

## Isfahan Twin Cohort: A Ten-Year Longitudinal Prospective Study Based on A Twin Registry

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### Original Article

#### Abstract

**BACKGROUND:** The Isfahan Twin Cohort (ITC) aims to provide a comprehensive understanding of the interplay between genetics and environment in the development of Non-Communicable Diseases (NCDs). As a type of specialized epidemiological investigation, twin studies are designed to quantify the contribution of genetics to a particular phenotype when confronted with environmental factors. In this context, the present study aims to present a detailed overview of the ITC methodology.

**METHOD:** The ITC is a prospective longitudinal study started in 2020. Data collection, including the demographics, socioeconomic status, health-related habits, medical history, and zygosity of the participants, was performed using validated questionnaires. Moreover, anthropometric measurements and blood pressure assessments were performed by a trained nurse. Also, fasting blood and morning urine samples were collected during a morning visit, and biochemical investigations were conducted at the central laboratory of the Isfahan Cardiovascular Research Institute. The participants underwent follow-up telephone interviews biannually, in which brief questionnaires were filled out on the changes in the lifestyle factors of the participants, such as diet, physical activity, psychological factors, and smoking habits. The second and final follow-up visit will include complete assessments, including blood and biological sample collections, similar to the baseline assessment.

**RESULTS:** The ITR has registered a total of 112 (n=224) monozygotic and 291 (n=582) dizygotic twin pairs during two years. The age range of the participants is 1 month to 56 years. Until November 2020 / 2021, the registered twins were categorized by age and included 48 pairs (n=96) in the infant group (monozygotic: 7 pairs, dizygotic: 41 pairs); 283 pairs (n=566) in the early childhood, late childhood, and adolescent groups (monozygotic: 74 pairs, dizygotic: 209 pairs); and 72 pairs (n=144) in the adult group (monozygotic: 31 pairs, dizygotic: 41 pairs).

**CONCLUSIONS:** The cohort is being prospectively followed with plans to investigate the clinical utility of the newly developed biomarkers and gene-environmental interactions in the future.

**Keywords:** Twin, Cohort, Longitudinal, Cardiometabolic

*Date of submission: 2022-Jan-15, Date of acceptance:2022-Mar-13*

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## Introduction

Several twin registries have been established all around the world <sup>1</sup>. For example, as the oldest twin registry in the world, the Danish Twin Registry was established in 1954 and included twins born between 1870 and 1910 <sup>2</sup>. In countries like Sweden <sup>3</sup>, Denmark <sup>2</sup>, Norway <sup>4</sup>, Finland <sup>5</sup>, Australia <sup>6</sup>, Sri Lanka <sup>7</sup>, and the United Kingdom <sup>8</sup>, twin registries cover the entire twin population in the country. Moreover, Swedish Twin Registry is the world's largest twin registry, including about 85,000 pairs of twins <sup>3</sup>. These twin registries paved the way for the establishment of twin cohorts across Nordic countries, the Netherlands, Australia, and many other countries later. Also, the GenomEU twin study performed large-scale analyses of European twin population cohorts in order to investigate the meta-analyses on heritability studies and identify genes of common risk factors and traits, including genes related to lipids, Bone Marrow Index (BMI), migraine, and height <sup>9</sup>.

On the other hand, Non-Communicable Diseases (NCDs) account for considerable morbidity and mortality throughout the world, especially in the Eastern Mediterranean Region (EMR) <sup>10-12</sup>. It is believed that twin studies, as the main studies investigating the interaction of genetics and environment and their effects on phenotypic development <sup>13</sup>, can be of utmost importance in illustrating the pathogenesis of NCDs. Typically, the statistical evidence of the inheritance of peculiar characteristics is always questionable due to the confounding effect of similar environmental conditions <sup>14, 15</sup>. However, twin studies provide a solid foundation for the validity of any potential risk factor found for a specific characteristic or condition by controlling the genetic variations. Also, twin studies are considered a preferred investigational instrument for behavior geneticists, who mainly use them for assessing the heritability of characteristics and to quantify the effect of a communal environment (family) and unique environment (lifestyle) on a trait <sup>16, 17</sup>. Thus, considering the lack of twin data from the EMR and the high

prevalence of NCD in this area, which also includes Iran, our team started a longitudinal prospective study entitled the "Isfahan Twin Cohort" (ITC), which is based on the Isfahan Twins Registry (ITR), a local registry including twins, multiples, and their parents in Isfahan, Iran <sup>18</sup>. ITC is an ongoing longitudinal study aiming to follow up with twins for ten years in order to detect the prevalence of NCDs and their risk factors. The present study describes the methodology, follow-up, procedures, questionnaires, and data collection methods of the ITC.

