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How to Generate Unbiased Data in Molecular Genetic Studies in Patients with Early Onset Coronary Artery Disease or Premature Myocardial Infarction?

EDITORIAL COMMENT
EDİTÖRYAL YORUM

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Individuals with early-onset coronary artery disease (CAD) constitute a special patient population with different clinical features compared to those with older onset CAD.[1] Besides the traditional risk factors, non-traditional risk factors such as immune mediated diseases, psychological stressors, and genetic factors contribute to the risk of developing atherosclerosis at a young age (Table 1). Currently, early onset CAD and young myocardial infarction (MI) are rising threats leading to premature mortality worldwide.[1] Prevalence of early CAD in some countries like Turkiye, India, and Middle East North Africa region is already high and constitutes a major health problem.[2-5] The burden of premature MI has also affected many other countries including Australia, Canada, the United Kingdom, and the United States, where its incidence and mortality are increasing.[6-12] Therefore, unbiased genetic studies are extremely important in understanding the genetic basis of the early onset atherosclerosis and pathophysiology of young MI for the prevention of premature mortality and morbidity. Several mutations in the genes involved in lipoprotein metabolism, inflammation, thrombosis, and oxidation have been shown to be associated with early atherosclerosis and young MIs.[1,13-15]

In their recent work in *Archives of Turkish Society of Cardiology*, Kahya Eren et al[16] provide new evidence for the association between the micro-RNAs (miRNA)s and

Table 1. Etiological Factors for the Development of Early-onset CVD and Young MI

A. Atherosclerotic factors (frequency 80-85%)	B. Non-Atherosclerotic factors (frequency 15-20%)
Smoking	Anomalous coronary arteries
Male sex	Myocardial bridge
Family history of premature CVD	Patent foramen ovale (paradoxical embolism)
Dyslipidemias (Familial hypercholesterolemia, Familial combined hyperlipidemia, etc)	Connective tissue disorders (Behçet disease, Takayasu arteritis, Kawasaki disease, Giant cell arteritis)
Elevated lipoprotein (a)	Substance abuse (cocaine, marijuana etc.)
Obesity	Oral contraceptives
Diabetes mellitus	Radiotherapy
Hypertension	Infections (SARS-CoV-2, HIV, Chlamydia, Helicobacter pylori)
Environmental factors (air pollution etc)	Spontaneous dissection (Pregnancy, immune diseases, hyperhomocysteinemia etc)
	Thrombophilia (hereditary or acquired) (Factor V Leiden, Factor II G20210A, MTHFR mutations, Hyperhomocysteinemia, and Protein C, Protein S and Antithrombin III deficiency etc)

CVD: Cardiovascular disease, HIV: Human immunodeficiency virus, SARS-CoV-2: severe acute respiratory syndrome coronavirus 2, MI: myocardial infarction, MTHFR: Methylenetetrahydrofolate Reductase.

premature CAD. miRNAs are a class of short (approximately 22 nucleotides in length), noncoding, single stranded RNA molecules, that regulate their target genes' expression at the post-transcriptional level.[17] Therefore, they may regulate a wide range of biological and cellular processes by affecting the translation of proteins. miRNAs are detectable both in tissues and in the circulation and have become a research area of interest in the last two decades. With growing body of evidence, miRNAs have been emerged as potential diagnostic and predictive biomarkers as well as therapeutic targets of various pathological processes including cardiovascular disease.[18] Several miRNAs' levels have been shown to be associated with CAD, heart failure, pulmonary hypertension, atrial fibrillation, aneurisms etc. In their cross-sectional single-center study, Kahya Eren et al[16] have explored the circulating levels of 13 different miRNAs (endothelial cell related miR-126, -92a/b; vascular smooth muscle cell (VSMC) related miR-145; inflammation related miR-16, -21, -125b, -146a/b, -147b, -150, -155, and lipid metabolism-related miR-27b, -122, -370) in three groups with early-onset (n:30) versus late-onset (n:30) CAD versus healthy controls (n:31). They showed that plasma expressions of the lipid metabolism related (miR-27b, miR-122), inflammation related (miR-125b, miR-146a/b, miR-147b, miR-150, miR-155), and VSMC related miR-145 were significantly down- and endothelial cell related miR-126 was significantly up-regulated in patients with early onset CAD compared to healthy controls. Whereas the circulating miRNA profile was also different between those early onset and late onset CAD.

