Tissue-specific multiOMICs analysis of atrial fibrillation

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Abstract

RATIONALE: Genome-wide association studies (GWAS) for atrial fibrillation (AF) have uncovered numerous disease-associated variants. The molecular mechanisms of genotype-phenotype relationships, especially consequences of sequence variants for mRNA and protein expression remain largely elusive. Novel multiOMICs approaches are needed for deciphering the underlying molecular networks. Recently, an omnigenic model postulated the existence of core genes, that accumulate trans regulatory effects and are directly linked to disease phenotypes. However, the existence and identity of AF-relevant core genes remain unknown.

OBJECTIVE: The aim was to systematically assess regulatory genetic variants and their downstream consequences on transcriptome, proteome and the disease phenotype AF on a genome-wide scale.

METHODS AND RESULTS: We integrated genomics, transcriptomics, and proteomics of human atrial tissue. We identified widespread effects of genetic variants on transcript (cis eQTL) and protein (cis pQTL) abundance of nearby genes with an overrepresentation of GWAS variants for AF. To investigate more complex genetic mechanisms we established a novel approach to identify candidates for AF core genes, as postulated by the omnigenic model. Our approach combined a polygenic risk score for AF with pathway enrichment analysis to uncover biological processes related to the genetic susceptibility of AF. Within these biological processes, we identified two trans eQTLs and six trans pQTLs for AF GWAS hits involved in cardiac contractile function or metabolism. Furthermore, we elucidated the role of the transcription factor NKX2-5 as a link between the GWAS SNP rs9481842 and thirteen putative AF core genes with a consistent downregulation in AF on proteomics level.

CONCLUSIONS: We established an integrative multiOMICs approach for the identification of AF core gene candidates and applied this technique to uncover trans-acting genetic networks in AF. We provide a valuable resource to investigate atrial tissue-specific regulatory variants for transcript and protein levels in the context of cardiovascular disease.

Key Words: omnigenic model, pQTL, atrial fibrillation, proteomics, human atrial tissue

INTRODUCTION

Genome-wide association studies (GWAS) have discovered thousands of disease-associated single nucleotide polymorphisms (SNPs) and improved our understanding of the genetic and phenotypic relationships.[1] In this regard, GWAS have been applied to investigate atrial fibrillation (AF), which affects more than 30 million individuals worldwide.[2] Various genetic risk loci for AF have been identified[3, 4, 5] and have been integrated into genome-wide polygenic risk scores (PRS) for AF risk prediction.[6, 7] However, AF risk loci known so far explain less than half of the estimated disease heritability.[3] In addition, more than 95% of these GWAS variants are localized in noncoding regions[3] not directly affecting the protein sequence but possibly acting through gene-regulatory mechanisms which are mostly unknown.

A common approach to identify target genes of GWAS variants is to consider tissue-specific cis-acting expression quantitative trait loci (eQTL), where genetic variants affect the transcription of nearby genes. However, cis-eQTLs only explain a fraction of the identified AF risk loci. Therefore, trans-eQTLs, where the variant is distant to the target gene, and more complex genetic or epigenetic mechanisms need to be considered.[5, 6, 8, 9] To date, it remains difficult to quantify the contribution of cis and trans variants to the heritability of complex diseases such as AF.

Recently, the contribution of trans effects to the genetic architecture of complex traits was theoretically assessed by the omnigenic model.[10] Based on this model, it was estimated that trans genetic effects explain at least 70% of the disease heritability by indirect propagation through gene regulatory networks.[10] Within these networks, multiple trans effects can accumulate on just a few central genes, so-called core genes, which in turn are functionally related to a phenotype. Identifying those core genes by trans eQTLs remains challenging due to the small effect size of each individual locus[11, 12] and the associated large multiple testing burden. Since a PRS summarizes the genetic risk information, it can act as a proxy for the accumulation of trans effects in one score.[12] By correlating the score with transcript expression (eQTS), the propagation of trans effects to mRNA level[12] can be evaluated. However, not only transcript abundance, but also the abundance of translated proteins can determine phenotypic consequences. To date, little is known about genetic effects on protein levels (pQTLs), e.g. through post-transcriptional regulation,[10, 13, 14, 15, 16] especially in atrial tissues. It is both challenging and important to establish methods to identify AF core genes and to integrate data from multiple OMICs levels to improve the understanding of genotype-phenotype relationships.

Here, we present a multiOMICs analysis that uses genomics, transcriptomics, and proteomics of human atrial tissue to better understand how genetics are related to molecular mechanisms of AF. The first aim was to systematically integrate OMICs data and identify genome-wide cis-regulatory mechanisms on transcript as well as protein level. We reasoned that core genes are key for a better understanding of complex molecular pathomechanisms of AF. Therefore, the second aim was to identify candidate core genes for AF and the trans-acting regulatory networks that link them to AF GWAS loci. We developed a novel approach combining the correlation of gene expression with a PRS for AF[7, 12] and pathway enrichment analysis to identify AF-associated biological processes. Based on those processes, candidate core genes for targeted trans-QTL analyses with AF GWAS SNPs were selected. This approach allowed the identification of core genes, their molecular networks and downstream consequences in AF.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request. A detailed description of the methods and materials can be found in the online methods section.

Data and preprocessing

Patient cohort Patients were enrolled in the ongoing observational cohort study AFHRI-B (Atrial fibrillation in high risk individuals-biopsy). Participants were older than 18 years of age and were scheduled to undergo open

heart coronary bypass surgery. For the current analyses, N=118 patients with multiOMICs data were available. Cardiovascular risk factor information was collected by questionnaire and from medical records. Baseline blood samples were aliquoted and stored prior to surgery. Atrial appendage tissue remnants were collected when the extracorporeal circulation was started and shock frozen immediately. Follow-up for AF and other cardiovascular disease outcomes was by questionnaire and medical chart review. The observational cohort study was approved by Ethikkommission Ärztekammer Hamburg (PV3982). The study was performed in compliance with the Declaration of Helsinki. The study enrollment and follow-up procedures were in accordance with institutional guidelines. All participants provided written informed consent. Sex stratification of the results was not possible due to the inherently small number of women in the study (Suppl Table S1). Analyses were adjusted for sex where appropriate.

