

# Genetics, transcriptomics, metagenomics, and metabolomics in the pathogenesis and prediction of atrial fibrillation

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## KEYWORDS

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The primary cellular substrates of atrial fibrillation (AF) and the mechanisms underlying AF onset remain poorly characterized and therefore, its risk assessment lacks precision. While the use of omics may enable discovery of novel AF risk factors and narrow down the cellular pathways involved in AF pathogenesis, the work is far from complete. Large-scale genome-wide association studies and transcriptomic analyses that allow an unbiased, non-candidate-gene-based delineation of molecular changes associated with AF in humans have identified at least 150 genetic loci associated with AF. However, only few of these loci have been thoroughly mechanistically dissected, indicating that much remains to be discovered for targeted diagnostics and therapeutics. Metabolomics and metagenomics, on the other hand, add to the understanding of AF downstream of the primary substrate and integrate the signalling of environmental and host factors, respectively. These two rapidly developing fields have already provided several correlates of prevalent and incident AF that require additional validation in external cohorts and experimental studies. In this review, we take a look at the recent developments in genetics, transcriptomics, metagenomics, and metabolomics and how they may aid in improving the discovery of AF risk factors and shed light into the molecular mechanisms leading to AF onset.

## Introduction

Atrial fibrillation (AF) risk assessment on the individual level still lacks precision. Even the best AF risk scores achieve a c-statistic (a measure of discrimination also known as the area under the receiver operating characteristic curve) of approximately only 0.80.<sup>1</sup> This

means that a 20% probability still exists for the risk prediction model to be unable to discriminate an individual likely to develop cardiovascular disease (CVD) on follow-up from one less likely to do so. In addition to risk factors and correlates, the molecular mechanisms underlying AF onset remain to a large extent controversial or unknown despite recent developments. This review focuses on how ‘omics’, in this case, genetics, transcriptomics, metagenomics, and metabolomics, are helping to improve AF risk

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factor discovery and to elucidate the mechanisms underlying AF.

### The missing links of AF genetics

Linkage analysis in families with many affected individuals and a clear hereditary pattern has helped to identify several mutations associated with AF, such as those affecting *KCNQ1*, *NPPA*, *MYL4*, *TBX5*, and *TTN*.<sup>2</sup> Although rare by nature and thereby having only a small impact on the global AF burden, analysis of these hereditary forms of AF has been most informative and aided greatly the understanding of AF disease biology and the potential molecular mechanisms of variant action. For example, the AF-linked mutations for the ion channel encoding gene, *KCNQ1*, have been shown to result in gain of channel function and likely shorten the atrial refractory period.<sup>3</sup> For atrial natriuretic peptide, *NPPA*, a frame-shift mutation removes a stop codon and leads to an extended mutant protein that can bypass degradation and thereby achieve increased activity and higher circulating levels.<sup>4</sup> *In vivo* experiments have shown this to lead to changes in atrial electrophysiology (i.e. shorter duration of monophasic action potential and effective refractory period) that could promote AF. Autosomal recessive mutations in atrial-specific myosin light chain, *MYL4*, can lead to early-onset AF through abnormal F-actin binding region and consequent disruption of the sarcomere and enlargement of the atria.<sup>5</sup> And finally, a gain-of-function mutation in the transcription factor, *TBX5*, causes developmental disorder that leads to heart and limb malformations through enhanced binding of the mutated *TBX5* to DNA and up-regulation of downstream targets, including *NPPA* and *GJA5*, a component of gap junctions that itself

carries AF-associated variants.<sup>6-12</sup> Together, these studies provide an anchor to disease biology and help to shape the potential mechanisms of action for AF variants more broadly.

Genome-wide association studies (GWAS) have further helped to provide insights into the onset and progression of human CVD.<sup>13</sup> The first AF-associated locus (4q25), near *PITX2*, was reported in 2007.<sup>14-16</sup> Since then, multiple studies have identified new susceptibility loci,<sup>2,14,17-29</sup> and linked them to putative genes ([Table 1](#); [Supplementary material online, Table S1](#)).<sup>17</sup> However, the major challenge with GWAS approach remains the same—instead of specific causal genes, it identifies a region of interest. In the case of *PITX2* locus, the link between the non-coding variants and the affected gene was obvious due to the exceptionally strong link, but for most of the 150 AF loci uncovered by GWAS ([Table 1](#); [Supplementary material online, Table S1](#)),<sup>17</sup> the causal variants remain unknown, as they may reside far away from the affected genes and have moderate effects, making their mechanisms of function less obvious. In addition, the variants may also affect both the expression and function of the target gene through different mechanisms and multiple variants, as illustrated by *NKX2-5*. The gene encoding transcription factor *NKX2-5* has been associated with ECG traits and is an example of an AF-linked transcription factor whose function is affected by regulatory variants. A recent study by Benaglio *et al.*<sup>30</sup> identified ~2000 single-nucleotide variants associated with allele-specific effects on *NKX2-5* binding sites across the genome. They experimentally confirmed two variants that modulate target gene expression through differential transcription factor binding in cardiac cells, concluding them as putative functional variants underlying the

