Lor from 3% to 5%, and Turkman, Bakhtiari, Qashghaei, and Balouch each with 2%.¹³

Iran-premature coronary artery disease (I-PAD) study aims to investigate the frequency of premature CAD and its risk factors according to different ethnicities in Iran, so the results can be a gateway to some preventive and treatment strategies. This report addresses the rationale, methodology, and some earlier results of I-PAD study.

Materials and Methods

I-PAD is a multi-centric case-control study which is ongoing on Iranian patients with different ethnicities. Patients are men and women who underwent coronary angiography. We recruited our patients from hospitals with catheterization laboratories in different cities. Patients are selected from across the country based on the distribution of different ethnicities (Fars, Azari, Kurd, Arab,

Lor, Gilak, Balouch, Turkman, Qashqaei, and Bakhtiari). Each ethnicity was considered positive if the patient and his/her parents had the same ethnicity. We included questions on ethnicity of the patients and their parents after obtaining their consent to participate in the study. If a patient indicated other ethnicity of even one of his/her parents, we excluded him from the study. Sampling will continue through convenience sampling until our study sample reaches 4000 individuals, depending on the percentage of different ethnicities in the population.

Inclusion criteria consist of patients who underwent coronary angiography, age ≤ 70 or ≤ 60 years for women and men, respectively, being related to one of the ethnicities considered in our study, and being familiar with their parent's ethnicity. Having an occlusion of at least single coronary artery equal and above 75% or left main coronary of equal or more than 50% and normal coronary artery were our criteria for case and control groups, respectively. Previous history of documented coronary artery disease such as coronary artery bypass surgery, balloon angioplasty, or percutaneous coronary intervention (PCI) was considered as exclusion criterion.

We calculated our sample size in each group with an error of the first type of 0.05, power of 0.8, and considering an odds ratio (OR) = 1.30. Our sample is about four-thousand patients. Individuals are recruited using convenience sampling in reference hospitals in more than twelve cities in Iran. The total sample was divided proportionally according to the distribution of each ethnic group. Calculated sample for each ethnicity is as follows: 2000 Fars, 510 Azari, 400 Kurd, 250 Gilak, 140 Arab, 140 Lor, 140 Turkman, 140 Bakhtiari, 140 Qashghaei, and 140 Balouch patients.

Initially, we designated focal points who were the heads or one of the authorities of cardiac catheterization centers in each city with one major ethnicity. In addition, we asked them to establish their team who could recruit patients according to our inclusion criteria and complete our questionnaires. We organized necessary training sessions for the executive team on how to interview patients and complete questionnaires. Furthermore, we developed a study practical manual and sent to all teams following our training sessions. We provided all necessary information on I-PAD study to patients who met our inclusion criteria. Then we obtained written consent forms from the patients.

Interviewers in hospitals completed our

questionnaires that included questions on demographics like age, sex, ethnicity, religion, education, income, marriage status, and lifestyle behaviors such as any type of smoking, alcohol or drug use, and nutrition via Food Frequency Questionnaire (FFQ), physical activity by International Physical activity Questionnaire (IPAQ), anxiety level through Hospital Anxiety and Depression Scale (HADS), sleep disorders using the Pittsburgh Sleep Quality Index (PSQI), personal and family history of illnesses and used medications. Questionnaires' reliability and validity were assessed if they were not done before. Individuals removed shoes and heavy cloths prior to anthropometric measurements, then height, waist, hip, neck, and thigh circumferences were measured in all patients, according to standard protocols.

Biobank: Blood, urine, stool, and saliva samples were taken from each participant. The patient should have been fasting for at least 12 hours. Blood samples were taken to measure triglyceride (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), and fasting blood sugar (FBS). Also, after the necessary procedures, in the shortest possible time, serum, buffy coat, plasma, whole blood, saliva, urine, and feces were taken of each patient and stored in specific cryoboxes then in a -80 °C freezer. Isfahan central core laboratory (Isfahan, Iran) executive team was responsible for receiving and storing the biosamples sent from different cities properly.

