

Significance of Molecular Pathology in Unexplained Sudden Cardiac Deaths: Epigenetics of Cardiomyopathies

Açıklanamayan Ani Kardiyak Ölümlerinde Moleküler Patolojinin Önemi: Kardiyomiyopatilerin Epigenetiği

Yasin Hasan BALCIOĞLU,^a
Filiz Ekim ÇEVİK,^b
Faruk AŞICIOĞLU^b

^aDepartment of Forensic Psychiatry,
Bakırköy Prof. Dr. Mazhar Osman
Mental Health and Neurological Diseases
Training and Research Hospital,

^bDepartment of Medical Sciences,
İstanbul University
Institute of Forensic Sciences,
İstanbul

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Yazışma Adresi/Correspondence:
Yasin Hasan BALCIOĞLU
Bakırköy Prof. Dr. Mazhar Osman
Mental Health and Neurological Diseases
Training and Research Hospital,
Department of Forensic Psychiatry,
İstanbul,
TURKEY/TÜRKİYE
yasinhasanbalcioglu@bakirkoyruhsinir.gov.tr

ABSTRACT Cardiomyopathy, a prominent cause of lethal ventricular arrhythmias and heart failure, may be defined as a primary weakness of the myocardium, impaired myocardial perfusion, or an infiltrative process. Cardiomyopathies are not random in the general population, particularly hypertrophic cardiomyopathy, arrhythmogenic right ventricular cardiomyopathy, and dilated cardiomyopathy are ought to be kept in mind, occasionally, when investigating sudden deaths of young adults. Cardiomyopathies are associated with multiple genetic and modifiable risk factors; however, given environmental and genetic influences yet to explain precise pathophysiology. Epigenetic mechanisms would endeavour to address some of the gaps in our current knowledge regarding the interaction between nature and nurture in the development of cardiomyopathies.

Keywords: Cardiomyopathies; epigenesis, genetic; death, sudden, cardiac; pathology, molecular; autopsy

ÖZET Kalp yetmezliğinin ve ölümcül ventriküler aritminin önemli bir sebebi olan kardiyomiyopati, birinci derecede miyokardiyumun zayıflaması, bozulmuş miyokardiyal perfüzyon ya da bir infiltratif süreç olarak tanımlanabilir. Kardiyomiyopatiler toplumda nadir değildir ve özellikle hipertropik kardiyomiyopati, aritmojenik sağ ventriküler kardiyomiyopati ve dilate kardiyomiyopati varlığı ihtimali, ani genç ölümleri araştırılırken dikkate alınmalıdır. Kardiyomiyopatiler çeşitli genetik ve modifiye edilebilen risk faktörleriyle bağlantılıdır ancak bilinen çevresel ve genetik etkiler patofizyolojiyi tam olarak açıklamada henüz yeterli değildir. Epigenetik mekanizmalar kardiyomiyopatilerin ortaya çıkışındaki kalıtsal ve edinsel unsurların etkileşimini açıklayıcı mevcut bilgilerdeki bazı boşlukların tamamlanmasında umut verici olarak değerlendirilebilir.

Anahtar Kelimeler: Kardiyomiyopatiler; epigenezis, genetik; ölüm, ani, kardiyak; patoloji, moleküler; otopsi

Most sudden deaths are originated of a cardiac etiology and defined as sudden cardiac death (SCD). SCD constitutes one of the most important unresolved challenges in the practice of forensic pathology. SCD is usually described as an unpredicted instantaneous death or being preceded by prodromal acute cardiac symptoms up to 1 hour before death. The estimated incidence of SCD in the general population in Europe and the United States is between 50 and 100 per 100,000 annually.¹ The vast majority of sudden cardiac deaths are due to ischemic heart disease, hypertensive heart disease, and cerebrovascular disease. Those illnesses are pretty straightforward for forensic pathologists to reveal out, owing to their

characteristic anatomical findings, and a review of the relevant history when all other competing causes have been ruled out. Nevertheless, the death of an apparently healthy, often young person who lacks a medical history and significant findings after a comprehensive medicolegal investigation, occasionally challenges the pathologists. Typically, the “autopsy negative” death requires the most detailed level of study including investigation of the conditions leading up to death, the scene in which death occurred, gross and microscopic examination of important organs (brain, heart, lungs, liver, and kidneys), various ancillary laboratory studies (toxicologic assays, microbiologic cultures, and metabolic screening tests, etc.) as well as review of any prior medical records. Particularly, cardiac channelopathies and cardiomyopathies are known to play significant roles in sudden cardiac deaths. Autopsy findings unveil atherosclerotic coronary artery disease as the most common etiology of SCD, especially in older people; in contrast, the cardiomyopathies account for approximately half of SCD cases in people who are younger than 35 years of age (Figure 1).²