**Table 1** Classification based on biological processes and cell biology of the nearest genes in AF associate loci in genome-wide association studies

Process/compartament	Genes
Classification based on biological processes	
Cardiac and skeletal muscle function and integrity	AKAP6, CFL2, MYH6, MYH7, MYO18B, MYO1C, MYOCD, MYOT, MYOZ1, MYPN, PKP2, RBM20, SGCA, SSPN, SYNPO2L, TTN, TTN-AS, WIPF1
Mediation of developmental events	ARNT2, EPHA3, FGF5, GATA4, GTF2I, HAND2, LRRC10, NAV2, NKX2-5, SLIT3, SOX15, TBX5
Intracellular calcium handling in the heart	CALU, CAMK2D, CASQ2, PLN
Angiogenesis	TNFSF12, TNFSF12-TNFSF13
Hormone signalling	CGA, ESR2, IGF1R, NR3C1, THRB
Function of cardiac ion channels	HCN4, KCND3, KCNH2, KCNJ5, KCNN2, KCNN3, SCN10A, SCN5A, SLC9B1
Classification based on cell biology	
Cell polarity and epithelial to mesenchymal transition	<i>PITX2</i> , <i>WNT8A</i> , <i>SMAD7</i>
Cell-cell interaction	<i>GJA5</i> , <i>GJA1</i> , <i>CAV1</i> , <i>PHLDB2</i> , <i>PKP2</i> , <i>PHLDA1</i>
Microtubules	<i>MAPT</i> , <i>CEP68</i> , <i>TUBA8</i> , <i>REC114</i> , <i>DNAH10</i>
Transcription regulator	<i>ZNF462</i> , <i>ZFHX3</i> , <i>HSF2</i> , <i>NKX2-5</i> , <i>CASZ1</i> , <i>TLE3</i> , <i>GTF2I</i> , <i>DPF3</i> , <i>SCMH1</i> , <i>NR3C1</i> , <i>ZNF292</i> , <i>KDM1B</i> , <i>PRDM8</i>
RNA binding	<i>RPS2</i> , <i>RBM20</i> , <i>POLR2A</i>
Cytoskeleton	<i>SSPN</i> , <i>PHLDB2</i> , <i>CFL2</i> , <i>WIPF1</i>
Post-translational regulation	<i>PKP2</i> , <i>USP3</i>
Golgi	<i>GORAB</i> , <i>COG5</i> , <i>GOSR2</i> , <i>GOPC</i>

electrocardiographic GWAS signals. Compelling evidence from several large-scale studies suggests that such regulatory variants (i.e. variants affecting transcription factor binding to cis-regulatory elements thereby altering target gene expression cell-type-specifically) may encompass substantial fraction of the non-coding variants with unknown functions.<sup>31-36</sup>

Another major limitation with GWAS approach is that it does not capture all known variants in the genome but usually utilizes genotyping arrays that assess hundreds of thousands of genetic variants throughout the genome from which a greater number of single-nucleotide polymorphisms can be imputed. Therefore, the discovery of rare and unknown variants is limited. Despite the firm links of familial mutations to AF, only a few of these loci have been identified in GWAS.<sup>17,37</sup> For example, *TTN* was first identified of having loss-of-function mutations in familial early-onset AF and shortly after, a similar finding was made among unrelated individuals with early-onset AF, and finally, exome sequencing data confirmed a similar strong association for loss-of-function variation in the general AF population, with markedly higher penetrance among polygenic *TTN* mutation carriers.<sup>38-40</sup> Although the loss-of-function approach used in this case provides a considerable advantage over GWAS by establishing a direct link from gene function to disease and directionality for the effect, it would miss the gain-of-function mutations such as those identified for *TBX5*.