## Materials and Methods

### *Participant Recruitment*

ITC is a 10-year prospective longitudinal study cohort started in 2020, with the related methodology and questionnaire having been approved by the Human Research Ethics Committee of Isfahan University of Medical Sciences (Ethics approval number: IR.mui.med.rec.1399.169). Moreover, the methodology, aims, and details of the ITR have been published elsewhere <sup>18</sup>.

The study population included all twins registered in the ITR, while the inclusion criteria of the ITR were Iranian nationality and residency in Isfahan City. Also, the study population was divided into the following groups: an infant group aged 1-24 months, an early childhood group aged 2-6 years, a late childhood group aged 7-12 years, an adolescent group aged 12-18 years, an adult group aged older than 18 years, and the mothers or caregivers responsible for the minor twins who completed the ITC questionnaires.

### *Data Collection*

The ITC used validated questionnaires to collect data regarding demographics, socioeconomic status, health-related habits, and medical histories. The eating habits were assessed using the Food Frequency Questionnaire (FFQ). The initial section of this questionnaire included questions on the typical amount of food consumed by the participants and included all food groups, such as fats and oils, fruit and

vegetables, dairy products, meats, bread and rice, beverages, snacks, and fast foods<sup>19</sup>.

Moreover, the physical activity level of the patients was assessed using the International Physical Activity Questionnaire - Short Form (IPAQ-SF), which was filled out for each patient separately by a trained nutritionist. This 100-item questionnaire is used in cross-sectional studies to evaluate the physical activity in the last seven days and includes four types of activities: vigorous activities (e.g. aerobics), moderate-intensity activities (e.g. leisure cycling and walking), and sitting<sup>20</sup>. Also, participants who were current smokers or had a smoking history of more than one pack per day were classified as smokers.

On the other hand, the psychological health of the participants was evaluated using the Iranian-validated version of the Hospital Anxiety and Depression Scale (HADS)<sup>21</sup>, which includes 50 items in two subscales: anxiety and depression. A score of 0-7 is classified as normal, while scores between 8 and 10 are considered borderline, and a score of 11 or higher on either subscale indicates a significant case of psychological morbidity. Moreover, the total quality of life and emotional functioning were assessed using the EORTC QLQ-C30 subscales<sup>22</sup>, which includes a 4-point subscale for rating the items related to emotional functioning and two 7-point items for total quality of life.

In order to determine zygosity, a set of standardized items were used to evaluate various aspects of physical resemblance between the twins regarding the extent to which the twins were often mistaken by parents, relatives, and strangers<sup>23</sup>. Moreover, a verified questionnaire was developed and used for female twins regarding menstrual, menopausal, or gynecological health issues<sup>24</sup>. Also, participants were requested to provide their medical records, including past hospital admissions, past medical history, previous or current use of medications, past surgical history, and results of paraclinical investigations. The participants had a positive family history if they had any history of CVD, malignancies, or diabetes in their first-degree relatives before 55 years of age in men and 65 years of age in

women.

All participants were weighed with light clothes on using a scale with an accuracy of 0.5 kg, and their height was measured while standing barefoot with shoulders in a natural position. Height measurement was performed using a non-elastic tape with an accuracy of 0.5 cm<sup>25</sup>. Moreover, the Waist Circumference (WC) of the participants was measured using a tape in the middle of the distance between the lower rib margin and the iliac crest, while hip circumference was considered at the maximum circumference over the buttocks<sup>26</sup>. Also, the blood pressure level of each patient was assessed thrice while in a sitting position, and the mean values were recorded<sup>27</sup>.

