Single cell transcriptomic meta-analysis uncovers sample-specific molecular heterogeneity in atrial fibrillation

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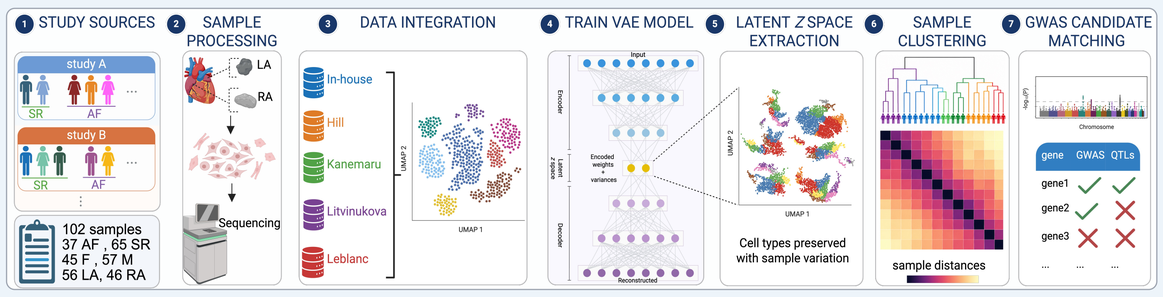
1. Background and Aim

Atrial fibrillation (AF) is a common arrhythmia and a major contributor to stroke and heart failure. Although AF is multifactorial and clinically heterogeneous, and current treatment strategies are effective in some patients but not all. Transcriptomic studies often summarize disease effects as an average AF-vs-SR contrast. This approach may overlook patient-specific molecular states that contribute to variable disease presentation and treatment response.

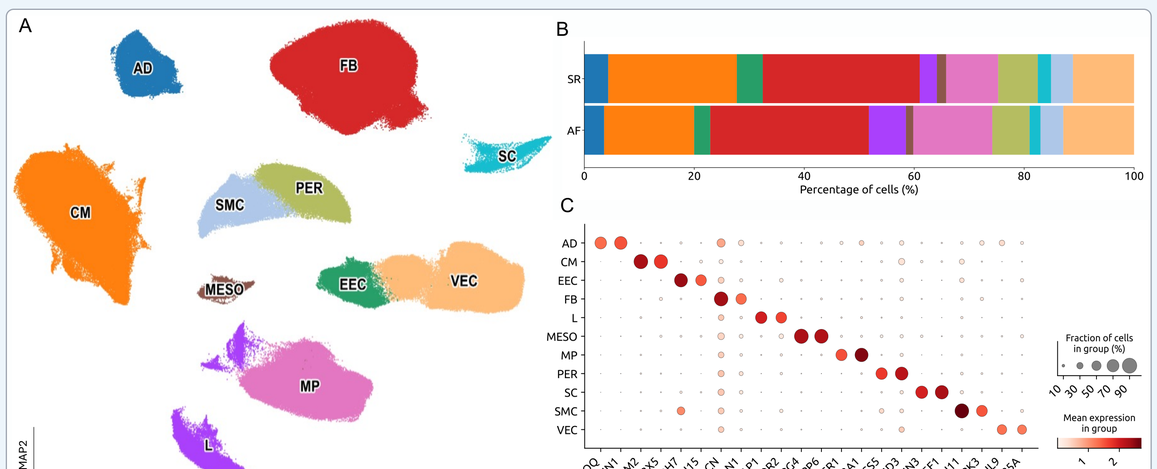
Here, we aimed to characterize AF-associated transcriptional heterogeneity across individuals and cardiac cell types using integrated human atrial snRNA-seq data.