Quality assurance and quality control: To ensure the quality of the project, several steps were taken, some of which are briefly described here. Initially, the design of questionnaires and data collection forms used in this study was approved and finalized by an expert team in each field. In each questionnaire, detailed, clear, and purpose-based questions were considered. The team included cardiologists, a nutritionist, a psychiatrist, an epidemiologist, a biobank expert, and a statistician.

Then, a comprehensive questionnaire and laboratory protocol were designed, and training sessions for questioners and project executives in different cities were held in Isfahan center to make the implementation of the project more uniform.

Random checking of the data entry in the database was done by a separate expert to check the accuracy of the entered data. Pilot design also helped researchers to identify existing defects, and greatly improved the quality of the study. A unique identity document (ID) for each patient was used on the questionnaire and all biosamples, and it was

used throughout the whole study process.

The quality control unit of the Isfahan Cardiovascular Research Institute which is a World Health Organization (WHO) collaborating center was responsible for periodic monitoring and inspections to ensure the best possible performance. The committee members conducted field visits to ensure the highest study performance and high-quality data collection and management.

Performing multiple interim analysis, once every two weeks at the beginning of the project and then, once a month while executing the project, receiving the necessary feedback, and making changes to each of the steps as necessary are among other measures that have been taken.

This study was reviewed and approved by the Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.REC.1396.2.055). A written informed consent was obtained from all patients and the Declaration of Helsinki was considered. Patients were provided with a complete description of the study and all their questions were answered. To keep the confidentiality, the IDs assigned to each patient were specific codes that did not correspond to the patient's specification, such as his or her national code. All people involved in this study who somehow had access to patient information were given the necessary training in this regard. Access to patient data was limited as much as possible. The outcome of data was not reported individually and patient information was gradually analyzed and disseminated.

The analysis was done in two descriptive and analytical sections. The prevalence of risk factors in the two groups of patients with premature CAD and the other is reported by ethnicity. Quantitative variables in different groups are reported as mean \pm standard deviation (SD). Univariate comparisons between case and control groups was performed according to the type of risk factor by comparison of means (independent t-test or Mann-Whitney) based on statistical assumptions or chi-square test based on qualitative variables. Effect size determination based on different risk factors was extracted by reporting ORs in logistic regression modeling. Conditional and unconditional logistic regression modeling were performed considering the confounders' elimination strategy. The receiver operating characteristic (ROC) curve was used to evaluate the difference between the case and control groups, based on risk factors. All analyses were performed at 5% error level, using Stata software (version 14, Stata Corporation, College Station, TX, USA).

Results

The number of patients enrolled in the study by February 2020 was 2071. The participants were patients with ethnicities such as Fars, Azari, Kurd, Lor, Bakhtiari, Qashqaei, Balouch, Arab, and Gilak. 1137 (54.91%) of the patients were men and 934 (45.09%) of the patients were women (Table 1).

Our enrollment is completed for Bakhtiari and Fars ethnicity; however, around 67% of originally calculated Kurd, 27% of Lor, 84% of Qashghaei, 7% of Arab, and 2% of Gilak, Balouch, and Turk patients were completed to date. The cities of Isfahan, Birjand, Yazd, Shahrekord, Rasht, and Orumiyeh are recruiting patients in a way to complete the sample according to ethnicities. Each city focused on one ethnicity or several specific ethnicities (Figure 1).

