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Genome-wide association study identifies 18 novel loci associated with left atrial volume and function

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Aims

Left atrial (LA) volume and function impose significant impact on cardiovascular pathogenesis if compromised. We aimed at investigating the genetic architecture of LA volume and function using cardiac magnetic resonance imaging data.

Methods and results

We used the UK Biobank, which is a large prospective population study with available phenotypic and genetic data. On a subset of 35 658 European individuals, we performed genome-wide association studies on five volumetric and functional LA variables, generated using a machine learning algorithm. In total, we identified 18 novel genetic loci, mapped to genes with known roles in cardiomyopathy (e.g. *MYO18B*, *TTN*, *DSP*, *ANKRD1*) and arrhythmia (e.g. *TTN*, *CASQ2*, *MYO18B*, *C9orf3*). We observed high genetic correlation between LA volume and function and stroke, which was most pronounced for LA passive emptying fraction ($r_g = 0.40$, $P = 4 \times 10^{-6}$). To investigate whether the genetic risk of atrial fibrillation (AF) is associated with LA traits that precede overt AF, we produced a polygenic risk score for AF. We found that polygenic risk for AF is associated with increased LA volume and decreased LA function in participants without AF [LA_{max} 0.25 (mL/m²)/standard deviation (SD), 95% confidence interval (CI) (0.15; 0.36), $P = 5.13 \times 10^{-6}$; LA_{min} 0.21 (mL/m²)/SD, 95% CI (0.15; 0.28), $P = 1.86 \times 10^{-10}$; LA active emptying fraction -0.35%/SD, 95% CI (-0.43; -0.26), $P = 3.14 \times 10^{-14}$].

Conclusion

We report on 18 genetic loci associated with LA volume and function and show evidence for several plausible candidate genes important for LA structure.

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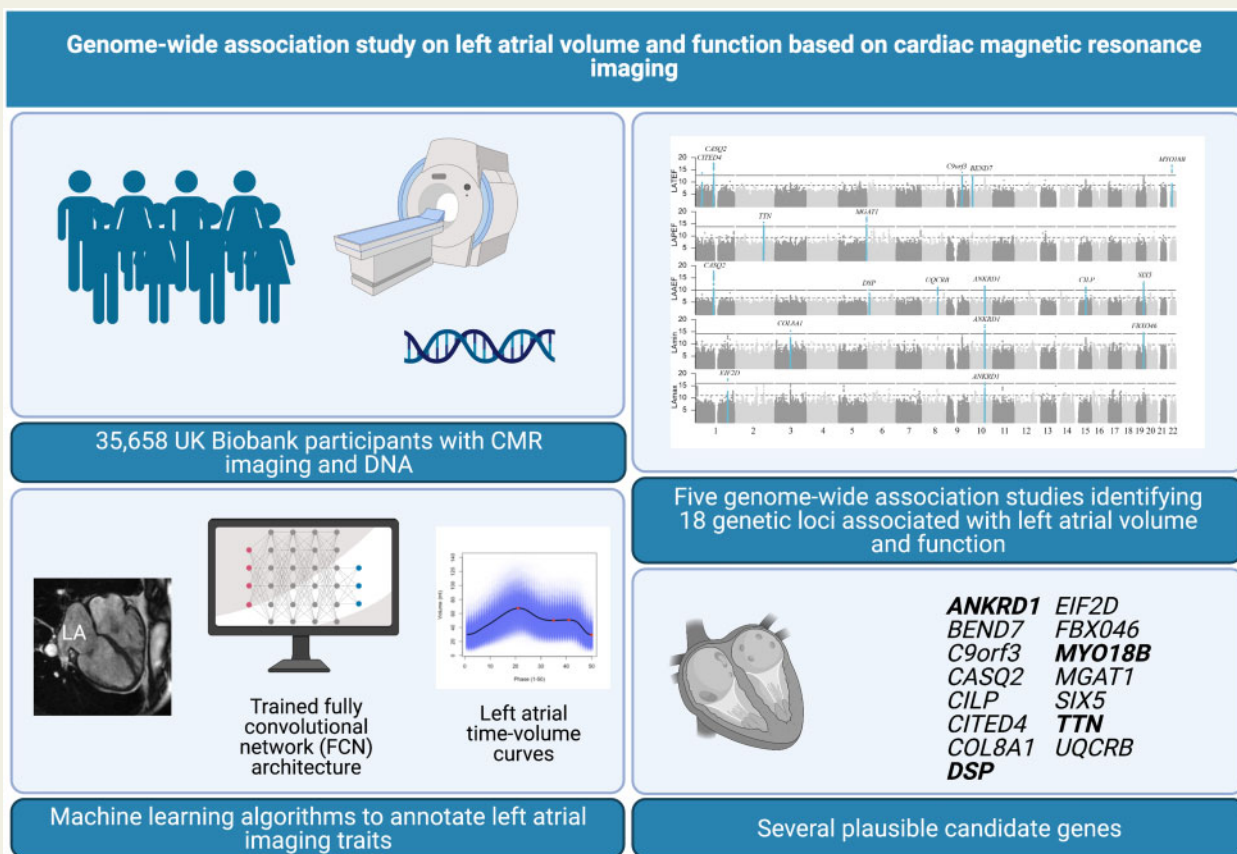
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Graphical Abstract