2. Study Design



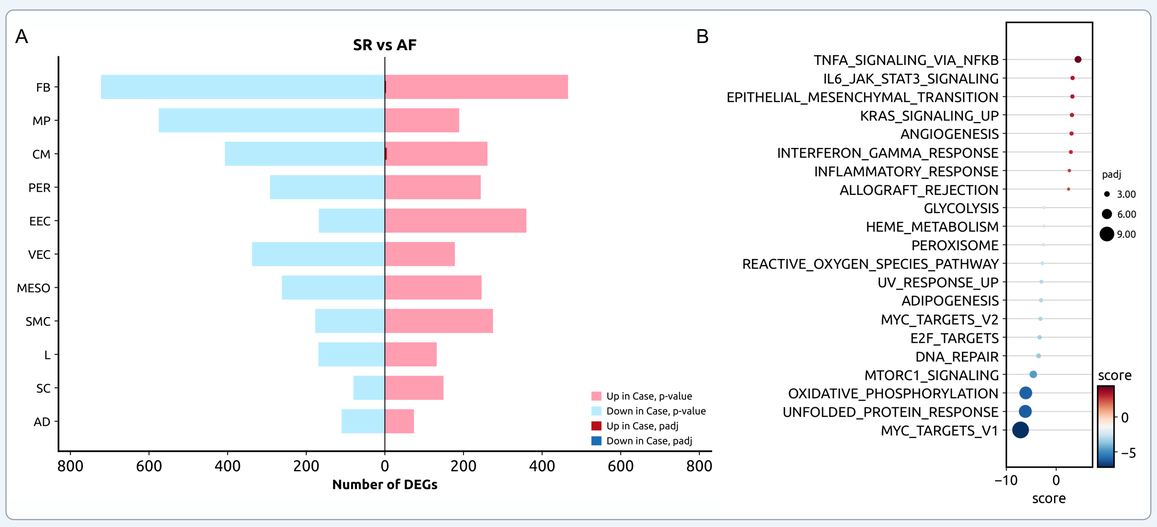
Schematic overview of the study design. Human atrial snRNA-seq datasets from in-house and four public studies (Hill et al.[1], Kanemaru et al.[2], Leblanc et al.[3], and Litvinukova et al.[4])were integrated across disease status, sex, and atrial region. After sample processing and cell-type annotation, cell-type-specific expression profiles were used to train a variational autoencoder (VAE) model and extract latent representations. Sample-level clustering of the latent space was then used to identify molecularly similar AF/SR samples and prioritize candidate genes with potential genome wide association studies (GWAS) or quantitative trait loci (QTL) support.

3. Integrated Cell Atlas



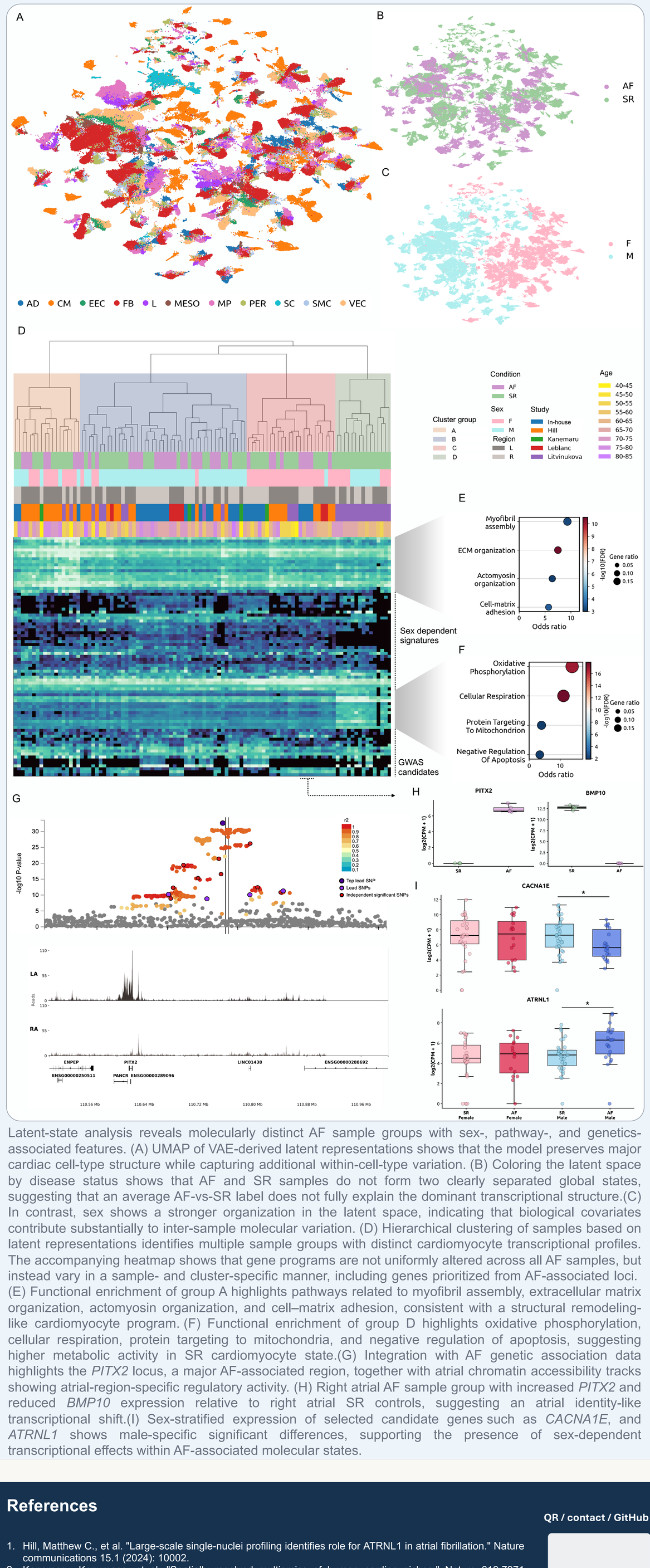
Integrated cell atlas after cross-study data harmonization
(A) UMAP embedding of integrated human atrial nuclei grouped by major cardiac cell types. (B) Comparison of cell-type proportions between AF and SR samples. (C) Canonical marker gene expression confirming cell-type annotation across atrial cell populations; dot size represents the fraction of expressing cells and color intensity represents mean expression.
AD, adipocytes; CM, cardiomyocytes; EEC, endocardial endothelial cells; FB, fibroblasts; L, lymphocytes; MESO, mesothelial cells; MP, myeloid phagocytes; PER, pericytes; SC, Schwann cells; SMC, smooth muscle cells; VEC, vascular endothelial cells.

