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Commentary

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The burden of somatic mutations in the aging heart

Brenda Gerull[1,2], Ruping Chen[1,2]

[1]Comprehensive Heart Failure Center, Department of Cardiovascular Genetics, University Hospital Würzburg, Würzburg 97078, Germany.
[2]Department of Medicine I, University Hospital Würzburg, Würzburg 97078, Germany.

Correspondence to: Dr. Brenda Gerull, Comprehensive Heart Failure Center and Department of Medicine I, University Hospital Würzburg, Am Schwarzenberg 15, Würzburg 97078, Germany. E-mail: Gerull_B@ukw.de

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Somatic mutations, which occur in the cells of somatic tissue after fertilization, can robustly be acquired as early as the first cellular division during embryogenesis[1]. Such mutations have been extensively studied in oncogenesis as they are the root cause of essentially all cancers. However, somatic mutations can and do occur in non-cancerous cells throughout the lifespan[2]. Comparative analysis of the mutational landscape across 16 mammalian species reveals that species-specific somatic mutation rates are inversely correlated with lifespan. In addition, increasing evidence suggests that somatic mutations cause genetic instability, which is a hallmark of aging[3]. Tissues accumulate somatic mutations linearly with aging but at variable rates in a tissue and cell-type-dependent manner, and the accumulation rates are consistently elevated in prenatal cells relative to postnatal cells[2]. Moreover, organisms evolve complex DNA repair systems and maintenance mechanisms to deal with DNA damage. However, with aging, DNA repair networks lose efficiency, which can accentuate the accumulation of somatic mutations[3].

Most investigations into cardiovascular disease genetics focus on germline mutations. However, several studies have demonstrated that the accumulation of somatic mutations accompanies cardiovascular aging and can lead to a variety of manifestations in cardiovascular disease (CVD), ranging from conduction abnormalities to the development of atherosclerotic coronary disease[1]. The elderly population is particularly susceptible to cardiovascular disease, but there is an incomplete understanding of how aging promotes CVD.

A recent study from Choudhury *et al.*, published in *Nature Aging*, aimed to evaluate the genome-wide burden of somatic single nucleotide variants (sSNVs)[4]. The authors employed single-cell whole-genome sequencing to analyze 56 individual human cardiomyocytes from 12 postmortem hearts, spanning an age range of 0.4 to 82 years. Surprisingly, the study revealed that cardiomyocytes accumulate sSNVs with age at rates even higher than non-dividing neurons and dividing lymphocytes, but similar to highly active metabolic hepatocytes. Additionally, the accumulation of sSNVs in cardiomyocytes increased with age, regardless of the nuclear ploidy, after normalizing to the corresponding genomic size[4].

Cardiomyocytes, a cell type that rarely undergoes cell division, were thought to have uncommon somatic mutations. To gain insight into the molecular mechanisms involved in sSNV formation, the authors deconvoluted the sSNV profiles of all cardiomyocytes into mutation signatures using non-negative matrix factorization (NMF). Cardiomyocyte sSNVs exhibited distinctive mutational signatures, with C>T and T>C mutations predominantly identified during the aging process, which were classified as Signature A. Signature A reflected faulty repair of deamination of methylated cytosine to thymine at CpG sites[4], which is the most common and reliable mechanism for somatic mutation formation[1]. Consistently, C>T transitions were robustly accumulated at CpG sites in old mouse hearts compared to young mouse hearts[5]. Signature C, prominently enriched in C>A mutations, reflected faulty repair of 8-oxo-7,8-dihydroguanine (8-oxoG)[4]. The heart, a highly metabolic organ, produces a significant amount of reactive oxygen species (ROS). 8-oxoG, the product of oxidative attack on the most oxidizable guanine nucleotide, can pair with adenine during DNA replication, generating G/C>T/A somatic mutations, and is mutagenic[2]. The base excision repair (BER) proteins OGG1 and MUTYH are responsible for removing 8-oxoG and 8-oxoG:A mispairs. If both fail, nucleotide excision repair (NER) can eliminate 8-oxoG lesions from the genome[2]. Signature D, which reflects a defect in mismatch repair (MMR), only accumulates in aged cardiomyocytes among the four examined cell types, indicating a distinct mutational process in the heart[4]. The analysis of mutational signatures suggested a model of oxidative stress-related mutation burden, which might overwhelm the NER, BER and MMR machinery in cardiomyocytes with aging.