FORENSIC PATHOLOGICAL ASPECTS OF CARDIOMYOPATHIES

Cardiomyopathy, a prominent cause of lethal ventricular arrhythmias and heart failure, may be defined as a primary weakness of the myocardium, impaired myocardial perfusion, or an infiltrative process. Cardiomyopathies are not uncommon in the general population, particularly, hypertrophic cardiomyopathy (HCM), arrhythmogenic right ventricular cardiomyopathy (ARVC) and dilated cardiomyopathy (DCM) are ought to be kept in mind when investigating sudden deaths of young adults. It is important to be aware of the fact that pathognomonic gross and histopathological changes of cardiac tissue for cardiomyopathies may be concealed; causing difficulties in accurate diagnosing at autopsy.³ Through the advances of molecular testing technologies, the depth of our understanding of cardiovascular disease-related sudden unexplained death has been remarkably improved over the past decade. Molecular diagnostics are directly impacting the practices of autopsy pathologists and medical examiners. In the past few years, over 100 cardiomyopathy-related genes have

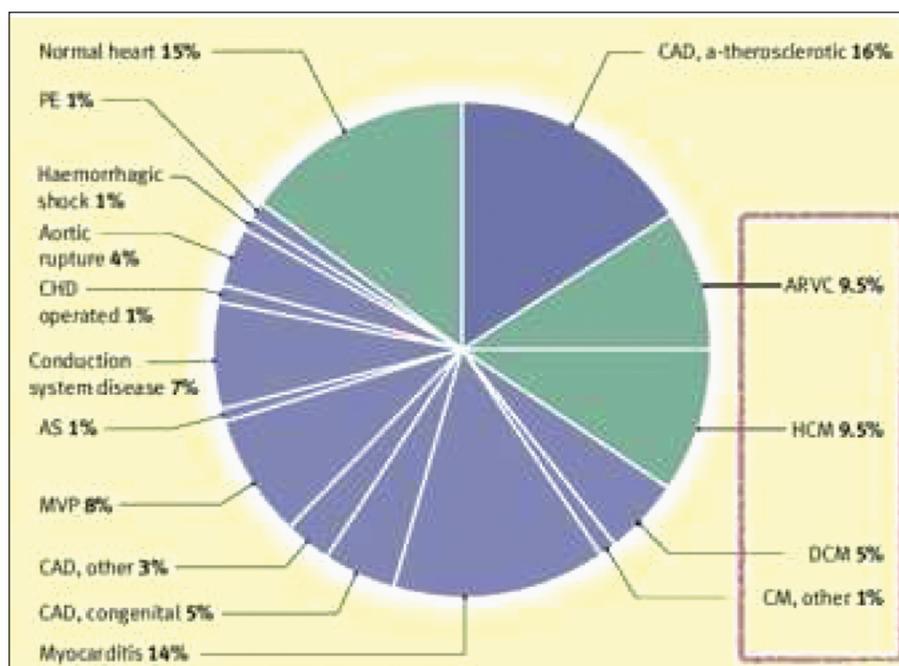


FIGURE 1: Causes of SCD in the young population (<35 years of age).

been identified (Table 1).³ Postmortem screening of the major genes involved in cardiomyopathies can help determine the cause of death and may help to evaluate the potential risk of relatives.

Cardiomyopathies are associated with predisposing genes, also known to be accompanied by altered gene expressions. Shifted gene expression resulting in structural change referred to as cardiac remodeling. Dysregulation of coding or non-coding genes is involved in several cardiovascular diseases including cardiomyopathies.⁴ The mechanisms underlying the control of these coding or non-coding genes are becoming increasingly well recognized. However, the discovery of epigenetic mechanisms has made the field of gene expression much more complicated. The term of “epigenetic” is defined as heritable modifications in gene expression that cannot be explained by changes in DNA sequence. Such a process is mainly characterized by DNA methylation and histone modifications and has a profound role in heart failure and arrhythmias. Thus, a clarification of the epigenetic

mechanisms may lead to further understanding of the pathophysiology of human diseases including cardiomyopathies.⁵

HYPERTROPHIC CARDIOMYOPATHY

HCM is characterized at a gross examination by either asymmetrical or symmetrical left ventricular hypertrophy, which is not explained by left ventricular pressure overload.² It was found to be as a consequence of missense mutations in the genes encoding sarcomeric proteins such as MYH7 and MYBPC3.⁴ Due to genetic heterogeneity and variable phenotype, genotype-phenotype correlations remain complex and studies involving a large cohort of unrelated HCM patients, have recommended paying considerable attention before assigning a prognostic significance to any particular mutation. Pathological hypertrophy is a maladaptive growth of the heart in response to stress. Chronic stress-induced specific histone modifications (histone acetyltransferase (HAT) activity and histone deacetylase (HDAC) activity) regulates car-

TABLE 1: Identified cardiomyopathy-associated genes.