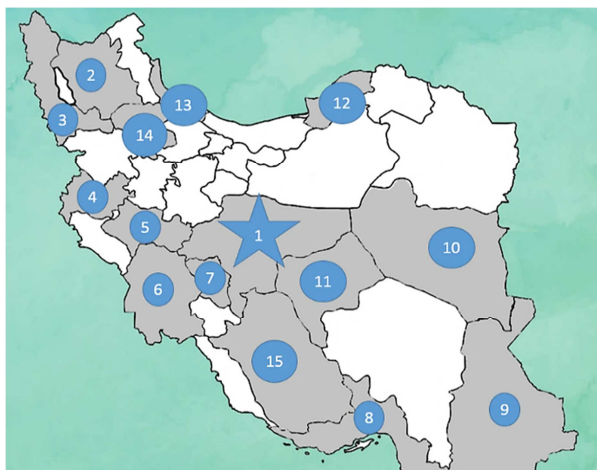


Figure 1. Cities included: Isfahan (1), Tabriz and Maragheh (2), Orumiyeh (3), Kermanshah (4), Khorramabad (5), Ahvaz (6), Shahrekord (7), Bandar Abbas (8), Zahedan and Zabol (9), Birjand (10), Yazd (11), Gorgan (12), Rasht (13), Zanjan (14), Shiraz (15)

To date, about 39.6% of the patients were normal. Also, about 26.0% were with one-vessel disease (1VD), 15.0% with two-vessel disease (2VD), and 15.2% with three-vessel disease (3VD).

Discussion

Currently, premature CAD is one of the most important and prevalent diseases in the world with a growing trend. At the same time, it is one of the diseases that can be reduced by preventive strategies.^{1-3,5} The instructions and guidelines available for dealing with heart disease risk factors and their treatment are the same across our country, and even in most countries around the world, as this is one of the biggest problems in the field of disease prevention and treatment. Because patients of different races and ethnicities may have different risk factors or the relative risk of each risk factor may differ in various populations, that can be due to behavioral, environmental, and genetic differences, as well as different gene-environment interactions. In fact, a risk factor may be more important in one ethnicity and less crucial in another.^{9-11,13}

There are different ethnicities living in Iran with different lifestyles;¹³ yet, no comprehensive study has been conducted to investigate the different cardiovascular risk factors among these ethnicities. The present study (I-PAD) is one of the largest studies in our country and the region on ethnic differences in terms of extent and sample size. This study investigates the risk factors of premature CAD among people of different ethnicities in more than ten cities of Iran. Although there have been limited studies in the past, none has comprehensively covered most Iranian ethnicities or ethnicities were not assured as in our study.^{9,10}

Table 1. Frequency distribution of different ethnicities by age and sex

Variables Ethnicities	Total (n)	Age (year)		Sex		Report of angiography	
		Mean ± SD	Men [n (%)]	Women [n (%)]	Normal [n (%)]	Premature CAD [n (%)]	
Fars	1441	53.66 ± 7.33	818 (56.80)	623 (43.20)	559 (40.65)	816 (59.35)	
Azari	8	51.00 ± 9.68	6 (75.00)	2 (25.00)	3 (37.50)	5 (62.50)	
Kurd	271	53.89 ± 7.93	120 (44.30)	151 (55.70)	114 (42.54)	154 (57.46)	
Lor	39	49.26 ± 10.40	23 (59.00)	16 (41.00)	20 (54.05)	17 (45.95)	
Bakhtiari	178	52.66 ± 7.66	99 (55.60)	79 (44.40)	64 (38.32)	103 (61.68)	
Qashqaei	118	53.77 ± 7.30	62 (52.50)	56 (47.50)	52 (45.61)	62 (54.39)	
Gilak	3	53.00 ± 6.08	1 (33.30)	2 (66.70)	1 (33.33)	2 (66.67)	
Arab	10	52.70 ± 6.21	7 (30.00)	3 (70.00)	3 (30.00)	7 (70.00)	
Turkman	0	-	0 (0)	0 (0)	0 (0)	0 (0)	
Balouch	3	56.33 ± 4.04	1 (33.30)	2 (66.70)	1 (33.33)	2 (66.67)	
Total	2071	53.51 ± 7.52	1137 (54.91)	934 (45.09)	817 (41.16)	1168 (58.84)	

CAD: Coronary artery disease; SD: Standard deviation

One such study by Jahangiry et al. was a cross-sectional study of 3506 participants, aged 30-70 years in Naqadeh (northwestern Iran, including two ethnicities, Kurd and Azari). Patients were evaluated for cardiometabolic risk factors associated with metabolic syndrome. Sixty percent of the study population were Azari. The researchers recommended that the identification of different components of the metabolic syndrome based on two studied ethnicities would be an appropriate step in determining interventional strategies, considering different ethnicities in Iran.¹⁰ Our study, however, examines more ethnicities and risk factors in a national sample representing ten ethnicities in Iran that comprise more than 95% of the total population.