The small number of the study population and the lack of data about the non-traditional risk factors such as thrombophilia, drug abuse are among the limitations as acknowledged by the authors. It's well known that traditional risk factors account for almost the 80-85% of premature MIs, while 15-20% of are due to non-atherosclerotic risk factors.[1,13] Non-atherosclerotic factors can be detected only in 5% of MIs at older ages.[1,13] miRNAs are extremely affected by cardiovascular risk factors but also by environmental and non-traditional factors which are also known to be associated with cardiovascular events in young age groups. Therefore, genetic and molecular protocols targeting young CAD/MI populations should cover environmental and non-atherosclerotic risk factors as much as possible to obtain unbiased data.

In the present study,[16] only a selected number of miRNAs that are known be related to endothelial cells, VSMC, inflammation, or lipid metabolism were analyzed. However, miRNAs can concurrently regulate several other pathways which were not discussed here. Moreover, most miRNA studies do not cover a related work on associated genes. For example, early-onset CAD is known to have strong correlation with genetic mutations of cholesterol metabolism such as familial hypercholesterolemia. Without adjusting for lipid levels, anti-lipid therapy, and the presence of even clinical familial hypercholesterolemia, or high lipoprotein(a) levels, it's impossible to interpret the aberrations of lipid metabolism related miRNAs' expressions.

In the present study Kahya Eren et al[16] have studied 30 consecutive patients with early onset CAD (onset of disease <40 years old), 31 age- and sex -matched healthy controls, and 30 patients with late onset CAD (age at 1st coronary event >55 years old in males and >65 years old in females). Using two control groups for comparison of very young CADs is an important strength of the study.

The most challenging aspect of designing molecular genetic studies on young MI or premature CAD is defining the selection criteria for the control group. Often, age and gender matched healthy volunteers are preferred as the control groups.[1] However, an age matched control group could easily lead to a selection bias. Because there is no guarantee that a young control subject included in the study will not suffer from MI after two days, two months, or two years. Therefore, to overcome misclassification bias, control groups should be chosen from healthy individuals over the age that is accepted as age criteria for young MI or CAD. While choosing the healthy control subjects matching for family history of early onset CAD, might help to betray the misclassification bias. Furthermore, studies comparing young study populations with age matched control groups may incorrectly predict an existing risk due to the small number of subjects as the expected incidence of events is relatively low at younger ages.

An important fact to be considered when interpreting the results of an early-onset CAD study is the lack of a consensus for a universally accepted age cut-off for defining young age.[1] Therefore, there is a significant disparity in the literature; some studies vary from ≤40 to ≤55 years of age and others define young MI as <45 years of age.[19] But most researchers prefer to use an age cut-off of 40 to 45 years to describe "young" patients with CAD or acute MI. These patients can be divided into two groups, based on the age of the first MI or cardiovascular event. The classification of patients who had their first event earlier than 40 years of age as *'very young or very early MI/CAD'* patients, and those who had the first event between the ages 40 and 50 as *'young or early MI/CAD'* patients, may appear to be more rationale. This approach may also oversee the important inconsistencies in sex, race, and genetics. However, defining the early-onset or late onset CAD age could be still challenging without considering the menopausal age in women.

In conclusion, molecular genetic studies including miRNAs are important in understanding the genetic basis of early onset atherosclerosis and pathophysiology of young MI. However, studies on young populations should cover data and analysis on non-atherosclerotic risk factors, and control groups should be chosen from those over the age cut-off defined as the age criteria of young or early onset, namely who completed the young ages without MI or diagnosis of CAD, to obtain unbiased data.

Declaration of Interests: The authors declare no conflicts of interest.

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