Diseases	Genes	Functions	Testing sensitivity & limitation
HCM	<i>ACTC1, GIA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, MYOZ2, PRKAG2, TNNT2, TNN13, TNNC1, CSRP3, TTN, ACTN2, and TPM1, PLN</i>	Components of sarcomere. e.g., myosin heavy and light chains	Approximately 50-60% of HCM patients are positive
ARVC	<i>PKP2, DSP, DSG2, TGFB3, JUP, RyR2, and TMEM43</i>	Components of Desmosome, Ca ²⁺ regulation, and transforming growth factor	Approximately 50-60% or ARVD patients are positive
DCM	<i>TTN, ANKRD1, ACTC, LDB3, LMNA, MYBPC3, MYH7, MYH6, PLN, SCN5A, TAZ, TNNC1, TNN13, TNNT2, BAG3, ANKRD1, RBM20, TPM1, CSRP3, CTF1, DES, EMD, LDB3, etc.</i>	Components of sarcomere. Z-disk, nuclear lamina proteins, and intermediate filaments, RNA-binding proteins, molecular chaperone regulatory proteins	Approximately 25% of DCM patients are positive
LVNC	<i>ACTC1, LDB3, LMNA, MYBPC3, MYH7, TAZ, and TNN2</i>	Components of sarcomere and nuclear lamina proteins	Approximately 25% of LVNC patients are positive

HCM: Hypertrophic cardiomyopathy; ARVC: Arrhythmogenic right ventricular cardiomyopathy; DCM: Idiopathic dilated cardiomyopathy; LVNC: Left ventricular noncompaction cardiomyopathy.

diac growth and remodeling with the support of the findings, which are showing that histone acetylation\deacetylation is critical to hypertrophic signaling pathways in heart muscle.⁶

ARRHYTHMOGENIC RIGHT VENTRICULAR CARDIOMYOPATHY

ARVC is increasingly designated as arrhythmogenic cardiomyopathy because biventricular and left-dominant forms are recognized. Around 40% of patients with ARVC carry pathogenic mutations in the similar genes as observed for other cardiomyopathies such as HCM.⁷ Mutation of autosomal desmoplakin (DSP) and plakophilin (PKP2) genes are often responsible for ARVC.² Insufficient methylation of histone H3 protein in adult cardiomyocytes was found to lead the down-regulation of Kv channel-interacting protein 2 (Kcnp2), which is repressed in heart failure and has a role in arrhythmogenesis; however, genotype-phenotype studies are still limited in ARVC.⁷

DILATED CARDIOMYOPATHY

DCM is commonly identified at forensic autopsy incidentally. Death may be seen at any stage of the progressive process of cardiac enlargement, ventricular chamber enlargement, thinning of the left ventricular wall and contractile dysfunction. Studies have shown that up to 48% of DCM cases may be inherited. Genome-wide mapping and exome sequencing in a unique affected family has identified GATAD1, encoding the GATA zinc finger domain containing protein 1, as another pathogenic gene causing autosomal recessive DCM.⁷ On the other hand, cardiac-specific deletion of Dot1L, a catalyzer for H3 methylation, increases lethality at the postnatal and adult stages and causes dilation of the cardiac chambers in mice. Cardiac remodeling in Dot1L deficient mice is associated with reactivation of fetal cardiac genes, increased fibrosis, and enhanced apoptosis. Dot1L knockout mice have increased volume of the cardiac chambers and reduced contractility. These alterations are reminiscent of patients with dilated cardiomyopathy.⁶

MICRO RNA - KEY REGULATOR FOR THE HEART REMODELING

With respect to covalent modification of DNA and histones, the role of micro ribonucleic-acids (micro RNA/miRNA) in heart failure has been more extensively investigated. Over the last few years, a handful of reports have demonstrated an important role in the pathogenesis of cardiomyopathies.⁷ miRNAs are single-strand non-coding RNAs, first described in *Caenorhabditis elegans* as a regulator of gene expression. The mechanisms involved in miRNA-mediated silencing of gene expression are yet under controversies. However, accumulating data suggests that alterations in many individual miRNAs correlate with the development and progression of human diseases, including cardiovascular diseases such as cardiomyopathies.⁵ Dysregulation of the miRNA biogenesis machinery causes aberrant miRNA maturation and function. This condition leads to several diseases including cardio vascular disease. Dicer is a major enzyme involved in the maturation of miRNA, it is obvious that altered Dicer expression results in abnormal miRNA profile. Although the miRNA pattern is globally affected in patients with heart diseases, such as cardiomyopathies, some miRNAs have been found to be more frequently disturbed than others. Pathological changes in miRNA expression were shown in hypertrophic myositis in mice.⁸ Particularly miRNA-133, which is abundantly expressed in the heart muscle, plays a critical role in the hypertrophy. In addition, miRNA-133 post-transcriptionally regulates the connective tissue growth factor (CTGF), which is a key protein in myocardial fibrosis process.⁷ Beyond its morphological remodeling regulation function, it has found that up-regulation of miRNA-133 is associated with a propensity to arrhythmia.⁹