## Genomics and transcriptomics as tools for uncovering AF mechanisms

The general dissection of the molecular changes underlying AF initiation and progress in human bulk right and left atrial tissue has identified pathway changes in mechanotransduction, extracellular matrix remodelling, ion channel signalling, oxidative stress, apoptosis, fibrosis, and structural tissue organization under both developmental and inflammatory signalling.<sup>41,42</sup> These profiling studies generally credit the development of AF to deregulation of ion channels, calcium handling, structural remodelling, or autonomic neural regulation, and as such, AF is seen as a consequence of other cardiovascular pathologies.<sup>42</sup> However, the overlap of the results in these studies is not overwhelming at the molecular level, indicating that much remains to be discovered for targeted diagnostics and therapeutics.

In a recent paper by Hulsmans *et al.*,<sup>43</sup> first single-cell characterization of human left atrial tissue from five control participants and seven patients with chronic AF was performed. The study reported inflammatory monocyte and *SPP1*<sup>+</sup> macrophage expansion in atrial fibrillation and confirmed the findings in an *in vivo* mouse model combining hypertension, obesity, and mitral valve regurgitation to create enlarged, fibrosed, and fibrillation-prone atria. Single-cell transcriptome of the model recapitulated the human tissue findings and inhibition of monocyte migration reduced arrhythmia, as did deletion of *Spp1*, which was identified as the signal that promotes AF through local crosstalk with immune and stromal cells. Although the study was limited in its coverage in terms of pathway enrichments and cell types, as only six major non-cardiomyocyte populations

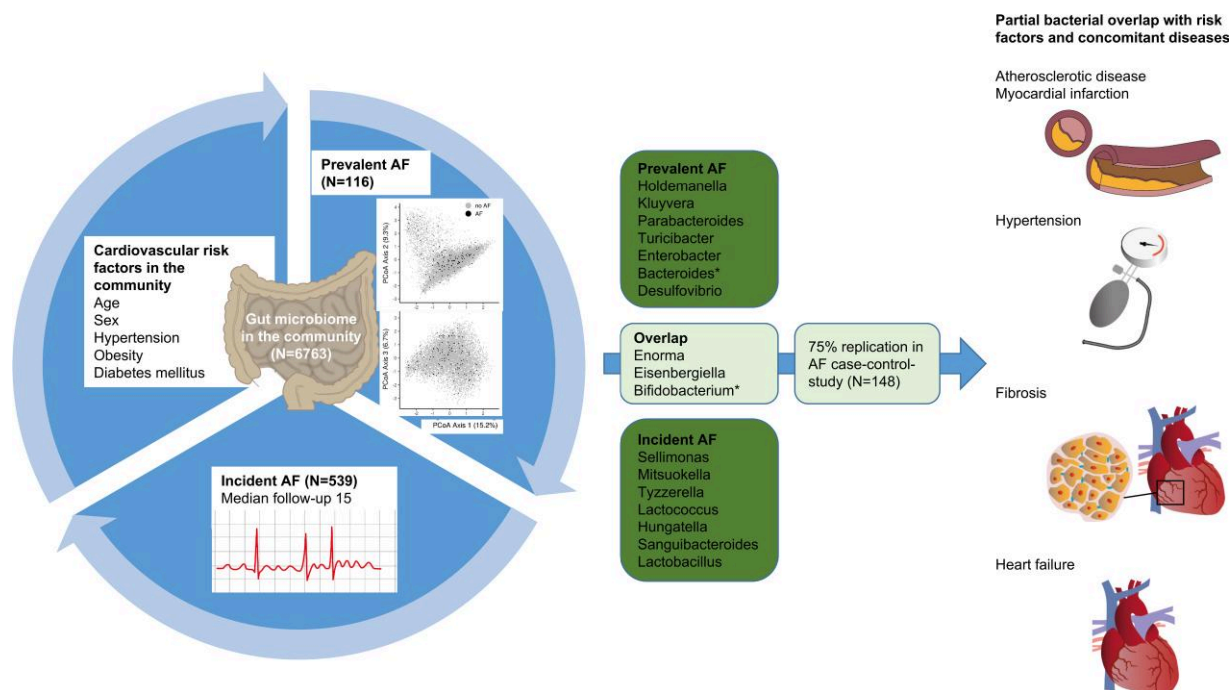
were captured, it clearly demonstrated the power of single-cell dissection of molecular mechanisms in finding putative diagnostic and therapeutic targets.