4. Average AF-vs-SR Signal



Average AF-vs-SR transcriptional signal across cardiac cell types.
(A) Cell-type-resolved differential expression showed widespread nominal AF-associated changes, but relatively few genes passed FDR correction, indicating that average AF-vs-SR contrasts capture only a modest shared disease signal.
(B) Hallmark pathway enrichment in cardiomyocytes highlighted pathway-level trends, including general inflammatory and stress-related programs, based on ranked AF-vs-SR transcriptional changes.

5. VAE Latent Heterogeneity



Latent-state analysis reveals molecularly distinct AF sample groups with sex-, pathway-, and genetics-associated features. (A) UMAP of VAE-derived latent representations shows that the model preserves major cardiac cell-type structure while capturing additional within-cell-type variation. (B) Coloring the latent space by disease status shows that AF and SR samples do not form two clearly separated global states, suggesting that an average AF-vs-SR label does not fully explain the dominant transcriptional structure.(C) In contrast, sex shows a stronger organization in the latent space, indicating that biological covariates contribute substantially to inter-sample molecular variation. (D) Hierarchical clustering of samples based on latent representations identifies multiple sample groups with distinct cardiomyocyte transcriptional profiles. The accompanying heatmap shows that gene programs are not uniformly altered across all AF samples, but instead vary in a sample- and cluster-specific manner, including genes prioritized from AF-associated loci. (E) Functional enrichment of group A highlights pathways related to myofibril assembly, extracellular matrix organization, actomyosin organization, and cell–matrix adhesion, consistent with a structural remodeling-like cardiomyocyte program. (F) Functional enrichment of group D highlights oxidative phosphorylation, cellular respiration, protein targeting to mitochondria, and negative regulation of apoptosis, suggesting higher metabolic activity in SR cardiomyocyte state.(G) Integration with AF genetic association data highlights the *PITX2* locus, a major AF-associated region, together with atrial chromatin accessibility tracks showing atrial-region-specific regulatory activity. (H) Right atrial AF sample group with increased *PITX2* and reduced *BMP10* expression relative to right atrial SR controls, suggesting an atrial identity-like transcriptional shift.(I) Sex-stratified expression of selected candidate genes such as *CACNA1E*, and *ATRNL1* shows male-specific significant differences, supporting the presence of sex-dependent transcriptional effects within AF-associated molecular states.

Summary

Human atrial snRNA-seq meta-analysis enabled cell-type-resolved analysis of AF/SR transcriptional variation across 102 samples.

Average AF-vs-SR comparisons captured only a modest shared disease signal, while VAE latent states revealed strong sample-specific heterogeneity.

AF samples separated into distinct cardiomyocyte molecular programs associated with sex, structural remodeling, mitochondrial metabolism, and AF genetic loci.

References

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