Genotypes and PRS for AF The genotype data was generated using the Affymetrix GeneChips Genome-Wide Human SNP Array 6.0, imputed based on the multi-population reference haplotypes from 1000 genomes Phase 3 genotypes[17, 18] and filtered for Hardy-Weinberg equilibrium P$>1 \times 10^{-4}$ resulting in 5 050 128 SNPs for 83 individuals. The polygenic risk score was calculated based on the LDpred omnigenic score published by Khera and colleagues[7] for our AFHRI cohort data and unrelated 1000 genomes[17, 18] CEU individuals to compute PRS percentiles across all 490 individuals (1000 genomes: 407, AFHRI cohort: 83). The percentiles for the 83 samples from the AFHRI cohort were used for further analysis.

mRNA and protein measurements The mRNA data was generated from human heart atrial appendage tissue samples using Affymetrix HuGene 2.0 ST Arrays and processed with the R Bioconductor package oligo.[19] The mean of multiple transcript clusters annotated to the same gene symbol was used to derive gene level expression values for 26 376 genes in 102 samples. RIN-scores were used as technical covariate in further analyses. Mass spectrometry analysis was performed on human heart atrial appendage tissue samples on a LC-ESI-MS/MS instrument (LTQ Orbitrap Velos). The Elucidator workflow was used to derive protein intensities by summing over all isotope groups with the same peptide annotation for all peptides annotated to one protein. Intensities for 1 419 proteins were quantified for 96 samples, median-normalized and log10-transformed. The original sample protein concentration was used as a technical covariate in further analyses. A fibroblast-score[20] was used to account for differences in cell type composition of tissue samples (see online methods).

Protein-per-mRNA ratios and residuals mRNA and protein measurements were already per-sample quantile-normalized and log-transformed. Both mRNA and protein measurements were additionally quantile-normalized per gene and the ratio was computed as the difference between protein and transcript values. Per-sample quantile-normalized, log-transformed mRNA and protein values were used to compute residuals. mRNA residuals were derived as the residuals from a linear model explaining mRNA by protein levels, i.e. mRNA $\sim \beta_0 + \beta_1 \cdot$protein $+ \varepsilon$. Protein residuals were derived as the residuals from a linear model explaining protein by mRNA levels, i.e. protein $\sim \beta_0 + \beta_1$ mRNA $+ \varepsilon$.

Cis analyses

QTL mapping Analyses were performed using R 3.4.1. (https://www.r-project.org). One to 30 PEER factors[21] were computed for transcriptomics, proteomics, and residuals using the R package PEER[21] to account for unknown factors in gene expression data. QTLs were calculated with a cis range of 1×10^6 bp and a linear, additive genotype model using the R package MatrixEQTL.[22] Expression quantitative trait loci (eQTL), protein quantitative trait loci (pQTL), expression residual quantitative trait loci (res eQTL), protein residual quantitative trait loci (res pQTL) and protein-per-mRNA ratio quantitative trait loci (ratioQTL) analyses were performed for per-sample-and-gene quantile normalized expression values[23, 24] using only PEER factors without additional covariates (12 PEER factors for eQTLs, 10 PEER factors for pQTLs, 8 PEER factors for res eQTLs and 12 PEER factors for res pQTLs). For ratioQTLs, 9 PEER factors and the fibroblast-score were used as covariates.

Definition of functional QTL categories Shared eQTL / pQTL were defined as SNP-gene pairs with a significant eQTL (FDR<0.05), pQTL (FDR<0.05) and no res eQTL (FDR>0.05) or res pQTL (FDR>0.05), i.e. genetic regulation is observable on transcriptomics and proteomics level and variation corresponding to the SNP influence in one OMIC level can be explained and therefore removed by the variation in the other OMIC level. Independent eQTLs were defined as SNP-gene pairs with a significant eQTL (FDR<0.05) and res eQTL (FDR<0.05) but no pQTL (FDR>0.05) and no res pQTL (FDR>0.05), i.e. regulation of SNP is only affecting transcript levels, not proteins. Also, the res eQTL disappears, if the SNP influences protein levels too much, for example a pQTL that barely missed the significance threshold. Independent pQTLs were defined as SNP-gene pairs with a significant pQTL (FDR<0.05) and res pQTL (FDR<0.05) but no eQTL (FDR>0.05) and no res eQTL (FDR>0.05), i.e. regulation of SNP is only affecting protein levels, not transcripts, i.e. by post-transcriptional regulation.

Enrichment of functional elements Functional genomic annotations (see online methods) of the top 5 QTL hits per gene were compared to a background distribution (100 background SNPs per QTL SNP) matched for MAF (difference $\leq$ 0.05) and distance to transcription start site (TSS) (difference $\leq$ 1 000bp) by computing a Fisher test on the contingency tables.

GWAS overlap and enrichments All hits for cardiovascular traits in the GWAS catalog[25] were annotated with proxies using SNiPA[26] (EUR population, R^2>0.8). Each original GWAS SNP was annotated with the proxy-gene pair for strongest QTL (P<1×10^{-5}). General enrichment of GWAS hits in QTLs was evaluated using a Fisher's exact test (cross tables SNP with significant QTL (P<1×10^{-5}) versus SNP (or R^2>0.8 proxy) annotated in the GWAS catalog).

Trans analyses

PRS correlations (eQTS, pQTS) Transcriptomics and proteomics ranking based on PRS correlations (eQTS, pQTS) were evaluated using summary statistics for β_1 from a linear regression model (equivalent to comparing the nested models with/without the PRS, sysBP=systolic blood pressure, CRP=C-reactive protein, NT-proBNP=N-terminal prohormone of brain natriuretic peptide):

$$\text{eQTS: mRNA} \sim \beta_0 + \beta_1 \cdot \text{PRS} + \beta_2 \cdot \text{age} + \beta_3 \cdot \text{sex} + \beta_4 \cdot \text{BMI} + \beta_5 \cdot \text{sysBP} + \beta_6 \cdot \text{CRP} + \beta_7 \cdot \text{NT-proNBP} + \\ + \beta_8 \cdot \text{RIN} + \varepsilon$$

$$\text{pQTS: protein} \sim \beta_0 + \beta_1 \cdot \text{PRS} + \beta_2 \cdot \text{age} + \beta_3 \cdot \text{sex} + \beta_4 \cdot \text{BMI} + \beta_5 \cdot \text{sysBP} + \beta_6 \cdot \text{CRP} + \beta_7 \cdot \text{NT-proNBP} + \\ + \beta_8 \cdot \text{protein conc.} + \varepsilon$$

Pathway enrichment analysis and trans QTL candidate selection Gene set enrichment analysis (GSEA)[27] was performed on eQTS/pQTS T-values using the Bioconductor R package fgsea[28] and MSigDB gene sets for GeneOntology biological processes (v6.1)[27, 29, 30] with a size between 15/5 and 500 genes. Leading edge genes were considered drivers of the enrichments of gene sets. A gene set was considered significantly enriched with a FDR<0.001 (Benjamini-Hochberg procedure) on transcript level and a FDR<0.2 on proteomics level. All leading edge genes that appeared ten or more times on transcript and two or more times on protein level were selected as trans QTL gene candidates for trans QTL computations.

Trans QTL calculations Trans QTLs were calculated using the R package MatrixEQTL.[22] An additive linear model was evaluated for 175 SNPs for AF in the GWAS catalog,[25] using additional covariates age, sex, BMI, systolic blood pressure, the fibroblast-score and RIN-score/protein concentration for transcripts/proteins.