Genome-wide association study on left atrial volume and function based on cardiac magnetic resonance imaging. Imaging data from 35 658 individuals were exposed to machine learning algorithms to annotate five left atrial traits. A total of 18 novel genetic loci were associated with left atrial volume and function. Of these, 15 constituted unique loci. Genes marked with bold have previously been associated with cardiomyopathy.

Keywords

Cardiac magnetic resonance • UK Biobank • GWAS • Left atrium

Translational perspective

Atrial cardiomyopathy has been suggested to play an important role in the development of atrial fibrillation (AF). Using machine-learning algorithms, we segmented and annotated the left atrium (LA) in cardiac magnetic resonance data from 35 658 individuals. We identified 18 novel genetic loci associated with LA volume and function, many near well-established cardiomyopathy and arrhythmia genes. We observed high genetic correlation between LA volume and function and stroke. We found that the genetic risk of AF influences LA structure prior to diagnosis of AF, suggesting an intrinsic relationship.

Introduction

The left atrium (LA) serves primarily as a reservoir chamber of blood entering the heart from the pulmonary veins and is important for enhancing ventricular filling. Changes in left atrial (LA) volume and function have been associated with impaired cardiac performance,¹ arrhythmogenesis,² risk of stroke,³ and mortality.⁴ Left atrial

assessment is part of the standard echocardiographic examination⁵ and changes in the LA are traditionally considered secondary to other cardiovascular diseases. However, the pathology behind changes in LA volume and function and the association with atrial fibrillation (AF), heart failure (HF), and risk of stroke is poorly characterized.^{6,7,8} An increasing interest in LA pathology and atrial tissue prompted the need for defining atrial cardiomyopathy.⁹ In a recent

expert consensus document, written by internationally recognized experts, atrial cardiomyopathy was defined as structural, architectural, contractile, or electrophysiological changes affecting the atria with the potential to produce clinically relevant manifestations.⁶

Cardiac magnetic resonance (CMR) imaging is at present the gold standard for assessing cardiac volumes, with a superior accuracy and precision compared with other imaging modalities such as echocardiography.¹⁰ The clinical use of CMR is currently expanding due to lowered costs and increased accessibility, but the widespread use of the CMR modality is still limited.

Genetics play a major role in ventricular cardiomyopathies and could also play a role in several of the pathophysiological mechanisms behind atrial cardiomyopathy,⁶ but large-scale genetic studies of LA volume and function are lacking. Prior genome-wide association studies (GWAS) on cardiac chamber volumes and function have been limited to echocardiographic measurements¹¹ or CMR imaging-derived left ventricular (LV) phenotypes.¹²

UK Biobank (UKBB) provides extensive phenotypic and genotypic data on more than ~500 000 individuals from the British general population.¹³ At the time of this study, a subset of UKBB participants had CMR imaging data available, which enabled the coupling of genetic data with these state-of-the-art imaging variables. Using a validated machine learning algorithm to annotate the LA, we performed GWAS on five LA volume and function traits.

Methods

UK Biobank

UK Biobank is a large, population-based, prospective cohort study with ~500 000 participants aged 40–69 years at the time of recruitment (2006–10). The biobank holds a variety of health-related information on participants, including health records, imaging data, lifestyle indicators, cognitive function, biomarkers from blood and urine, imputed genome-wide genotypes, information on population structure, relatedness, and genotype-level quality. A detailed description of data collection and study protocol has been provided previously by Bycroft *et al.*¹³

All participants gave written informed consent and the study complies with the Declaration of Helsinki.

Derivation of cardiac magnetic resonance measurements

The UKBB CMR protocol has previously been described in detail.¹⁴ In brief, CMR scans were performed using a 1.5 Tesla scanner (MAGNETOM Aera, Syngo Platform VD13A, Siemens Healthcare, Erlangen, Germany) using an 18-channel body coil. Long-axis cines and a complete short axis stack of balanced steady-state free precession cines were acquired at one slice per breath hold.