To understand how mutations formed and accumulated in hearts with aging, the authors further utilized RNA-seq expression data from the GTEx portal. They observed an overall downregulated gene expression for the core components of the MMR complex, NER, and BER pathway. Interestingly, the MMR pathway exhibited a faster reduction in gene expression compared to the NER and BER pathways in aged hearts, suggesting a specific impairment of the MMR pathway responsible for Signature D mutations in cardiomyoytes[4].

Finally, the authors proposed a prediction model of the effect of sSNVs on the abundance of knock-out (KO) tetraploid cardiomyocytes. They utilized imaging flow cytometry and karyotyping techniques to investigate polyploidization in cardiomyocytes and its relationship with age. The findings indicated that polyploidization in cardiomyocytes initiates during the neonatal stage and becomes more prevalent as individuals age[4]. Consistently, the study by Kirillova *et al.* observed that neonatal hearts possess approximately 60% of cardiomyocytes with a single-nucleated diploid nucleus[6]. However, as individuals grow older, the proportion of adult cardiomyocytes with a single-nucleated diploid nucleus decreases to 30%. Instead, 70% of adult cardiomyocytes exhibit polyploidy, with 40% having a single-nucleated polyploid nucleus and 30% possessing bi-nucleated diploid or polyploid nuclei[6]. The authors pointed out that tetraploid or higher ploidy cardiomyocytes could better tolerate the damage compared to diploid cardiomyocytes when mutations affect both alleles. Polyploidization in cardiomyocytes may provide a mechanism of genetic compensation to withstand oxidative stress by increasing cell size and metabolic production, ultimately minimizing the complete KO of essential genes during aging[4]. Additionally, aside

from the protective effect of polyploidization in cardiomyocytes, more sSNVs can be generated through DNA endoreplication errors during the polyploidization formation.

Genomic instability is a common hallmark of aging across species and in various organs. Notably, age-related mutations and their effects on the cardiovascular system are more prevalent in the blood and bone marrow rather than in the heart and vasculature. This phenomenon, known as clonal hematopoiesis of indeterminate potential (CHIP), is primarily caused by somatic mutations in hematopoietic stem/progenitor transcriptional regulators such as DNA (cytosine-5)-methyltransferase 3A (DNMT3A), methylcytosine dioxygenase TET2 (TET2), polycomb group protein ASXL1 (ASXL1) and tyrosine-protein kinase JAK2 (JAK2)[7]. CHIP contributes to a wide spectrum of CVDs, including atherosclerosis, pathological cardiac remodeling, and heart failure, primarily through an inflammatory response. The accumulated rates of somatic mutations vary substantially between cell types and contexts, and aged hearts consist of a higher proportion of myeloid cells and fewer cardiomyocytes[7].

In addition, to sSNVs, somatic mutations encompass other types, such as deletions and insertions, and they are pervasive in the nuclear genome (nDNA). However, in the highly metabolic active heart, somatic mutations in the mitochondrial genome (mtDNA) should not be overlooked. As we know, the primary source of ROS production is the respiratory chain inside the mitochondria. Interestingly, mtDNA mutations in aged cells arise from an imperfect replication process executed by polymerase γ (POLG) rather than oxidative lesions[8]. The absence of an accumulation of oxidative damage-linked mtDNA mutations with age suggests a lifelong dynamic clearance of either the oxidative lesions or oxidized mtDNA[9]. Since mtDNA replicates independently of nuclear DNA, apparent heteroplasmies can increase with aging, even in the absence of cell division in cardiomyocytes. The authors identified inefficient DNA repair pathways, including NER, BER and MMR, in aged hearts using data from the GTEx portal[4]. Studies in humans and other long-lived species have revealed that enhanced DNA repair mechanisms coevolve with increased longevity.

In summary, the authors observed an increased presence of sSNVs in aging cardiomyocytes, particularly the cardiomyocyte-specific Signature D. This mutation type indicates a defect in the repair of damage involving almost all mismatches, which can be attributed to a faster reduction in MMR pathway-related genes compared to those of the NER and BER pathways. Furthermore, polyploidization in cardiomyocytes initiates early in life and becomes more prevalent with age, which may confer better tolerance to the detrimental effects of mutations in cardiomyocytes compared to diploid cardiomyocytes when both alleles are affected[4] [Figure 1].