TF (NKX2-5) target definition selection To investigate the link between a GWAS hit to target genes through a trans-eQTL-regulated transcription factor (TF), we considered only genes with transcriptomics and proteomics measurements and at least one functional TF BS (see online methods TF BS).
We first evaluated three types of associations:

1. **Association of GWAS SNP with target transcript (trans eQTL):**
   target transcript $\beta_0 + \beta_1 \cdot$SNP $+ \beta_2 \cdot$fibroblast-score $+ \beta_3 \cdot$RIN $+ \varepsilon$
2. **Independent effects of the SNP on target transcript, that are not mediated by the TF transcript:**
   target transcript $\beta_0 + \beta_1 \cdot$SNP $+ \beta_2 \cdot$TF transcript $+ \beta_3 \cdot$fibroblast-score $+ \beta_4 \cdot$RIN $+ \varepsilon$
3. **Association of target protein with TF transcript:**
   target protein $\beta_0 + \beta_1 \cdot$TF transcript $+ \beta_2 \cdot$fibroblast-score $+ \beta_3 \cdot$protein conc. $+ \varepsilon$

Next, we selected only genes with a significant, positive association of the SNP (relative to the allele associated with higher TF expression) with the target transcript (in 1.): β_1<0, P(β_1)<0.05) that vanished, when including the TF transcript in the model (in 2.): P(β_1)>0.2). This was used to ensure that the SNP was not influencing the target directly, but acting through the TF. For the remaining subset, we ranked all targets based on the association of the target protein with the TF transcript and performed FDR correction (Benjamini-Hochberg) on the P values from model (3.) P(β_1) to determine significantly associated genes. Based on this, all targets with FDR<0.05 were defined as functional NKX2-5 targets.

Differential proteome analysis for AF Summary statistics for β_1 (AF) in the following linear model (N=78) were used:

$$\text{protein} \sim \beta_0 + \beta_1 \cdot \text{AF} + \beta_2 \cdot \text{sex} + \beta_3 \cdot \text{age} + \beta_4 \cdot \text{BMI} + \beta_5 \cdot \text{diabetes} + \beta_6 \cdot \text{sysBP} + \beta_7 \cdot \text{hypertension medication} + \\ + \beta_8 \cdot \text{myocardial infarction} + \beta_9 \cdot \text{smoking status} + \beta_{10} \cdot \text{fibroblast-score} + \beta_{11} \cdot \text{protein conc.} + \varepsilon$$

RESULTS

Cis QTL analysis

We analyzed disease-independent effects of genetic variants on transcript (cis eQTLs) and protein levels (cis pQTL) of nearby genes. All cis QTLs were calculated using expression values for 16 306 genes and 1 337 proteins (Table 1) with additional PEER factors to adjust for relevant covariates such as technical confounders and cardiovascular risk factors (see methods and Suppl Figure S1,S2). We assessed the replication rate of these eQTLs in GTEx[24] atrial appendage tissue. Effect sizes for the best eQTL (P$<1 \times 10^{-5}$) per gene showed a correlation of 0.83 (P$=3.6 \times 10^{67}$) in GTEx, 66% replicated (GTEx: P$<1 \times 10^{-5}$) and 88% showed concordant allelic effects (see also see also Suppl "Cis QTL replication", Suppl Figure S3,S4,S5). Furthermore, correlations between transcript and protein abundances were comparable to previous studies[14] (Suppl Figure S6,S7, Suppl Table S2) indicating high quality of the proteomics measurements.

Cis regulatory patterns specific to atrial tissue

We first sought to functionally characterize the cis regulatory variants. Local genetic variation can lead to different modulations in mRNA and protein abundance which are commonly attributed to transcriptional and post-transcriptional regulation.[14, 15, 31] Protein abundances are suggested as more direct determinants for phenotypic consequences of expression QTLs[14] emphasizing the need to integrate mRNA and proteomic measurements to better understand functional genotype-phenotype relationships. Thus, for the following cis QTL analyses, we focused only on genes with both transcriptomics and proteomics measurements (1 243 genes, Table 1). As observed previously,[14, 15, 31] significant eQTLs and pQTLs (FDR<0.05) differ considerably, as only 8.2% of significant SNP-gene associations are shared between mRNA and proteins (Figure 1, Suppl Table S3).

Divergence of mRNA from protein abundance can arise through diverse molecular mechanisms which we additionally analyzed by calculating protein-per-mRNA ratios (ratioQTLs) (see methods). To identify shared as well as independent effects on mRNA and protein abundance, three simple regulatory categories were analyzed (see methods, Figure 2, Suppl Figure S8, Suppl Table S4):

(i) For 11 genes, 430 shared eQTLs / pQTLs were identified, where SNPs affect both mRNA expression and the respective protein abundance as depicted in Figure 2 A. The corresponding variants were primarily enriched in cis-regulatory elements such as active transcription start sites (TssA) (Suppl Figure S8 A, Suppl Figure S9).

(ii) For 37 genes, 1 593 independent eQTLs were identified, where only transcript levels are associated, but the respective protein abundance is independent of the genotype (Figure 2 B). Corresponding variants were enriched in elements regulating transcription, e.g. transcription factor binding site (TF BS) or enhancer regions, and within splicing regions (Suppl Figure S8 B, Suppl Figure S9 A). Possible mechanisms involved in unchanged protein levels remain largely elusive and range from adaptation of translational rate to protein degradation and long-noncoding RNAs.[32, 33]

(iii) For 21 genes, 1 083 independent pQTLs were identified, where the SNP affects only protein abundance (Figure 2 C). pQTL variants were enriched for exonic regions and although not significantly, in binding sites of RNA binding proteins (RBP) (Suppl Figure S8 C, Suppl Figure S9 A), where they may influence mRNA translation resulting in an independent pQTL association.[34]

Altogether, we confirmed that QTL variants corresponding to different categories tended to cluster in distinct genomic regions (Suppl Figure S9, Suppl "Enrichment of functional elements").[14, 23, 35] By integrating matched transcriptomics and proteomics data, we were able to differentiate functional regulatory mechanisms not observable by transcriptomics data only.

GWAS overlap and enrichment

In order to investigate genotype-phenotype relationships in the context of cardiovascular disease, we used our cis QTL data (FDR<0.05) to annotate GWAS variants for phenotypes either related to cardiovascular measurements or cardiovascular disease (Figure 3 A).

Of all the overlaps between GWAS hits and cis QTLs, AF-related loci were most abundant (17 eQTLs, four also with pQTL, see Suppl Table S5,S6). Furthermore, we found an independent pQTL overlapping with the GWAS hit for creatine kinase levels (Figure 3 B). This genetic effect was not detected on mRNA level illustrating the importance of proteomics data. In addition, we systematically assessed whether significant QTLs are enriched at GWAS loci in the hierarchical groups cardiovascular traits, arrhythmias and AF. We identified a strong significant overrepresentation (P<1e-5) of eQTLs at GWAS hits for all three groups, and a significant overrepresentation (P<0.05) of pQTLs in variants annotated with arrhythmias and AF (Figure 3 C). We showed widespread effects of cis-acting variants on gene expression and protein abundance in atrial tissue and a possible relation to AF.

