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Somatic Mutations and Atrial Fibrillation: The End or Just the Beginning?

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Atrial fibrillation (AF) is a common, morbid, and heritable arrhythmia. Well-documented Mendelian[1] and polygenic[2] contributions to the inherited basis for AF exist. In contrast, the potential contribution of somatic or acquired mutations in atrial tissue has not been extensively explored for AF. The hypothesis that somatic mutations may underlie AF is particularly intriguing when considering that AF is largely a tissue-specific disease (albeit influenced by systemic modulating factors such as inflammation or autonomic tone). Thus, it stands to reason that mutations specific to atrial or pulmonary venous tissue might be sufficient to initiate the complex cascade of molecular events leading to AF.

In 2006, Gollob et al. reported that somatic mutations in *GJA5*, a gene encoding a cardiac gap junction protein, connexin 40, were found in cardiac tissue from 3 of 15 patients with idiopathic AF who had undergone surgical AF ablation.[3] This observation supported the notion that somatic mutations might underlie a substantial proportion of AF. In a subsequent report, Gollob and colleagues identified a mutation in *GJA1* or connexin 43, which again supported somatic mosaicism as a mechanism of AF.[4]

In this issue of *Circulation Cardiovascular Genetics*, Roberts et al.[5] describe their extensive effort to identify somatic mutations in a sample of 34 patients who underwent surgical left atrial appendage excision, 25 of whom had AF and 9 of whom did not. Of the 25 patients with AF, 20 underwent surgery specifically for an AF ablation. The authors performed targeted sequencing of 560 candidate genes using next generation sequencing of both peripheral lymphocytes and excised left atrial appendage tissue. After filtering and removing variants deemed most likely to represent artifacts, they identified a total of 5 variants present in the left atrial appendage tissue but not circulating lymphocytes. Of these, 3 were in AF samples, and 2 were in control samples, a difference that was not statistically significant.

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Among these discordant variants, none were located in *GJA1* or *GJA5*. Interestingly, Sanger sequencing failed to confirm any of these somatic mutations in the atrial tissue samples.

Perhaps the most important conclusion to draw from this study is the fact that identifying somatic mosaicism is complex, and maximizing the ability to distinguish between true variants and artifacts requires a careful study design. First, sample processing can be essential for minimizing the introduction of artifacts. The authors recognize and discuss this challenge, highlighting the fact that in the prior reports implicating somatic mutations in *GJA1* and *GJA5* in AF pathogenesis, DNA was amplified from tissue that was fixed in formalin and paraffin-embedded,[3,4] a process that can be associated with sequence errors.[6] In the present report,[5] the tissue samples were flash frozen, thereby minimizing the possibility of such artifacts.

Second, careful bioinformatic filtering and variant processing are essential to the process. In the present report, the authors first identified 8,710 discordant variants. Computational analyses ultimately reduced this number to the 5 possible variants, but as noted, none were confirmed by Sanger sequencing. With such large potential for artifact, rigorous algorithms are necessary to preserve true discordant variants, and reject likely artifacts, for downstream analysis.

Third, and among the most important elements, is that studies need to be designed in concert with best epidemiological practices to facilitate discovery of disease susceptibility loci. Study design considerations, such as sampling cases and controls from extremes of the disease susceptibility spectrum, using large sample sizes, and employing replication samples are fundamental to maximizing power and ensuring study validity.

Even with the elegant nature of the current work, it is important to note that a well-powered study designed to identify somatic mutations underlying AF would require a considerably larger sample size. For example, assuming an evenly split case and control sample, and a 1% background somatic mutation rate in controls, about 200 subjects would be necessary to identify a somatic mutation proportion of about 10% in AF cases with P=0.05 and 80% power using a simple allelic test between cases and controls. The sample size requirements increase to nearly 600 subjects with a somatic mutation rate of 5% in cases. Moreover, the sample size requirements increase exceedingly as one adjusts the significance threshold to protect against false positive findings that may result from multiple hypothesis testing, as may occur when testing whether specific genes are more likely to harbor somatic mutations. In the current report 560 candidate genes were considered, whereas future efforts would ideally include all of the ~20,000 genes in the exome and potentially noncoding genomic regions as well. However, the sample sizes required for such an approach are likely to be prohibitive. As the authors have noted, these study design considerations are particularly challenging given the difficulty of obtaining cardiopulmonary tissue from optimal cases and controls.

What then, do the results of conflicting studies tell us about the potential for somatic mutations to contribute to AF? Whereas it remains possible that somatic mosaicism may contribute to AF susceptibility, it seems that mosaicism is not the predominant mechanism.

It may be however, that a final assessment of the contribution of somatic mosaicism to AF pathogenesis cannot be conducted until a larger number of samples are available for study. Until then, the current work serves as a milestone and perhaps a new beginning rather than an end to the potential role of somatic variation in AF.

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