Cardiac magnetic resonance DICOM images on >43 000 individuals were converted into NiftI format. In an automated image analysis, the NiftI images were quality controlled and followed by annotation of cardiac imaging traits from the segmentations, using a trained fully convolutional network architecture. The method is described in detail by Bai *et al.*,¹⁵ see URLs for source code and model availability. The reported mean Dice metric for LA segmentation of the model was 0.93 for LA (2-ch) and 0.95 for LA (4-ch).¹⁵ The following traits were derived as described below: indexed LA maximum (LA_{max}) and LA minimum (LA_{min}) volumes, LA active emptying fraction (LAAEF), LA passive emptying fraction (LAPEF), and LA total emptying fraction (LATEF).

Annotations of cardiac atrial imaging traits were defined by the identification of the global maximum LA volume in a phase, followed by a local minimum (LA mid-diastolic volume; LA_{mdv}), a subsequent local maximum (LA volume before atrial contraction; LA_{bac}) and lastly a global minimum volume (Supplementary material online, Figure S1). Cardiac magnetic resonance images were excluded if any of these points could not be identified. Left atrial volumes were indexed by body surface area, calculated on each participant using Du Bois formula,¹⁶ denoted LA_{max} and LA_{min} (mL/m²). Left atrial passive emptying fraction was calculated as $(LA_{max} - LA_{mdv})/LA_{max} * 100$, LAAEF as $(LA_{bac} - LA_{min})/LA_{bac} * 100$ and LATEF as $(LA_{max} - LA_{min})/LA_{max} * 100$. See Supplementary material online, Methods for details on CMR quality control. A subset of images was manually segmented and the reliability between automatic and manual segmentation was evaluated (Supplementary material online, Methods).

Study population

From the individuals with available CMR images, we identified an ethnically homogeneous subpopulation using principal component (PC) analyses. UK Biobank provides quality control sheets with information such as relatedness and PCs. We removed individuals with sex mismatch, heterozygosity rate outliers, missing genotypes or excess relatives.

We then calculated the mean and standard deviations (SD) of PC 1–5 given by individuals classified ‘white British ancestry’ in UKBB data field 22006. We included individuals with a sum total of less than 6 SDs in PC 1–5 who also self-reported ‘white’ ancestry (see Supplementary material online, Methods and Figure S6). To minimize confounding by pre-existing conditions, participants with previous myocardial infarction, diagnosis of HF or cardiomyopathy as well as body mass index (BMI) <16 or >40 kg/m² were excluded from analyses (see Supplementary material online, Methods for phenotype definitions).

Genetic analysis

UK Biobank version 3 is imputed with the haplotype reference consortium (HRC) reference panel and a merged UK10K and 1000 Genomes reference panel and has ~90 million imputed variants.

We extracted a subset of UKBB imputed autosomal genotypes from the selected study population. Info scores and minor allele frequencies (MAF) were calculated using QCTOOL on the subset of genotypes. Variants with a MAF <0.5% or info score <0.8 were excluded, leaving 10.6 million quality-controlled variants available for analysis. New PCs were generated with fastPCA v.2.0¹⁷ (see Supplementary material online, Methods for further details).

The GWASs were performed on autosomal chromosomes assuming an additive genetic model based on genotype dosages with BOLT-LMM v.2,¹⁸ which employs a linear mixed model algorithm. For the model single nucleotide polymorphisms (SNPs) used to assess random effect in BOLT, we had 581 883 hard called genotypes with a MAF > 0.5%, call rate > 95% and linkage disequilibrium (LD) < 0.9. Single nucleotide polymorphisms residing in the major histocompatibility (MHC) complex (chr6:28–35 Mb) or the chromosome 8 inversion (chr8:7–13 Mb) were removed from the analyses. Outcomes were rank-based inverse transformed residuals obtained by regressing LA traits on the covariates sex, age, PC 1–10, genotyping array (UKBB and UK BiLEVE array) and assessment centre (UKBB data field 54, instance 2).

To test the reproducibility and robustness of our results, we conducted three sensitivity analyses: excluding individuals with AF diagnosis, excluding individuals diagnosed with valvular heart disease and adjusting for systolic blood pressure (SBP; see Supplementary material online, Methods for definition of covariates). Systolic blood pressure was corrected for antihypertensive treatment by adding 10 mmHg to the observed blood pressure.¹⁹

Variance component analysis

Single nucleotide polymorphism heritability (h_g^2) and genetic correlation (r_g) were estimated with the BOLT-REML algorithm for variance component analysis.²⁰ As input, we used the model SNPs described in the genetic analysis above. Sex, age, genotyping array, assessment centre, and PC 1–10 were used as covariates in the analysis. The variance explained by each sentinel SNP was calculated with effect allele frequency (f) and beta (B) estimates, in SD^{-1} , with the formula $R^2 = B^2(1-f)2f$.²¹ For each trait separately the total variance explained by the sentinel SNPs was calculated by summing the variance explained by each sentinel.