This study offers an initial view into the landscape of somatic mutations of human cardiomyocytes during the aging process. The specific mutational signature found in aged cardiomyocytes highlights the significant roles played by ROS and defective DNA repair pathways. This newfound understanding enhances our knowledge of the mechanisms behind cardiac aging. However, several questions remain unanswered. For instance, the study did not address other types of somatic mutations in aging cardiomyocytes, such as insertion-deletion mutations (Indels), and the role of somatic mutations in mitochondrial DNA (mtDNA) during the aging process. While tissues accumulate somatic mutations in a linear fashion with age, the rates of somatic mutations vary depending on the tissue and cell type[2]. For example, lymphocytes and endothelial cells exhibit the highest somatic mutation burden in aged lung tissue, which correlates with the high expression of senescence signatures in these cells[10]. Apart from cardiomyocytes, the human heart consists of various other cell types, including endothelial cells, fibroblasts, immune cells, and more. These are dividing cells that can capture accumulated somatic mutations during replication, particularly under

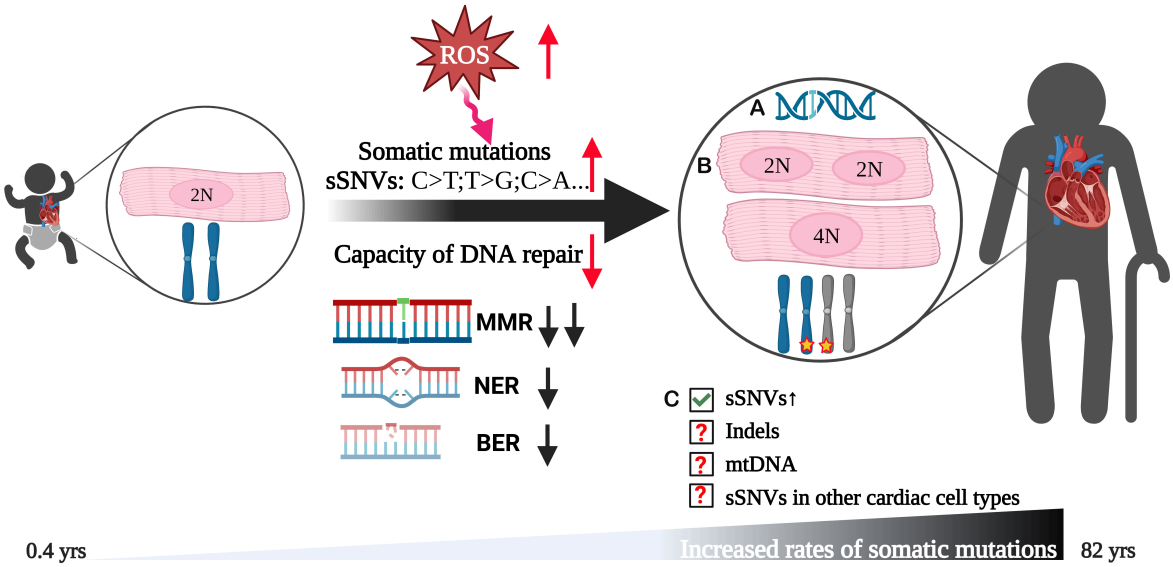

Figure 1. Somatic mutation burden in aging cardiomyocytes. (A) sSNVs increase in human cardiomyocytes and DNA repair pathways become defective with age, particularly the MMR pathway. (B) Polyploidization confers cardiomyocytes tolerance to deleterious effects of mutations compared to diploid cardiomyocytes when mutations affect both alleles. (C) Open questions include the presence of other types of somatic mutations, such as Indels and mutations in mtDNA, and the contributions of sSNVs in the other cardiac cells during the aging process and heart diseases. ROS: Reactive oxygen species; sSNVs: somatic single nucleotide variants; MMR: mismatch repair; NER: nucleotide excision repair; BER: base excision repair; Indels: Insertion-deletion mutations; mtDNA: mitochondrial DNA; 2N: diploid; 4N: tetraploid. The image was created with BioRender.

conditions of oxidative stress. Understanding the contribution of somatic mutations, such as sSNVs, in other cardiac cell types will be crucial [Figure 1]. Integrating these puzzle pieces into the aging process of the heart will shed light on potential treatments targeting age-related heart diseases.

DECLARATIONS

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

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Not applicable.

Consent for publication

Not applicable.

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