Trans QTL analysis

Establishing an AF core gene model

We further extended cis-regulatory analyses by investigating trans-effects. Specifically, we addressed a key hypothesis of the omnigenic model,[10] which postulates the existence of core genes. Core genes are central genes with trans-associations to AF GWAS loci, whose expression levels directly affect the disease phenotype. Here we sought to identify candidate core genes for AF to understand the contribution of trans-genetic effects in the pathology of AF. To prioritize genes satisfying the properties predicted by the omnigenic model, we evaluated the accumulation of trans-effects, their relevance in gene regulatory networks, and the association with AF by the following strategy (Figure 4):

1. We evaluated the cumulated trans-effects of AF-associated variants on expression by ranking genes based on their correlation of mRNA and protein abundance with the PRS[7] for AF (eQTS, pQTS).[12] Here, the PRS served as a proxy for an aggregation of AF-related trans effects (Suppl Figure S10) across the whole genome.
2. To identify genes sharing molecular function and representing biological networks that propagate trans-effects to core genes, pathway enrichment analysis (GSEA)[27, 28] was performed on the eQTS and pQTS rankings. Genes driving the enrichment of multiple gene sets were selected as core gene candidates.
3. The link between the core gene candidates and AF was established based on a significant trans eQTL or pQTL for an AF GWAS hit and further supported by differential protein abundance analysis.

Identification of AF core genes

Based on this strategy, we recovered processes functionally related to AF: On transcript level, 48 GO biological processes were enriched (adjusted P value <0.001, 200 GO terms at adjusted P value <0.2, Suppl Table S7) mostly related to heart muscle or energy metabolism, including the processes *Generation of precursor metabolites and energy*, *Regulation of cardiac muscle contraction*, *Regulation of heart rate*, *Cardiac muscle tissue development*. On protein level, 9 GO biological processes were enriched (adjusted P value <0.2, Suppl Table S8), the majority connected to metabolism with leading processes being *Small molecule metabolic process* and *Long chain fatty acid transport*.

Our pathway enrichment approach yielded 30 transcripts and 100 proteins as core gene candidates that we used to calculate trans QTLs with 175 AF GWAS SNPs. On mRNA level, we identified two trans eQTLs encoding for a cardiac structural protein (rs11658168 - *TNNT2*) and a transcription factor (rs9481842 - *NKX2-5*) (Table 2). On protein level, we discovered six trans pQTLs which are all connected to metabolism (rs13376333 - NDUFA9/NDUFB3/ADK, rs6666258 - ACOT9, rs11658168 - HIBADH, rs7987944 - TALDO1) (Table 2). Noticeably, four out of six identified genes encode for mitochondrial enzymes (ACOT9, HIBADH) or enzyme subunits (NDUFA9, NDUFB3). Half of the putative core genes have already been mentioned by other studies in the context of arrhythmias and other cardiovascular diseases (Suppl Table S11) which independently replicates the disease link.

NKX2-5 transcription factor network

In order to get more detailed information about complex molecular mechanisms underlying AF, we further analyzed the TF network of NKX2-5 (see Figure 5 A) since the TF has already been described in the context of cardiac development,[36] AF,[37, 38, 39] and congenital heart diseases.[40]

To evaluate the downstream effects of the SNP rs9481842 via the TF NKX2-5 in AF we analyzed the influence of NKX2-5 transcript levels on target genes by estimating NKX2-5 TF activity. We annotated NKX2-5 binding sites in promoter regions based on published iPSC-derived cardiomyocyte ChIPseq and promoter-capture HiC data to identify almost 10 000 target genes. The number of binding sites per gene in open chromatin regions were counted for each gene and the TF activity (TFA) was computed as the sum of target transcript expression weighted by the number of binding sites. We observed a high correlation between the SNP rs9481842 and the NKX2-5 transcript (cor=-0.43, P=1.4×10^{-4}, two-sided Pearson's correlation, Figure 5 B) as well as for the direct molecular link between the NKX2-5 transcript and the TF activity (cor=0.36, P=2.5×10^{-4}, two-sided Pearson's correlation, Suppl Figure S11). In addition, there was a weak association between SNP rs9481842 and the TF activity (cor=-0.13, P=0.145, one-sided Pearson's correlation, Figure 5 C) most likely attributed to the indirect link through NKX2-5. Partial correlation analysis further supported NKX2-5 being the causal link between SNP and target expression (Suppl Figure S11, Suppl Table S12).

Next, to elucidate the role of NKX2-5 as a link between the disease variant and AF, we further analyzed its effect on specific targets, which we also prioritized as putative core genes. Overall, we identified 13 functional targets that are significantly influenced by the SNP rs9481842 as well as NKX2-5 transcript levels on both mRNA and protein level (see methods for details, Suppl Table S13). For these 13 targets, we observed a consistent downregulation on mRNA and protein level with respect to the rs9481842 risk allele (Figure 5 D). As the core gene model predicts a

direct effect of core gene expression on the phenotype,[10] we evaluated the protein abundance of the NKX2-5 target genes in patients with AF compared to patients in sinus rhythm to assess functional connection to the disease. For all targets, AF cases showed lower protein levels (Figure 5 D). When adjusting for common risk factors of AF, five out of 13 targets showed a nominal P value smaller 0.05 (Table 3). More importantly, the identified target set collectively displayed a strong association with AF on proteomics level (GSEA P$=7.17 \times 10^{-5}$). This serves as independent validation of the disease link, since these genes were identified based on molecular data in our cohort in combination with public AF annotations without using the actual cohort phenotypes. Furthermore, the majority of the identified proteins are in fact involved in contractile function (MYL4, MYL7, TNNC1, TCAP) or metabolism (PPIF, CKM, AK1, PGAM2, CYC1, ETFB, ALDOA), two mechanisms linked to processes directly influencing AF. At this point, our identified putative core gene point to potential novel targets for further experimental research to better understand molecular consequences of genetics underlying AF.

DISCUSSION

We present a comprehensive multiOMICs analysis that integrates genomics, transcriptomics, and proteomics in human atrial tissue to better understand how genetics are related to molecular mechanisms of AF.