Correlation between left atrial and left ventricular traits

We compared the phenotypic correlation between LA traits using Pearson's correlation. Furthermore, we evaluated the correlation between LA traits and measurements of LV ejection fraction (LVEF), LV end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV). Left ventricular measurements were derived simultaneously to the annotation of the LA, as described by Bai *et al.*¹⁵

Genetic correlation of left atrial traits with other traits

Using summary statistics, we applied LD score regression (LDSC software) to estimate the genetic correlation between LA traits and 10 traits selected based on availability and relevance for cardiovascular disease [AF, type 2 diabetes (T2D), systolic and diastolic blood pressure, any stroke, any ischaemic stroke, cardioembolic stroke (CES), HF, overall health rating, BMI; [Supplementary material online, Data S2](#), stroke definitions from the MEGASTROKE Consortium].²² We excluded the MHC region and SNPs with MAF < 1%. Only phenotypes with a mean $\chi^2 > 1.02$ were considered. Pre-computed LD scores for HapMap3 SNPs were used in the regression (see URLs).

Biological and functional annotation

Loci were formed around sentinel SNPs. Sentinel SNPs were chosen by the lowest P -value. Variants that were nominally significant ($P < 0.05$) and within 1000 kb and a $r^2 > 0.1$ of the sentinel SNPs were assigned to a locus. The locus region was defined by the SNPs assigned to a locus. To identify independently associated variants within a locus, we performed conditional and joint analyses using GCTA-cojo v.1.91 with default settings.²³

Co-localization

We investigated co-localization with expression quantitative trait loci (eQTL) using *coloc*.²⁴ We tested all genes with a significant *cis*-eQTL association within 250 kb of the sentinel SNP in each locus. Pre-computed *cis*-eQTLs from LV and atrial appendage tissue were obtained from GTEx v.8 (see URLs). We used the *coloc.abf* function with default settings and tested variants intersecting both GTEx and GWAS datasets. We reported on eQTLs for genes with high evidence of co-localization (posterior probability > 0.75).

Transcriptome-wide analyses

We used MetaXcan v0.6²⁵ to investigate mediating effects of gene expression levels on LA traits. Using summary statistics and eQTL data (GTEx v.7 atrial appendage and LV heart tissue, see URLs) we performed gene-based association analyses of predicted gene expression and phenotype risk. We tested 5369 genes with available expression data in the LV and 5982 in the atrial appendage. A $P < 9.3 \times 10^{-6}$ was considered significant in the LV and a $P < 8.4 \times 10^{-6}$ was considered significant in atrial

appendage tissue. See [Supplementary material online, Methods](#) for further details.

FINEMAP

In an effort to identify plausible causal variants, we employed FINEMAP v1.4²⁶ at each of the 18 identified loci. All variants within 500 kb of a sentinel SNP were used to model each locus. Variants with a log₁₀ Bayes factor (log₁₀BF) > 2 were functionally annotated using snpEFF v.4.3²⁷ and dbNSFP v2.9.²⁸

Conditional analysis

Mediating effects on loci by AF or HF were analysed by performing conditional analyses with multi-trait conditional and joint analysis (mtCojo) from GCTA v1.92.²⁹ The mtCojo method is able to condition phenotype y on phenotype x using GWAS summary statistics. These estimates are free from collider bias, which otherwise can occur when adjusting for heritable covariates.³⁰ For estimation of LD structure, a reference sample set of 12 000 individuals without CMR imaging and free from AF or HF was randomly selected from UKBB. Left atrial traits summary statistics were adjusted on GWAS summary statistics of AF³¹ and HF.³²

Polygenetic risk score for atrial fibrillation

We derived a polygenetic risk score (PRS) for AF using stacked clumping and thresholding.³³ A penalized regression was used for stacking the clumping and threshold predictions. Therefore, the penalized regression was fitted on a training set and then predicted onto the CMR cohort. Unrelated UKBB participants with European ancestry and without available CMR ($n = 332\ 673$) were used for developing the PRS (the training set). Input beta estimates and P -values came from summary statistics from a GWAS on AF not containing the UKBB cohort.³⁴ We used SNPs with MAF > 1% and a grid of varying thresholds for P -values, base size for clumping window and LD (r^2) to derive intermediate PRSs ([Supplementary material online, Table S1](#)). The intermediate PRSs were stacked into one PRS, weighted by a penalized logistic regression. The polygenetic risk for AF was then calculated for each individual with available CMR and free of AF included in this study. Additionally, we employed a two-sample 2-stage least square (2SLS) regression in order to explore the impact of LA traits on AF, HF, and stroke ([Supplementary material online, Methods](#)).