In recent years, genomics has emerged as a way to bridge the gap between GWAS variants and their function, highlighting putative causal variants based on chromatin conformation, gene regulation and expression. However, the existing datasets largely arise from bulk tissue samples that represent a complex and variable mix of cell types, masking the signal for the less abundant cell types. To overcome the issue, two studies have used the combination of single-cell transcriptome and chromatin accessibility profiling in human cardiac tissue together with GWAS mapping, improving the cell type-resolution of the risk variant mapping.<sup>44,45</sup> These studies confirmed the assumption that the interrogation of the cell-type-specific genomic and transcriptional changes is a prerequisite for the understanding of the molecular mechanisms of variant action. In the paper by Hocker *et al.*,<sup>45</sup> the researchers described 16 451 differentially accessible cis-regulatory elements between the pooled atria and ventricles, most of them in cardiomyocytes, whereas the difference between the left and right sides was less pronounced, but stronger between the left and right atria (101 differentially accessible sites between left and right ventricles, and 2 687 between left and right atria). AF-associated variants showed significant enrichment in both atrial and ventricular cardiomyocytes but were not enriched in accessible chromatin of non-cardiac tissues, with the exception of endothelial cells. In contrast to comparisons between atria and ventricles, the differentially accessible regions resided primarily in cardiac fibroblasts. Taken together, the findings of the paper suggest that the causal risk variant mapping requires data from the whole heart, as differences exist both between the left-right and atrium-ventricle axes.

In a more recent paper by Selewa *et al.*,<sup>44</sup> a more thorough fine-mapping of AF risk variants was performed, identifying putative causal variants in 122 AF-associated loci and highlighting known AF risk genes ([Table 1](#); [Supplementary material online, Table S1](#)), such as transcription factors involved in cardiac development and atrial rhythm control (e.g. *NKX2-5*, *TBX5*, and *PITX2*), ion channels (e.g. *KCNN3*), and genes involved in muscle contraction (e.g. *TNN*), and several new ones, such as *ASA1*, *ATXN1*, *ERBB4*, *RPL3L*, *TUBA8*, *EPHA3*, *THRB*, *BEND5*, and *PKP2*. The study concluded that most uncovered AF risk variants did not colocalize with heart eQTLs, due to under-detection of cell-type-specific effects with bulk eQTL studies and over-detection of variants with effects in cell types shared across tissues, undermining the common strategy of annotating GWAS results using eQTLs, and partially explaining the lack of functional understanding of the disease-associated loci.

## The 'second genome' and AF

Many of the established AF risk factors have been linked to gut microbial dysbiosis and, conversely, gut microbiota derived metabolites have been associated with cardiovascular health. This has led to the hypothesis that gut microbiota is associated with AF pathogenesis.<sup>46</sup> The first evidence of this link was obtained in a Chinese



**Figure 1** The links of gut microbiota with prevalent and incident AF in the FINRISK 2002 cohort. Gut microbiota likely contributes to AF both directly and mediating effects through overlapping risk factors and diseases. Reprinted from Palmu *et al.* *eBioMedicine* 2023;91:104583. Copyright 2023, with permission from Elsevier.<sup>47</sup>

case-control study of 50 patients hospitalized with non-valvular AF.<sup>46</sup> Individuals with AF exhibited alterations in seven microbial genera, 96 serum metabolites and 63 stool metabolites compared to healthy controls. In particular, AF was associated with relative overgrowth of genera *Ruminococcus*, *Streptococcus*, and *Enterococcus* and a reduction of the genera *Faecalibacterium*, *Alistipes*, *Oscillibacter*, and *Bilophila*. The first large-scale observational study ( $n=6763$ ) was published in 2023 (Figure 1), in which nine microbial genera were associated with prevalent AF (*Bacteroides*, *Bifidobacterium*, *Eisenbergiella*, *Enorma*, *Enterobacter*, *Holdemanella*, *Kluivera*, *Parabacteroides*, and *Turicibacter*) and eight microbial genera with incident AF (*Bifidobacterium*, *Enorma*, *Hungatella*, *Lactococcus*, *Mitsuokella*, *Sanguibacteroides*, *Sellimonas*, *Tyzzerella*).<sup>47</sup> These results were replicated in an independent German case-control cohort, in which a consistent trend was observed for 56% microbial genera associated with prevalent AF and 75% of genera associated with incident AF.<sup>47</sup>