We found widespread genetic effects related to the expression of nearby genes on transcript and protein level. Our integrated eQTL and pQTL analysis allowed the distinction between functional regulatory mechanisms with consequences for mRNA and protein levels. For example, we found many genetic variants exclusively affecting mRNA or protein abundances contributing to a modest overlap between both molecular levels using stringent statistical criteria. Compared to other studies, a similar extent of co-regulation between mRNA and protein levels was previously documented by comparing cis pQTLs in human plasma to cis eQTLs in GTEx tissue[15] (see Suppl "Overlap of eQTLs and pQTLs", Suppl Table S3). We assume that the use of less stringent significance cutoffs or multiple testing correction as well as more sensitive measurement techniques might achieve a higher overlap as observed by Battle and colleagues for cell-type specific transcriptomics and proteomics in the same lymphoblastoid cell lines.[14] We and others[14] found that proteome specific pQTLs are enriched in the coding sequence, where post-transcriptional regulatory elements might be affected by sequence variants, which may at least partially explain the divergence between eQTLs and pQTLs. In line with prior studies,[14, 15, 31] we observed large differences in transcript and protein expression as well as their regulation, emphasizing the necessity and benefit of taking multiple molecular entities into account to investigate genotype-phenotype relationships.

To extend the cis QTL analysis, we assessed trans-associations by applying a candidate selection strategy based on the correlation of gene expression with a PRS, a concept termed eQTS.[12] PRS accumulate small genetic effects at many individual genome-wide loci. In the theoretical omnigenic model[10] it has been suggested that these loci are linked to the phenotype by weak trans effects on gene expression, which accumulate in so called core genes. It has been shown that this accumulation of trans effects would lead to strong eQTS associations for core genes.[12] Here we used eQTS and pQTS in combination with gene set enrichment analysis to identify core gene pathways and putative core genes for AF. As core genes are postulated to have trans associations with AF GWAS SNPs we subsequently performed a targeted trans QTL analysis. This strategy allowed the investigation of tissue-specific trans-acting genetic mechanisms underlying AF using a relatively small clinical dataset by reducing the multiple testing burden. The PRS-based gene set enrichment approach revealed cardiac-specific biological pathways associated with the genetic susceptibility for AF which are similar to results identified by Wang and colleagues.[41] We identified different pathways on transcriptome and proteome level which is probably not only caused by biological but also technological reasons like protein coverage. On transcriptome level, the majority of the identified pathways were involved in contractile function and metabolism. On proteome level, the proteins were all annotated to participate in metabolic regulation. These mechanisms have been reported by clinical and experimental studies to play a major role in the pathology of AF.[42, 43, 44] As expected, also the putative core genes identified by trans eQTLs and trans pQTLs were involved in those mechanisms. In addition, all identified transcripts and some of the proteins have been described in the context of arrhythmias or cardiovascular disease (Suppl Table S11). As observed for the cis QTL analysis, the trans analysis revealed similar differences between transcriptomics and proteomics level. Interestingly, none of the trans pQTLs had a significant association on expression level (see Suppl "Trans eQTL / pQTL overlap", Suppl Table S14). Differences between trans eQTLs

and pQTLs for the same gene have previously not been discussed in detail in the literature. Sun and colleagues[15] analyzed overlapping cis QTLs but not trans QTLs. Yao and colleagues analyzed overlaps of cis and trans eQTLs and pQTLs in plasma,[16] however no overlaps were found in trans. Suhre and colleagues validated plasma pQTL findings in other proteomics datasets, but did not evaluate corresponding trans eQTLs.[13] Possible reasons besides the small sample size might be the effect of other genetic variants, post-transcriptional regulation or environmental factors.

To investigate more complex molecular mechanisms underlying AF, we further analyzed the TF network of NKX2-5, since the TF has been described in the context of heart development and arrhythmias. For instance, Benaglio and colleagues discovered that changes in NKX2-5 binding can contribute to electrocardiographic phenotypes.[39] In addition, a NKX2-5 loss-of-function mutation has been reported to be associated with an increased susceptibility to familial AF[37] emphasizing its relevance in the pathology of AF. Although we did not detect NKX2-5 on protein level, we were able to investigate downstream effects by inferring NKX2-5 TF activity based on target transcript expression and functional binding sites. The high correlation between the estimated NKX2-5 TF activity and its mRNA levels shows that NKX2-5 modulates target gene expression. Thus, we also expect altered NKX2-5 protein abundance. These TF activity analyses could only be achieved because of tissue- and cell-type specific annotations, available for NKX2-5. Our analysis suggests that NKX2-5 acts as a transcriptional activator for the majority of genes. The role as activator is consistent with other studies.[36, 39] However, the TF can also function as a transcriptional repressor of genes like ISL1.[36, 45] We were able to detect strong effects of the *NKX2-5* transcript on various target transcript and protein levels. Most of the identified target genes are involved in contractile function or metabolism, two mechanisms highly linked to processes involved in AF. Our unique trans-QTL approach revealed direct disease-relevant associations between candidate core genes, NKX2-5 target genes and AF. This finding was confirmed by differential protein abundance between AF patients and controls, which serves as independent validation of the disease relevance of NKX2-5 target genes, since these genes were identified based on molecular data in our cohort in combination with public AF annotations without using the actual cohort phenotypes.

Overall, we successfully integrated multiOMICs data and established a unique approach to investigate not only cis- but also trans-regulatory effects. This provided a platform to generate hypotheses on functional interactions underlying the genetic associations that can be further experimentally investigated.

Limitations

We acknowledge some limitations that are attributed to common biological and technical factors. First, the use of human heart tissue came with several challenges including restricted sample sizes and heterogeneity of cellular composition. Importantly, changes in cell-type composition and structural remodeling have been described for the pathology of AF.[46] To take differences in the cellular composition into account, we used a fibroblast-score based on a fibroblast-specific gene signature to adjust expression levels in eQTL/pQTL analyses.[20] Second, expression data were generated using microarrays, however, to date, more information can be generated by RNA-seq. Third, human cardiac muscle tissue is dominated by mitochondrial and sarcomere proteins[47] which affects the detection of less abundant proteins such as TFs (e.g. NKX2-5). Therefore, missing TF coverage was not due to data quality but biological and technological restrictions. In addition, only limited functional genomic annotations specific for atrial tissue are currently available including TF-, miRNA- and RBP binding sites. Therefore, we integrated multiple sources to render functional annotations as reliable and accurate as possible. Replication in an independent dataset was not feasible, since this was the first study investigating pQTLs in atrial tissue.

Conclusion

In this study we suggest a novel, integrative analysis of genomics, transcriptomics and proteomics data of human atrial tissue to identify genome-wide genetic effects on molecular mechanisms in the context of AF. Our multiOMICs approach permits the identification of shared and independent effects of cis-acting variants on transcript expression and protein abundance. Furthermore, a unique approach has been established to successfully investigate complex genetic networks in AF even with a limited sample size. By providing these unique tissue-specific OMICs data as a

publicly accessible database in an interactive browser, we hope to extend the availability of valuable resources for hypothesis generation, experimental design and target prioritization.

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Disclosures

None (all authors).