EPIGENETICS AS A NOVEL RESEARCH FIELD IN FORENSIC MOLECULAR PATHOLOGY

Despite advances in the prevention and management of cardiovascular diseases, this group of multifactorial disorders remains a major cause of

mortality worldwide. Cardiomyopathy is associated with multiple genetic and modifiable risk factors; however, given environmental and genetic influences yet to explain precise pathophysiology. Epigenetics encourage to fill some of the gaps in our current knowledge regarding the interaction between nature and nurture in the development of cardiomyopathies. Epigenetic mechanisms include DNA methylation, histone modification, and microRNA alterations, which collectively enable the cell to respond quickly to environmental changes which result in pathogenic cardiovascular processes. Clinical guidelines pay attention to genetic screening in cardiomyopathies because it can identify relatives at risk of developing the disease and it also confirms the diagnosis of cardiomyopathy with the subtypes in postmortem studies. However, low quality of genetic testing can detect pathogenic variants in only about 50% of individuals with familial HCM, DCM, or ARVC.¹⁰ This limitation may be postulated as a result of poor understanding of genotype-phenotype relationships in cardiomyopathies. Silences and reactivations of the susceptible genes are more important for emerging of the disease. Thus, epigenetic alterations may be counted

as more promising diagnostic features to determine the diagnosis. Furthermore, due to their transmissibility to the following generation, epigenetic screening would be helpful for familial prevention. Today, postmortem epigenetic studies, such as quantitative and qualitative determination of up and down regulations in specific miRNA's, are unlikely to be common due to their complex technique and high cost, nevertheless their promising diagnostic value for unexplained sudden deaths caused by complex genotype-phenotype associated disorders such as cardiomyopathies is a promising field of forensic molecular pathology.

Conflict of Interest

Authors declared no conflict of interest or financial support.

Authorship Contributions

Idea/Concept: Yasin Hasan Balcioğlu; **Design:** Yasin Hasan Balcioğlu; **Control/supervision:** Yasin Hasan Balcioğlu, Filiz Ekim Çevik, Faruk Aşıcıoğlu; **Data collection and/or processing:** Yasin Hasan Balcioğlu, Filiz Ekim Çevik; **Analysis and/or Interpretation:** Yasin Hasan Balcioğlu; **Literature review:** Yasin Hasan Balcioğlu, Filiz Ekim Çevik; **Writing the article:** Yasin Hasan Balcioğlu; **Critical Review:** Faruk Aşıcıoğlu.

REFERENCES

- Miles CJ, Behr ER. The role of genetic testing in unexplained sudden death. *Transl Res* 2016;168:59-73.
- Basso C, Carturan E, Pilichou K, Corrado D, Thiene G. Sudden arrhythmic death and the cardiomyopathies: Molecular genetics and pathology. *Diagnostic Histopathol* 2010;16(1): 31-42.
- Tang Y, Stahl-Herz J, Sampson BA. Molecular diagnostics of cardiovascular diseases in sudden unexplained death. *Cardiovasc Pathol* 2014;23(1):1-4.
- Khraiweh B, Arif MA, Seumel GI, Ossowski S, Weigel D, Reski R, et al. Transcriptional Control of Gene Expression by MicroRNAs. *Cell* 2010;140(1):111-22.
- Asrih M, Steffens S. Emerging role of epigenetics and miRNA in diabetic cardiomyopathy. *Cardiovasc Pathol* 2013;22(2): 117-25.
- Mahmoud SA, Poizat C. Epigenetics and chromatin remodeling in adult cardiomyopathy. *J Pathol* 2013;231(2):147-57.
- Yang J, Xu WW, Hu SJ. Heart failure: Advanced development in genetics and epigenetics. *Biomed Res Int* 2015;2015:1-11.
- Cheng Y, Ji R, Yue J, Yang J, Liu X, Chen H, et al. MicroRNAs Are Aberrantly Expressed in Hypertrophic Heart: Do They Play a Role in Cardiac Hypertrophy? *Am J Pathol* 2007; 170(6):1831-40.
- Xiao J, Luo X, Lin H, Zhang Y, Lu Y, Wang N, et al. MicroRNA miR-133 represses HERG K⁺ channel expression contributing to QT prolongation in diabetic hearts. *J Biol Chem* 2007; 282(17):12363-7.
- Ordovás JM, Smith CE. Epigenetics and cardiovascular disease. *Nat Rev Cardiol* 2010; 7(9):510-9.