In addition to observational correlations, two Mendelian randomization studies have also reported on the potential causal pathways between gut microbiota and AF. Mao *et al.*<sup>48</sup> published the first report using GWAS summary statistics for AF and microbiota. The authors observed a positive association for genus *Ruminococcaceae* and a negative association for genus *Turicibacter* with AF that is consistent with a causal effect. Dai *et al.*<sup>49</sup> used GWAS meta-analysis of six contributing European and American studies for AF and the Dutch Microbiome Project for species level gut microbial GWAS summary statistics; the authors also used data from the FinnGen and UK Biobank projects for validation. The authors observed positive associations for genus *Holdemania* (validated in FinnGen

and UK Biobank) and species *Eubacterium ramulus* (insignificant in validation cohorts) with AF that is consistent with causal effect. A consistent association was observed for three of the four potentially causal taxa in one of the two previously published observational studies.<sup>46,47</sup>

Animal studies have also provided evidence on the pathophysiological pathway linking gut microbiota to AF. Faecal matter transplantation (FMT) from aged rats to young hosts led to increased levels of circulating lipopolysaccharide and up-regulated expression of NOD-like receptor protein (NLRP)-3 inflammasome promoting development of AF.<sup>50</sup> Conversely, selective inhibitors of the NLRP3 inflammasome and FMT from young rats to old hosts both reduced AF susceptibility.<sup>50</sup> In another study, cross-species FMT from AF patients to mice led to prolonged P wave duration, aggregated atrial electrical remodelling, and decreased circulating and faecal linolenic acid concentration compared to FMT from healthy donors.<sup>51</sup> Finally, an *in vitro* experiment has suggested that linoleic acid mediates a protective anti-inflammatory effect on mouse atrial myocytes against lipopolysaccharide/nigericin-induced injuries.<sup>51</sup> Increased lipopolysaccharide levels have consistently been linked with age, AF, and recurrence of AF after ablation in humans.<sup>50,52</sup>

Short-chain fatty acids (SCFAs) are gut microbial fermentation products of dietary fibres influencing cell signalling that can be absorbed to the circulation.<sup>53</sup> In a mouse model, the lack of dietary fibre-derived SCFAs was linked to AF susceptibility while supplementation of SCFAs attenuated the observed NLRP3 inflammasome activation.<sup>54</sup> Two Chinese human studies have also reported that AF is linked with lower number of SCFA

producing gut microbial taxa and reduced faecal SCFA levels.<sup>54,55</sup>

Gut microbial metabolism of cholines, phosphatidyl cholines, and L-carnitine produces trimethylamine (TMA) that is converted to trimethylamine-N-oxide (TMAO) in the liver.<sup>56</sup> Increased levels of TMAO are an independent risk factor for thrombus formation in AF.<sup>57</sup> Dietary sources of the TMA precursor include red meat, cheese, and egg yolk nutrients abundant in Western diet.<sup>58</sup> In a rat model, AF susceptibility was linked with reduced abundance of *Akkermansia muciniphila*, leading to increased levels of enzymes involved in TMA synthesis.<sup>59</sup> In two small cohort studies, AF was consistently linked to changes in gut microbial TMA production and plasma TMAO levels in humans.<sup>60,61</sup>

## Metabolic end-products and AF

In metabolomics, the metabolic responses of an organism to various stimuli (i.e. metabolites) are profiled most commonly using mass spectrometry.<sup>62</sup> This method can measure ionized molecules based on their mass-to-charge ratios and allows a wide range of metabolites to be recognized especially when used in tandem with liquid (or other) chromatography separation techniques. The circulating metabolomic profile is strongly associated with environmental factors (e.g. diet and exposure to xenobiotics), genetics, and the gut microbiome, providing valuable information on the host, host environment, and host microbiome.<sup>63</sup>

Several studies have studied the relation of circulating metabolites with incident AF (see [Supplementary material online, Table S2](#)). Tissue metabolomics, animal studies, and operative patients are out of scope of this review. The Atherosclerosis Risk in Communities (ARIC) was the first large study to link elevated levels of conjugated bile acids to incident AF in black individuals, followed by the Framingham Heart Study, which connected perturbations in glucose, fructose, and galactose metabolism to incident AF.<sup>64,65</sup> In a follow-up study with a more diverse sample of ARIC participants, the associations of the metabolites pseudouridine, uridine (from pyrimidine metabolism), and acisoga (a catabolic product of spermidine in the polyamine metabolism) with AF were also statistically significant. The results for acisoga and other spermidine metabolites, such as arginine, were later confirmed in the Malmö Diet and Cancer Study and the Prevención con Dieta Mediterránea (PREDIMED) trial.<sup>66,67</sup> Arginine may have antioxidative effects and could prevent cardiovascular dysfunction by increasing impaired nitric oxide synthesis as the reactive oxygen species (ROS) are neutralized.<sup>68</sup>