Author contributions

T.Z., R.S. and M.H. conceived the research, supervised the study and obtained funding. J.K., U.V., E.H., T.Z. performed experiments. R.S., C.S.B. and L.C. recruited the patient cohort and collected data. I.A. and M.H. developed computational methods and analyzed the data. B.G. and C.M. contributed to statistical results interpretation. M.O.S. analyzed genotype data. C.M. contributed to the analyses of transcriptomic data. I.A., J.K., T.Z. and M.H. wrote the manuscript with input from all authors. All authors made critical revisions of the manuscript and approved the final version of the manuscript.

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Tables

Table 1: Summary of tested data and discovered QTLs.
Results for a FDR<0.05 (according to Benjamini-Hochberg procedure) and P value $<1 \times 10^{-5}$.
FDR, false discovery rate; eQTL, expression quantitative trait loci; pQTL, protein quantitative trait loci; ratioQTL, ratio quantitative trait loci;

Results for all available transcriptomics and proteomics measurements:							
	Tested:			**FDR<0.05:**		**P<1$\times 10^{-5}$:**	
	SNPs	**Pairs**	**Genes**	**Pairs**	**Genes**	**Pairs**	**Genes**
eQTL	4 861 118	56 139 851	16 306	57 403	1 058	40 267	552
pQTL	2 323 504	4 508 654	1 337	4 081	91	2 543	45
Results only for genes with both transcriptomics and proteomics measurements:							
eQTL	2 249 758	4 198 168	1 243	4 603	124	3 218	64
pQTL	2 249 758	4 198 168	1 243	3 906	87	2 406	42
ratioQTL	2 249 758	4 198 168	1 243	563	16	575	18

Table 2: Trans QTL results.
Significant trans eQTLs and pQTLs for a FDR<0.2 (Benjamini-Hochberg procedure). Trans analyses were performed on 30 transcripts and 100 proteins for 175 variants associated with atrial fibrillation from the GWAS catalog.
QTL, quantitative trait loci; FDR, false discovery rate; Mutation known to affect cardiovascular phenotypes; **Mutation known to affect arrhythmias; $^{+}$Differential expression functional impairment for cardiovascular phenotypes; $^{++}$Differential expression or functional impairment for arrhythmias; For details to disease links in literature see Suppl Table S11;

Variant			**Gene**		**trans QTL**			
SNP	**Chr**	**Position**	**Symbol**	**Chr**	**QTL**	β	**P value**	**FDR**
rs11658168	chr17	7406134	*TNNT2**	chr1	transcript	-0.530	3.42×10^{-5}	0.115
rs9481842	chr6	118974798	*NKX2-5***	chr5	transcript	-0.608	4.37×10^{-5}	0.115
rs13376333	chr1	154814353	NDUFA9^{++}	chr12	protein	-0.767	1.06×10^{-5}	0.0567
rs6666258	chr1	154814268	ACOT9	chrX	protein	-0.819	1.90×10^{-5}	0.0567
rs13376333	chr1	154814353	NDUFB3*	chr2	protein	-0.905	2.01×10^{-5}	0.0567
rs11658168	chr17	7406134	HIBADH	chr7	protein	-0.513	3.17×10^{-5}	0.0567
rs13376333	chr1	154814353	ADK	chr10	protein	-0.877	4.97×10^{-5}	0.0791
rs7987944	chr13	23371474	TALDO1	chr11	protein	0.527	1.53×10^{-4}	0.191

Table 3: Putative core genes and functional targets with disease association.
Proteomics differential abundance results for prevalent AF, corrected for AF-related covariates sex, age, BMI, diabetes, systolic blood pressure, hypertension medication, myocardial infarction, and smoking status (see methods differential protein analysis).
AF, atrial fibrillation; QTL, quantitative trait loci; FDR, false discovery rate; Mutation known to affect cardiovascular phenotypes; **Mutation known to affect arrhythmias; $^{+}$Differential expression or functional impairment for cardiovascular phenotypes; $^{++}$Differential expression or functional impairment for arrhythmias.

		Protein AF association		
Gene	**Type**	β	**P value**	**FDR**
*TNNT2**	trans eQTL	-0.0609	0.113	1.00
*NKX2-5***	trans eQTL			
NDUFA9^{++}	trans pQTL	-0.0533	0.235	1.00
ACOT9	trans pQTL	0.00479	0.918	1.00
NDUFB3*	trans pQTL	-0.0631	0.182	1.00
HIBADH	trans pQTL	-0.0454	0.218	1.00
ADK	trans pQTL	-0.0211	0.689	1.00
TALDO1	trans pQTL	0.0193	0.629	1.00
PPIF	NKX2-5 target	-0.0342	0.261	1.00
MYL4**	NKX2-5 target	-0.0270	0.509	1.00
CKM^{++}	NKX2-5 target	-0.0875	0.00705	0.134
MYL7	NKX2-5 target	-0.0421	0.304	1.00
PGAM2^{++}	NKX2-5 target	-0.1750	0.000452	0.00903
TNNC1*	NKX2-5 target	-0.0557	0.0929	1.00
CYC1	NKX2-5 target	-0.0946	0.0360	0.613
ETFB^{++}	NKX2-5 target	-0.0553	0.105	1.00
PRDX5	NKX2-5 target	-0.0524	0.0789	1.00
AK1	NKX2-5 target	-0.0669	0.0341	0.613
ALDOA^{++}	NKX2-5 target	-0.0646	0.0341	0.613
TCAP*	NKX2-5 target	-0.0178	0.779	1.00
TOM1L2	NKX2-5 target	-0.0771	0.0849	1.00