Mendelian randomization analyses

We used Mendelian randomization to investigate causal effects between the five exposures AF,³¹ HF,³² SBP, coronary artery disease (CAD), and T2D and LA traits. We employed the inverse-variance weighted method³⁵ as primary model, and Mendelian randomization-Egger regression, weighted median and mode-based estimates as sensitivity analyses. All analyses were performed using the R package TwoSampleMR.³⁶ The MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method was used to assess whether any detectable effect was mediated through outliers. We selected SNPs that were genome-wide significant ($P < 5 \times 10^{-8}$), and removed SNPs in LD using default settings ($r^2 < 0.001$) in order to obtain independent variants. A $P < 0.05/5$ traits = 0.01 was considered statistically significant. See [Supplementary material online, Methods](#) for references to MR tools.

Associations between left ventricular loci and left atrial traits

To assess the role of LV loci in LA traits, we queried the genetic variants associated with LV by Aung *et al.*¹² in our summary statistics. To correct

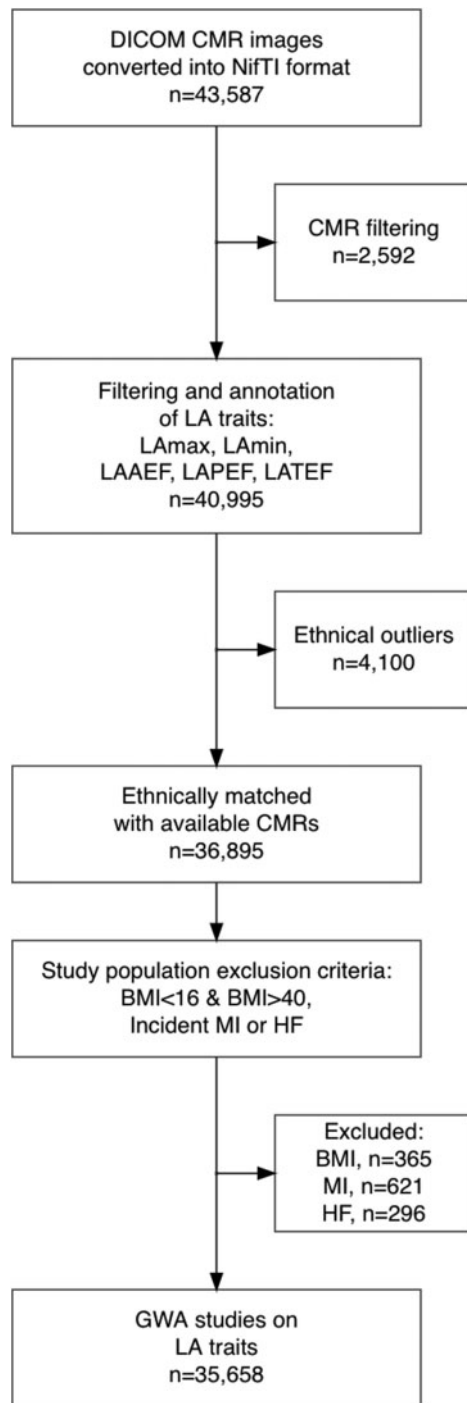


Figure 1 Sample inclusion flowchart. Please note that some individuals may fulfil multiple exclusion criteria and can be listed multiple times in the exclusion boxes, e.g. having both extreme BMI and MI. BMI, body mass index; CMR, cardiac magnetic resonance; GWAS, genome-wide association study; HF, heart failure; LA, left atrial; LAAEF, left atrial active emptying fraction; LAPEF, left atrial passive emptying fraction; LATEF, left atrial total emptying fraction; LAV, left atrial volume; MAF, minor allele frequency; MI, myocardial infarction.

for multiple testing, variants with a $P < 0.05 / (11 \text{ loci} * 5 \text{ LA traits}) = 0.0009$ were considered significantly associated with LA traits.

Results

The overall study design is illustrated in *Figure 1*. Baseline characteristics of the study population are shown in *Table 1* and summary of CMR traits in *Table 2*. A comprehensive description of the selection of study population and covariates is available in [Supplementary material online, Methods](#).

A total of 35 658 individuals were included. The median age was 64 years (IQR 58–70), average BMI was 26 (SD = 4) and 52% were women. Biplane LA_{\max} had a mean of 39 mL/m² (SD = 11 mL/m²), biplane LA_{\min} had a mean of 16 mL/m² (SD = 6 mL/m²), and LATEF 61% (SD = 8%), which are comparable to previous studies on reference values on the UKBB cohort³⁷ ([Supplementary material online, Figure S2](#)). The reproducibility of the included images with automatic and manual measurements was high (intraclass correlation coefficient $[ICC]_{\text{lamax}} = 0.93$, $ICC_{\text{lamin}} = 0.88$, $n = 50$; [Supplementary material online, Methods](#)).