In addition to the polyamine metabolites, the results with carnitines have been most consistent, suggesting a protective role against the onset of AF.<sup>67,69-71</sup> Carnitines are an essential part of the cardiomyocyte energy metabolism, transporting fatty acids for use in mitochondrial energy production, while preventing ROS formation.<sup>72</sup> There is also evidence for their cardioprotective abilities, blood pressure benefit, and reduction in left ventricle dilation after an acute myocardial infarction.<sup>73-75</sup> However, a recent Mendelian randomization study found conflicting evidence, suggesting cardiovascular harm on a multitude of cardiovascular endpoints—including AF—with genetic variants predicting L-carnitine levels.<sup>76</sup> It is also worth

noting that large-scale intervention data are lacking. Fatty acids and other lipids have also been linked to AF in several studies,<sup>77-79</sup> but these results must be considered with caution due to potential confounding effects. In addition, several lysophosphatidylcholines (LysoPC) and cholesterol esters have been linked to AF in many recent studies.<sup>69,77,78,80,81</sup> LysoPC species are antiatherogenic, have anti-inflammatory responses, and reduce metabolic syndrome progression,<sup>82</sup> and the concentrations of these metabolites have been lower in AF patients than in controls.<sup>81,82</sup> There is also evidence on the connection between other inflammatory biomarkers and AF. In a PREDIMED follow-up study, the only significant predictor of AF was quinolinic acid, an inflammation-inducing metabolite from the tryptophan-kynurenine pathway.<sup>83</sup>

## Conclusions and future perspectives

The continuously decreasing sequencing costs enable the transition towards large-scale sequencing studies, polygenic risk assessment in and across diverse ethnicities in both sexes,<sup>17,84</sup> discovery of structural genetic variation, and integration of data from large-scale cell-based assays, and single-cell profiling of patient samples. Computational genetic fine-mapping utilizing these different data layers will in the future facilitate the discovery of disease-relevant conditions, such as cell types and cell states, and open a way for precision medicine. The key challenges hampering the genetic fine-mapping efforts include strong linkage disequilibrium among variants that can limit statistical power and resolution of the mapping, genetic signals at affected regions that commonly harbour many variants acting together that make the process of simultaneously searching for multiple causal variants computationally heavy, and the confounding bias hidden in GWAS summary statistics, such as socioeconomic status and geographic clustering, that can produce spurious signals.<sup>85</sup> Despite the challenges, more powerful computational approaches are emerging to help the prioritization of putative causal variants underlying complex multifactorial traits and diseases. However, much remains unknown regarding the AF-associated variant effects on the expression, processing, and function of the non-coding genome, including the non-coding RNAs, such as microRNAs, long non-coding RNAs, and circular RNAs.

Although our review highlights the benefits of single-cell-resolution data over bulk tissues, the picture remains incomplete. In existing single-cell studies on AF, the number of samples has been low, sex-differences have not been considered, cells were profiled from post-mortem samples, and the focus has been on 'healthy' tissue, potentially missing disease-prevalent cell types, cell states, and signalling that may further elucidate the disease mechanisms. Current studies suggest cardiomyocytes as the main mediator cell type of AF risk.<sup>44,45</sup> However, this is yet to be validated in larger datasets that address the previously mentioned limitations.<sup>86</sup>

Gut microbiota is a novel risk factor that has been linked with AF in observational studies, pre-clinical models, and Mendelian randomization studies. While the gut microbiota likely contributes to AF both directly and through overlapping risk factors and diseases, our understanding of the pathophysiology of the phenomenon is still dependent upon future observational and experimental research in

this rapidly developing field. In addition to environment and genetics, gut microbiota is also one of the key factors regulating the circulating human metabolome. Currently, there is budding evidence on the links between circulating metabolites, such as carnitine and LysoPC species, with prevalent AF, but the heterogeneous methods and populations of metabolomics studies have provided somewhat inconsistent results. Furthermore, metabolic changes in AF can arise from multiple reasons and do not yet permit any conclusions on their arrhythmogenic or preventive potential.<sup>87</sup>

In conclusion, many of the omics methods are still rapidly developing, but provide a great opportunity to discover novel risk markers and mechanisms of AF.

## Supplementary material

Supplementary material is available at *European Heart Journal Supplements* online.

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## Data availability

No new data were generated or analysed in support of this research.

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