Figures with Figure Legends

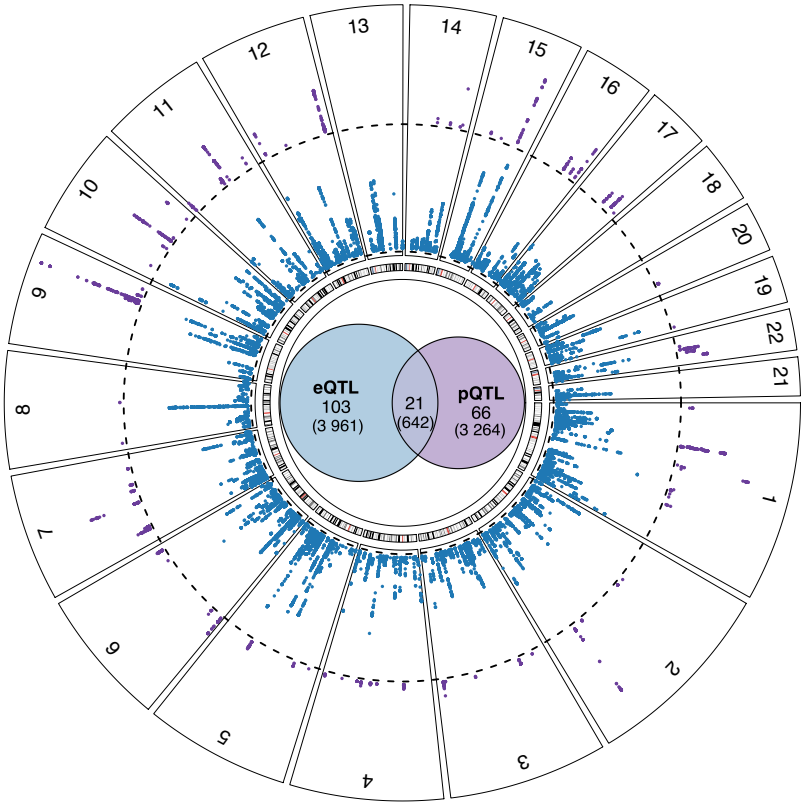

Figure 1: Significant cis eQTLs, cis pQTLs and their overlap.
Depicted are the significant cis eQTLs (blue) and pQTLs (purple) at a FDR cutoff of 0.05 (dotted line). Considering only genes with both transcriptomics and proteomics measurements, we visualized the overlap of significant eQTLs and pQTLs in the circle center. In total, 124 and 87 genes had at least one significant eQTL or pQTL, respectively. Only 21 of those genes had at least one overlapping eQTL and pQTL. The numbers in brackets represent the number of significant SNP-gene pairs. eQTL, expression quantitative trait loci; pQTL, protein quantitative trait loci; FDR, false discovery rate;

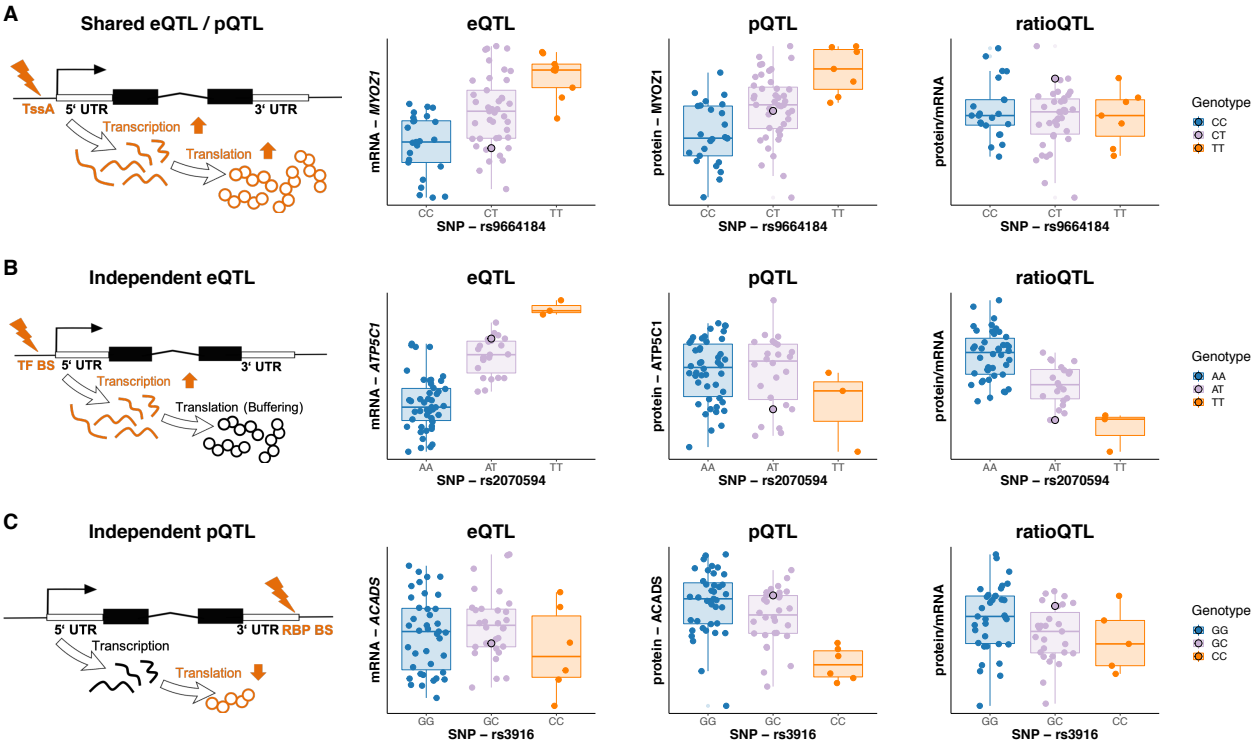

Figure 2: Different genetic regulatory patterns derived by multiOMICs QTL integration.
A: Shared eQTLs / pQTLs represent QTLs, where the effect of transcriptional regulation translates into mRNA and protein abundances exemplified by the significant SNP - gene pair rs9664184 - MYOZ1. No corresponding ratio QTL can be observed as the genetic variation is shared across both OMICs levels.
B: Independent eQTLs depict variants with regulation on mRNA but not on protein level displayed by the significant SNP - transcript pair rs2070594 - *ATP5C1.*
C: Independent pQTLs represent variants that show regulation only on protein level as shown for the SNP-protein pair rs3916 - ACADS. Genetic influence is not observable on transcript level.
eQTL, expression quantitative trait loci; pQTL, protein quantitative trait loci; ratioQTL, ratio quantitative trait loci; TssA, active transcription start site; UTR, untranslated region; TFBS, transcription factor binding site; RBP, RNA binding protein;