All participants older than 6 years underwent blood sampling (10 mL) from the antecubital vein. Moreover, 3 fresh blood spots were collected from infants (0-18 months) on the guttery papers. Also, 4 tubes of fasting blood and one morning urine sample were taken from each patient during a morning visit. All biochemical tests were performed in the central laboratory of the Isfahan Cardiovascular Research Institute.

The Fasting Blood Sugar (FBS), total cholesterol, Triglyceride (TG), and High-Density Lipoprotein (HDL) were assessed using a Hitachi 902 Analyzer (Japan) and standard enzymatic method, while Low-Density Lipoprotein (LDL) levels were directly assessed. Moreover, the levels of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), and C-reactive protein (quantitative) were assessed using commercial kits (Pars Azmon, Iran). Also, a Sysmex-XK-21 device (Japan) was used for Complete Blood Count (CBC) assessment.

The biological samples, including whole blood, DNA, serum, plasma, buffy coat, and stool coat, were stored at -70 °C in a freezer, while nail and hair samples were kept at room temperature. Moreover, the parents of the early and late childhood groups filled out a brief questionnaire and gave whole blood samples. The DNA was isolated from the peripheral lymphocytes using the standard salting-out method<sup>28</sup>.

*Follow-up*

Following baseline assessments, the participants attend biannual follow-up visits, when summarized questionnaires are used to assess lifestyle-related factors, such as physical activity, psychological factors, smoking habits, and dietary habits. Moreover, in case of developing a new NCD or risk factor or using any medication due to CVD risk factors, such as diabetes, hypertension, headache, and stroke, the participants are instructed to visit the twin clinic and report their new health-related event

while bringing their medical records for the past two years, including past hospital admissions, past medical history, previous or current use of medications, past surgical history, and results of paraclinical investigations. Following two years after the baseline visit, the second phase of the study is started through telephone interviews and the last follow-up visit. Following 10 years, the participants undergo complete assessments similar to the baseline evaluation, which is accompanied by biological samplings. The flowchart of the study is presented in Figure 1.