Identification of loci associated with left atrial volume and function

We identified 18 novel loci associated with LA volume and function (LA_{\max} $n = 2$; LA_{\min} $n = 3$; LAAEF $n = 6$; LAPEF $n = 2$; LATEF $n = 5$, *Figure 2*, [Supplementary material online, Data S3](#)). Of these, 15 constituted unique loci. Several loci were associated with multiple LA traits, e.g. rs9664170 in proximity of *ANKRD1* reached genome-wide significance in LA_{\max} , LA_{\min} , and LAAEF (MAF = 41%, $LA_{\max}/LA_{\min}/LAAEF$ $P = 4.3 \times 10^{-8}/6.8 \times 10^{-10}/3.4 \times 10^{-9}$). There was little evidence of population stratification or cryptic relatedness ([Supplementary material online, Figures S6 and S7](#) and *Table S2*). Regional plots for each locus are shown in [Supplementary material online, Figure S8](#). We conducted three sensitivity analyses, to test the robustness and reproducibility of our results. The main results did not materially change, when we excluded individuals with AF, valvular heart disease or when adjusting for blood pressure ([Supplementary material online, Data S4](#)). Moreover, nominally significant variants ($P < 0.05$) in the reported loci correlated with beta estimates obtained in the sensitivity models (min $r > 0.98$).

Variance explained by loci was 0.29/0.18/0.63/0.21/0.50% for $LA_{\max}/LA_{\min}/LAAEF/LAPEF/LATEF$, respectively ([Supplementary material online, Data S5](#)).

Heritability and genetic correlation of left atrial traits

Heritability estimates were $h_g^2 = 0.24/0.22/0.17/0.18/0.16$ for $LA_{\max}/LA_{\min}/LAAEF/LAPEF/LATEF$, respectively ([Supplementary material online, Figure S9](#) and *Data S6*). We observed a high genetic correlation between LA_{\max} and LA_{\min} ($r_g = 0.94$), as well as LAAEF and LATEF ($r_g = 0.92$). Left atrial emptying fractions had a negative genetic correlation with LA volumes, e.g. LATEF with LA_{\max}/\min ($r_g = -0.59/-0.84$). Left atrial traits had a lower genetic correlation with LV traits (LVEDV, LVESV, LVEF). The highest absolute genetic correlation with LV traits was observed between LA_{\max} and LVEDV ($r_g =$

Table 1 Baseline characteristics

N	35 658
Age, years, median (IQR)	64 (58–70)
Male sex, <i>n</i> (%)	16 979 (48)
Height, cm, mean (SD)	169 (9)
Weight, kg, mean (SD)	76 (10)
BMI, kg/m ² , mean (SD)	26 (4)
Body surface area, m ² , mean (SD)	1.86 (0.2)
Systolic/diastolic blood pressure, mmHg, mean (SD)	130 (18)/77 (10)
Pulse rate, b.p.m., mean (SD)	67 (11)
Stroke, <i>n</i> (%)	341 (1)
AF, <i>n</i> (%)	713 (2)
T2DM, <i>n</i> (%)	1070 (3)
CAD, <i>n</i> (%)	552 (1.5)
HT, <i>n</i> (%)	9272 (26)
Valvular heart disease, <i>n</i> (%)	102 (0.3)

AF, atrial fibrillation; BMI, body mass index; CAD, coronary artery disease; HT, hypertension; IQR, interquartile range; SD, standard deviation; T2DM, type 2 diabetes mellitus.

Table 2 Cardiac magnetic resonance variables

LA _{max}	38.9 (10.5)
LA _{min}	15.5 (6.3)
LA _{mdv}	25.9 (8.4)
LA _{bac}	28.9 (8.6)
LAAEF	47.5 (8.7)
LAPEF	33.9 (7.8)
LATEF	61.1 (7.9)
LVEF	59.7 (5.8)
LVEDV	148.0 (33.0)
LVESV	60.0 (18.3)

All data are presented as mean (SD). Volume measures for LA are indexed by BSA.

bac, before atrial contraction; BSA, body surface area; CMR, cardiac magnetic resonance; LA, left atrium; LATEF, left atrial active emptying fraction; LAPEF, left atrial passive emptying fraction; LATEF, left atrial total emptying fraction; LVEF, left ventricular ejection fraction; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; mdv, mid-diastolic volume.

0.36). Left ventricular end-diastolic volume also carried the largest heritable component ($h_g^2 = 0.39$).