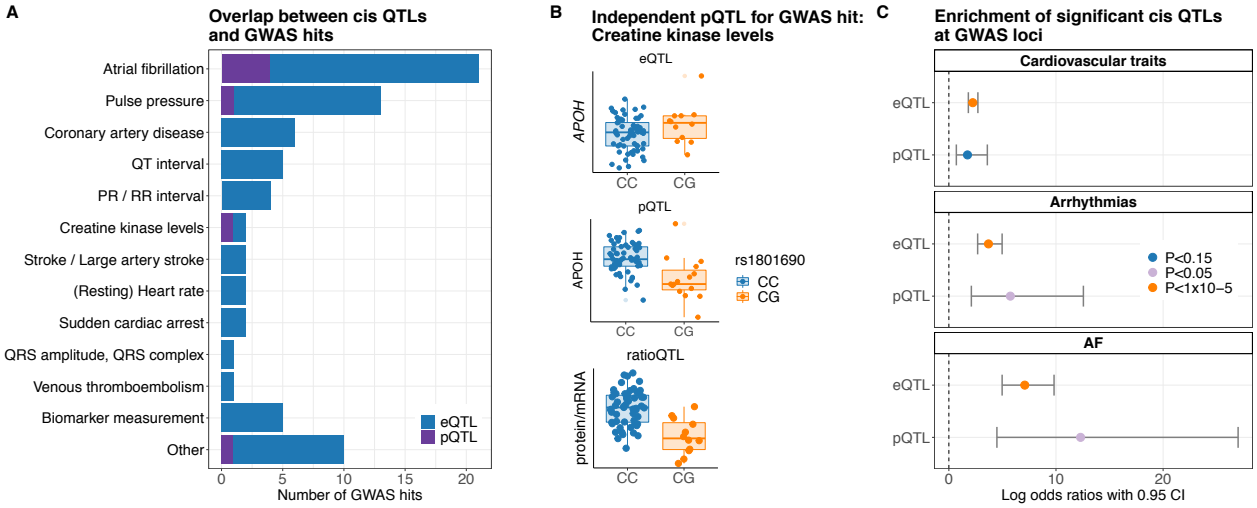

Figure 3: Overlap of cis QTL associations with GWAS hits annotated in the GWAS catalogue.
A: Overview of significant cis eQTLs and pQTLs (FDR<0.05) overlapping with GWAS hits for different disease traits.
B: Independent pQTL for GWAS hit creatine kinase levels. Shown are the significant cis eQTL, pQTL and ratio QTL for the SNP rs1801690 and the gene APOH (FDR<0.05).
C: For three different trait categories (cardiovascular traits, arrhythmias and atrial fibrillation) the enrichment of GWAS hits in significant cis QTLs (FDR<0.05) was evaluated. Enrichments were calculated using Fisher's test.
QTL, quantitative trait loci; eQTL, expression quantitative trait loci; pQTL, protein quantitative trait loci; ratioQTL, ratio quantitative trait loci; CI, confidence interval;

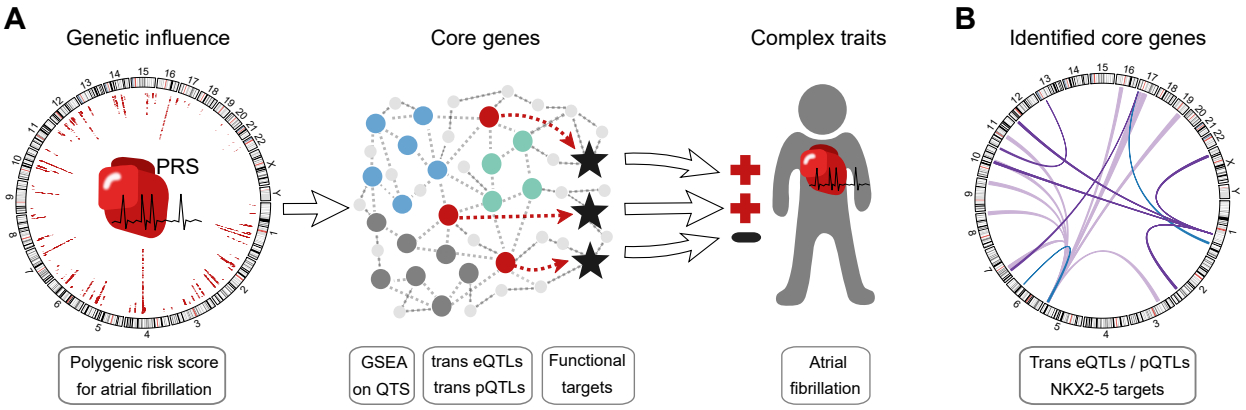

Figure 4: Graphical illustration of the strategy for trans QTL analysis to identify AF-relevant genes.
A: Overview: Based on patient-specific PRS values for AF correlated with transcript and protein expression, we performed GSEA to preselect genes for trans eQTL and pQTL analyses from the leading edge of enriched pathways. Core genes were identified as significant trans eQTLs or trans pQTLs. We further assessed their functional targets to investigate the genotype-phenotype relationship in the context of AF.
B: Identified core genes as trans eQTLs (blue), trans pQTLs (purple) (FDR<0.2) and functional NKX2-5 targets (light purple).
FDR, false discovery rate; AF, atrial fibrillation; GSEA, gene set enrichment analysis; PRS, genome-wide polygenic risk score; QTL, quantitative trait loci for mRNA (eQTL) or protein abundance (pQTL); blue, green or gray dots=core gene candidates; red dots=core genes with trans QTL; stars=functional targets of core genes;

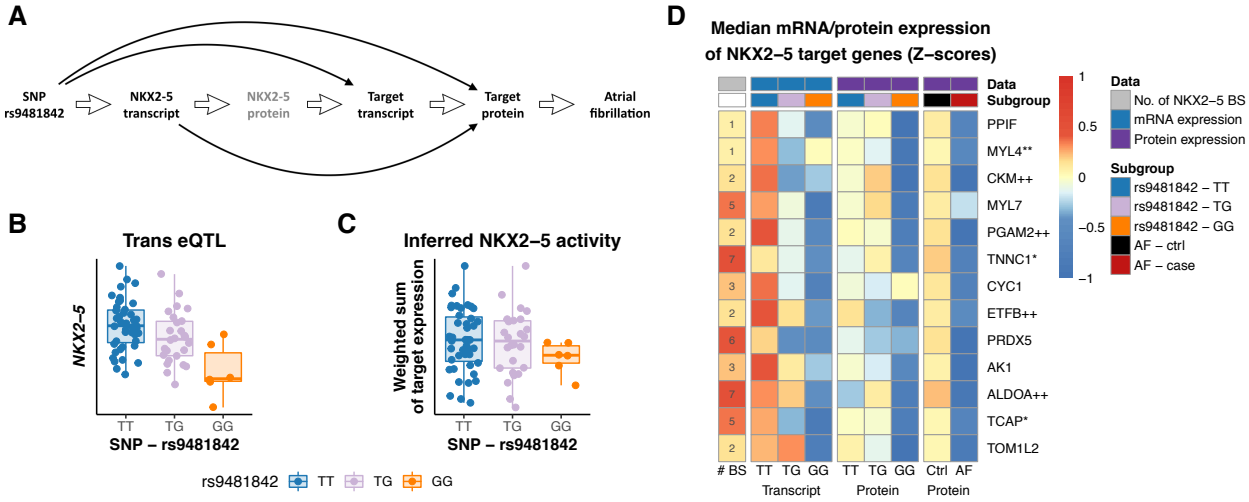

Figure 5: NKX2-5 activity controlled by AF GWAS variant rs9481842.
A: Graphial illustration of NKX2-5 TF target gene analysis in AF.
B: Strong trans eQTL of the SNP rs9481842 with the *NKX2-5* transcript.
C: NKX2-5 activity estimated based on target mRNA expression is still influenced by the rs9481842 genotype.
D: Depicted are functional NKX2-5 targets with the number of TF binding sites (column 1), trans eQTL strength (columns 2-4), trans pQTL strength (columns 5-7) and protein level in AF (columns 8-9). The color scale represents median transcript or protein Z-scores per group.
AF, atrial fibrillation; QTL, quantitative trait loci; BS, binding site;Mutation known to affect cardiovascular phenotypes; **Mutation known to affect arrhythmias; $^{+}$Differential expression or functional impairment for cardiovascular phenotypes; $^{++}$Differential expression or functional impairment for arrhythmias.