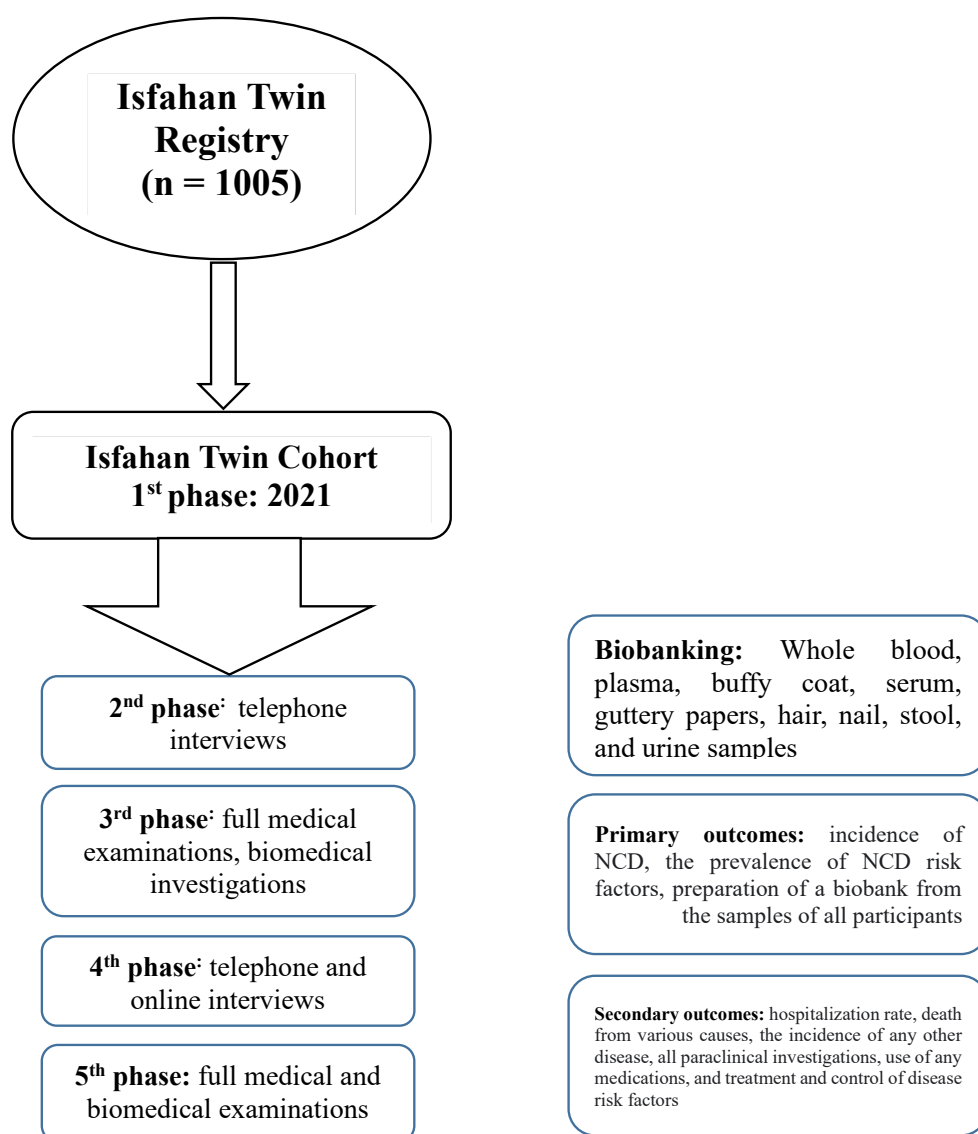


Figure 1. Flowchart of ITC

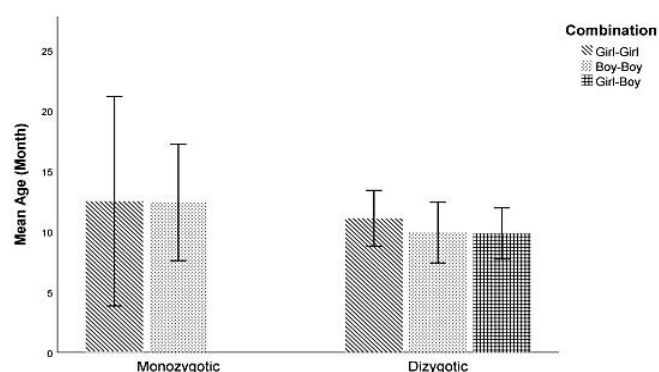
## Results

Until November 2021 / 2020, a total number of 1005 participants had been registered as the primary sample of ITC, including 403 pairs of twins (n=806), 59 sets of triplets (n=177), 3 sets of quadruples (n=12), and 2 sets of 10 quintuplets (n=10). The age range of the participants is 1 month to 56 years. Moreover, twins (n=806) include 112 monozygotic (n=224) and 291 dizygotic (n=582) pairs. Also,

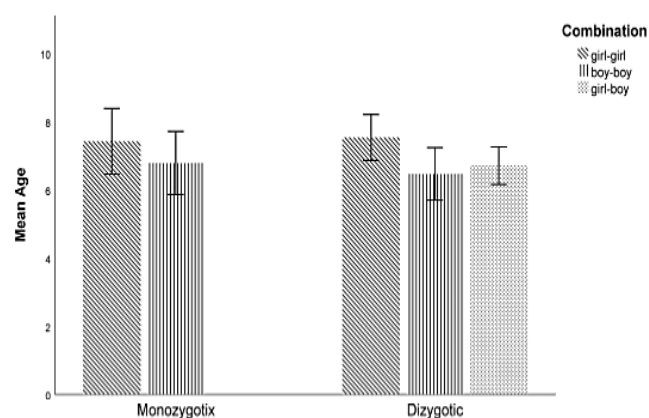
twin participants (n=806) include 48 pairs (n=96) in the infant group (monozygotic: 7 pairs, dizygotic: 41 pairs); 283 pairs (n=566) in the early childhood, late childhood, and adolescent groups (monozygotic: 74 pairs, dizygotic: 209 pairs); and 72 pairs (n=144) in the adult group (monozygotic: 31 pairs, dizygotic: 41 pairs). The prevalence of different multiple births in the study population is presented in Table 1.