Characterization of left atrial associated loci

We examined co-localization of loci and eQTLs by conducting a Bayesian co-localization analysis.²⁴ Using GTEx v.8 tissue samples from atrial appendage ($n = 372$) and LV ($n = 386$), we found evidence of co-localization (posterior probability > 0.75) between loci and eQTL for 10 genes (Supplementary material online, Data S7). Left atrial volume associated loci co-localized with *ANKRD1*. Left atrial

function associated loci co-localized with *C9orf3* (also known as *AOPEP*), *CASQ2*, *RP11-10A14.4*, *VANGL1*, and *RP11-236B18.2*. Loci associated with both LA function and volume co-localized with *BHMG1*, *DMWD*, *PCGF5*, and *SYMPK*. Interestingly, the genes *BHMG1*, *DMWD*, and *SYMPK* reside in the same gene dense on chromosome 19. We also investigated the effects of gene expression levels on LA traits in a transcriptome-wide analysis (TWAS) using MetaXcan. This revealed 20 associations between the predicted gene expression and LA volume/function (Supplementary material online, Methods, Figures S10 and S11, and Data S8–S17). Interestingly, the expression of *C9orf3* was associated with increased LA function and decreased LA volume in atrial appendage tissue samples (LA_{min}/LAAEF/LATEF, $P = 9.1 \times 10^{-8}/4.7 \times 10^{-6}/3.0 \times 10^{-7}$).

In a further effort to identify genes that might be causally linked to the associated loci, we used FINEMAP²⁶ to identify credibly causal alleles. We identified seven missense variants in genes associated with LA function (Supplementary material online, Data S18), e.g. missense variants associated with LAAEF in the genes *CASQ2*, *CILP*, *DSP*, and *SIX5*, and a missense variant in *TTN* associated with LAPEF. Two missense variants associated with LATEF were identified in *CASQ2* and *MYO18B*. Functional characterization and evidence linking genes to loci are summarized in Table 3.

Genetic correlation with cardiovascular traits

Using LD score regression, we investigated the genetic correlation of LA volume and function with selected cardiovascular diseases (Figure 3 for nominal significant associations, Supplementary material online, Data S19 for all associations). Atrial fibrillation, CES, and HF had higher genetic correlation estimates with LA_{min} than LA_{max} (LA_{min}-AF $r_g = 0.20$, $P = 3 \times 10^{-4}$; LA_{min}-CES $r_g = 0.32$, $P = 4 \times 10^{-3}$; LA_{min}-HF $r_g = 0.19$, $P = 4 \times 10^{-2}$). For LA functional traits, we observed the highest genetic correlation in LAPEF with all-cause stroke (AS) and HF ($r_g = 0.40$, $P = 4 \times 10^{-6}$; $r_g = 0.53$, $P = 6 \times 10^{-7}$), whereas the genetic correlation between LAPEF and AF did not reach nominal significance. Left atrial passive emptying fraction had higher genetic correlation estimates than other LA traits with overall health rating, DBP and SBP ($r_g = -0.33$, $P = 4 \times 10^{-12}$; $r_g = -0.32$, $P = 3 \times 10^{-11}$; $r_g = -0.28$, $P = 1 \times 10^{-8}$). Among LA functional traits, LATEF had the highest genetic correlation with AF and CES ($r_g = 0.26$, $P = 5 \times 10^{-5}$; $r_g = -0.38$, $P = 5 \times 10^{-3}$).

Structural effects of polygenetic risk for atrial fibrillation

To identify whether genetic risk of AF yields LA structural changes without AF diagnosis, we produced a PRS for AF. We calculated the estimated polygenetic risk for AF in each included individual with available CMR and without AF ($n = 34\ 945$, the test set), given our stated definition of AF (Supplementary material online, Methods). Fitting a linear model adjusted for age and sex, we found that the AF PRS was associated with decreased LAAEF [-0.35%/SD, 95% confidence interval (CI) (-0.43; -0.26), $P = 3.14 \times 10^{-14}$] and LATEF [-0.27 SD, 95% CI (-0.35; -0.19), $P = 4.57 \times 10^{-11}$], but not with LAPEF ($P = 0.54$). Atrial fibrillation PRS was also associated with increased LA_{max} and LA_{min} [LA_{max} 0.25 (mL/m²)/SD, 95% CI (0.15; 0.36), $P = 5.13 \times 10^{-6}$; LA_{min} 0.21 (mL/m²)/SD, 95% CI (0.15; 0.28), $P = 1.86$