In this study, the ethnicity of parents is considered in addition to individual ethnicity. In fact, in many studies, the boundary between the use of the word “ethnicity” and the word “race” is blurred, and sometimes the two words are used interchangeably.^{14,15} In studies of different races, it is much easier to separate races than to study different ethnicities. In addition, in most studies on ethnicity, participants report their own ethnicity and not that of their parents, which in many cases may cause problems in the final analysis.^{9-11,16,17} For example, a person who was born of an Azari mother and a Fars father may, for any reason, identify himself as a Fars or an Azari. Even a person born to parents of the same ethnicity, for whatever reason, presents herself/himself as an ethnicity that is the predominant ethnicity of the city where she/he lives. However, in the present study, one of the inclusion criteria was considered to be same parental ethnicity, in order to minimize such errors in data analysis. In addition, the paternal and maternal grandparents' ethnicity is also questioned although it is not included in the inclusion criteria, but it can be considered in later analyses.

Also, considering the dominance of Fars ethnicity in the country (between 50 and 70 percent of the population),¹³ Fars people from four different cities (Isfahan, Birjand, Yazd, Shahrekord) were included in this study to investigate possible differences and gain a better understanding of gene-environment interaction. These cities are located in different parts of Iran with probably different risk factors and different environments.

Establishment of biobank is also one of the most important steps in this study. Serum samples, buffy coat, plasma, whole blood, saliva, urine, and feces were taken from each patient. This biobank is able to hold samples for years to be used in future studies like epigenetics. In addition, one of the

advantages of this study was feasibility study in Isfahan. Thus, about 5% of all samples were initially studied in Isfahan. All glitches were carefully examined and resolved at each stage of the project.

This study has some limitations like any other study. There are, however, other ethnicities in Iran but their community is not large enough to be considered. In addition, they have no catheterization laboratory in their living areas and should travel to larger cities which made the process of diagnosis of their ethnicity more difficult.

Due to existing limitations, it was not possible to examine other ethnicities such as Taleshi, Georgian, Kurmanji, etc. However, the percentage of these ethnicities in the country is very low (less than 5%), and there may not be enough individual available to meet our criteria.

Conclusion

I-PAD is one of the largest studies of ethnicity in Iran and the Middle East area. Studying the prevalence of premature CAD and its risk factors among different ethnicities of the country can give a much better perspective to health care providers, as well as national health decision-makers. In addition, the biobank established in this project could be one of the most important sources for genetic and epigenetic studies in Iran in the coming years.

Acknowledgments

This study is supported by Ministry of Health and Medical Education of Iran and Isfahan University of Medical Sciences (grant number: 700/142). Authors would like to acknowledge participants who took part in the study. Also, they thank the staff of Isfahan Cardiovascular Research Institute and all coordinators and their team members in different cities.

Conflict of Interests

Authors have no conflict of interests.

References

1. Trevino KM, McConnell TR. Religiosity and religious coping in patients with cardiovascular disease: Change over time and associations with illness adjustment. *J Relig Health* 2014; 53(6): 1907-17.
2. Shams-Beyranvand M, Farzadfar F, Naderimaghani S, Tirani M, Maracy MR. Estimation of burden of ischemic heart diseases in Isfahan, Iran, 2014: Using incompleteness and misclassification adjustment models. *J Diabetes Metab Disord* 2017; 16: 12.