**Table 1.** Prevalence of multiple births at Isfahan Twin Registry

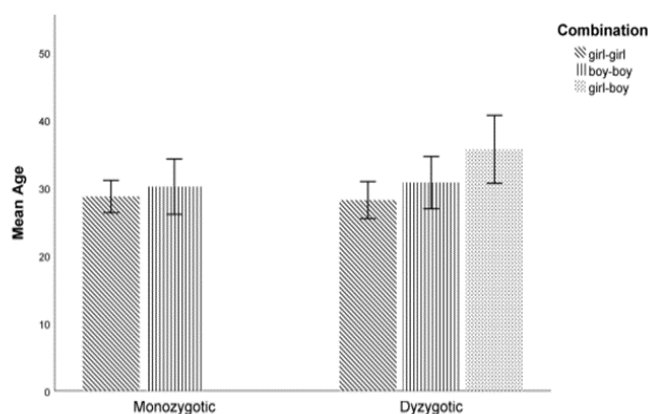
	Twins (n)		Triplets (n)		Quadruples (n)		Quintuplets (n)		Total
	Number of individuals	Number of sets	Number of individuals	Number of sets	Number of individuals	Number of sets	Number of individuals	Number of sets	
0-24 months	96	48	12	4	-	-	-	-	108
2-18 years	566	283	159	53	12	3	10	2	747
19 years ≤	144	72	6	2	-	-	-	-	150
Total	806	403	177	59	12	3	10	2	1005



A. Infant groups (1-24 months)



B. Early childhood, late childhood, and adolescent groups (2-18 years)

C. Adult group (19 years  $\geq$ )**Figure 2.** Zygosity in the ITC based on age and gender distribution.

## Discussion

It has been shown that twin longitudinal studies are beneficial for investigating the effects of genetics and environment on the early-onset intermediate phenotypes related to adult-onset diseases. Using a cross-lagged model, researchers can identify the genes sensitive to environmental factors that are associated with complex diseases. Moreover, the specific biomarkers of such genes can be identified as well <sup>29-31</sup>. It has been reported that in 2016, NCDs, such as cardiovascular diseases, cancer, chronic respiratory diseases, and diabetes, accounted for 41 million deaths globally (71% of the total annual death) <sup>11</sup>. Also, these diseases are a major cause of several adverse health outcomes in most countries <sup>32-34</sup>.

On the other hand, the ITC study has combined the twin study design with the longitudinal evaluation of exposure, biological samples, and phenotypic outcomes of twins in order to predict the future development of NCDs in study participants. Therefore, the ITC can address the exceptional conditions of exposure or outcomes characteristic of developing countries undergoing rapid Westernization in the incidence of a range of NCDs. Moreover, the ITC follows two future goals: The first one involves a descriptive analysis of twins reared apart, while the second one is combining classical twin studies with complex molecular genetics and epigenetic analyses. Despite the

progress made in twin studies, there is still an opportunity for future twin research and data mining. Our future objective is to follow the twins recruited in the ITC and use their data for designing related studies, such as cohort studies and matched case-control studies in disease/lifestyle-discordant twin pairs. Most importantly, we intend to establish an ITC-based twin health information platform and encourage researchers to submit proposals to work on the data obtained in the ITR and ITC.

## Acknowledgment

The present twin cohort study was funded by the Isfahan University of Medical Sciences and the Isfahan Cardiovascular Research Institute. Moreover, we would like to thank the workers of the laboratory of the Cardiovascular Research Institute, as well as the study participants for their time and involvement.

## Author Contribution

MG wrote the first draft of the manuscript, while MD prepared the tables and conducted the analyses. Moreover, EL, LS, and ZJ performed the data collection and assessments. Also, NM and NS commented on the first draft. All authors reviewed the statistical analysis. The datasets for the present study are available by official request.

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**How to cite this article:** Gharipour M, Dianatkhah M, Mohammadifard N, Khosravi E, Sadeghian L, Jabarzare Z, et al. **Isfahan twin cohort: a ten-year longitudinal prospective study based on a twin registry.** *ARYA Atheroscler* 2023; 19(2): 42-9.