3. Ebrahimi M, Kazemi-Bajestani SM, Ghayour-Mobarhan M, Ferns GA. Coronary artery disease and its risk factors status in Iran: A review. *Iran Red Crescent Med J* 2011; 13(9): 610-23.
4. Mohammad AM, Jehangeer HI, Shaikhow SK. Prevalence and risk factors of premature coronary artery disease in patients undergoing coronary angiography in Kurdistan, Iraq. *BMC Cardiovasc Disord* 2015; 15: 155.
5. Poorzand H, Tsarouhas K, Hozhabrossadati SA, Khorrampazhouh N, Bondarsahebi Y, Bacopoulou F, et al. Risk factors of premature coronary artery disease in Iran: A systematic review and meta-analysis. *Eur J Clin Invest* 2019; 49(7): e13124.
6. Alkhawam H, Zaiem F, Sogomonian R, El-Hunjul M, Al-Kateb M, Bakhsh MU, et al. Coronary artery disease in young adults. *Am J Med Sci* 2015; 350(6): 479-83.
7. Gerami H, Javadi M, Hosseini SK, Maljaei MB, Fakhrzadeh H. Coronary artery stenosis and associations with indicators of anthropometric and diet in patients undergoing coronary angiography. *J Diabetes Metab Disord* 2018; 17(2): 203-10.
8. Adeva-Andany MM, Martinez-Rodriguez J, Gonzalez-Lucan M, Fernandez-Fernandez C, Castro-Quintela E. Insulin resistance is a cardiovascular risk factor in humans. *Diabetes Metab Syndr* 2019; 13(2): 1449-55.
9. Veghari G, Sedaghat M, Maghsodlo S, Banihashem S, Moharloe P, Angizeh A, et al. Differences in the prevalence of obesity among Fars-native, Turkman, and Sisstanish Ethnic Groups in Iranian Northern Adults in 2010. *Int Cardiovasc Res J* 2013; 7(2): 56-61.
10. Jahangiry L, Khosravi-Far L, Sarbakhsh P, Kousha A, EntezarMahdi R, Ponnet K. Prevalence of metabolic syndrome and its determinants among Iranian adults: Evidence of IraPEN survey on a bi-ethnic population. *Sci Rep* 2019; 9(1): 7937.
11. Sun Z, Zheng L, Zhang X, Li J, Hu D, Sun Y. Ethnic differences in the incidence of hypertension among rural Chinese adults: results from Liaoning Province. *PLoS One* 2014; 9(1): e86867.
12. Abdalla M, Akwo EA, Bluemke DA, Lima JAC, Shimbo D, Maurer MS, et al. Association between reduced myocardial contraction fraction and cardiovascular disease outcomes: The Multi-Ethnic Study of Atherosclerosis. *Int J Cardiol* 2019; 293: 10-6.
13. Banihashemi K. Iranian human genome project: Overview of a research process among Iranian ethnicities. *Indian J Hum Genet* 2009; 15(3): 88-92.
14. Ma IW, Khan NA, Kang A, Zalunardo N, Palepu A. Systematic review identified suboptimal reporting and use of race/ethnicity in general medical journals. *J Clin Epidemiol* 2007; 60(6): 572-8.
15. Anderson KK, Flora N, Archie S, Morgan C, McKenzie K. Race, ethnicity, and the duration of untreated psychosis: A systematic review. *Soc Psychiatry Psychiatr Epidemiol* 2014; 49(7): 1161-74.
16. Hnin K, Mukherjee S, Antic NA, Catcheside P, Chai-Coetzer CL, McEvoy D, et al. The impact of ethnicity on the prevalence and severity of obstructive sleep apnea. *Sleep Med Rev* 2018; 41: 78-86.
17. Ou GJ, Su PC, Yu H, Ji X, Liu F, Wang SL, et al. HNA-3a and HNA-3b antigens among 9 ethnic populations and the Han population in Southwest China. *J Transl Med* 2018; 16(1): 67.