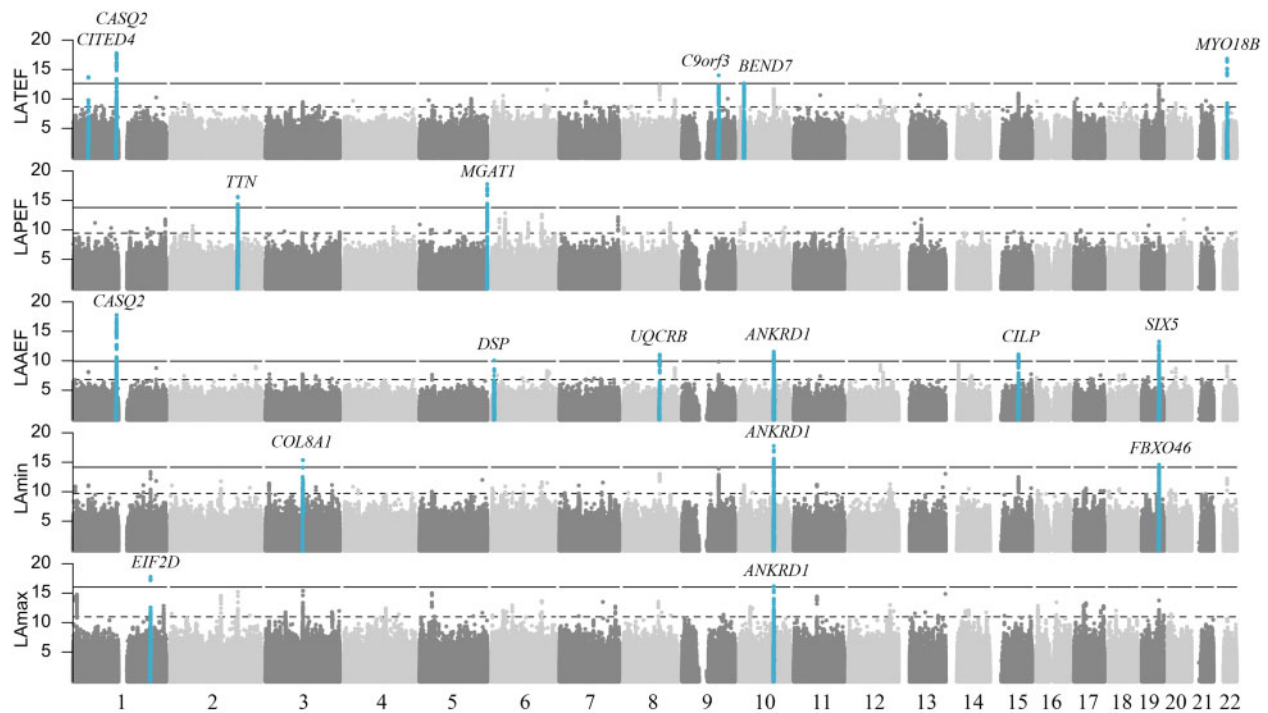


Figure 2 Genome-wide associations. Manhattan plots with the $-\log_{10}(P\text{-value})$ plotted on the y-axis and chromosomal position on the x-axis. Each locus is labelled with name of nearest gene to sentinel SNP. All sentinel SNPs are listed in [Supplementary material online, Data S3](#). LA, left atrial; LAAEF, left atrial active emptying fraction; LAPEF, left atrial passive emptying fraction; LATEF, left atrial total emptying fraction; SNP, single nucleotide polymorphism.

$\times 10^{-10}$]. Furthermore, 2SLS regression of sentinel SNP allele scores indicated that LA function has an impact on AF risk [LAAEF/SD OR = 0.69, 95% CI (0.57; 0.84), $P = 2 \times 10^{-4}$; LAPEF/SD OR = 0.43, 95% CI (0.31; 0.61), $P = 1.0 \times 10^{-6}$; LATEF/SD OR = 0.41, 95% CI (0.33; 0.51), $P = 1.0 \times 10^{-15}$; [Supplementary material online, Data S20](#)]. For LAAEF and LATEF, we also identified a nominal significant modification on the effect of AF-PRS for individuals with incident AF (LAAEF $P = 0.019$, LATEF $P = 0.036$; [Supplementary material online, Methods and Data S21](#)).

Conditional analyses and Mendelian randomization

To estimate whether the associations between genetics and LA traits were mediated by AF or HF, we performed a conditional analysis using mtCOJO.²⁹ All identified sentinel SNPs with their respective LA trait were conditioned on either AF or HF, using publicly available summary statistics. We did not observe any mediating effect on beta estimates greater than the estimated standard error, which suggests that SNP effect sizes are independent from AF and HF ([Supplementary material online, Figure S12 and Data S22](#)).

Next, we estimated the causal effects of AF, HF, SBP, CAD, and T2D on LA traits using MR.

We found supporting evidence for a casual effect of SBP on all LA traits ([Supplementary material online, Data S23](#)). Systolic blood pressure increases LA volume and decreases LA function (beta $LA_{\max}/$

$LA_{\min}/LAAEF/LAPEF/LATEF = 0.23/0.21/-0.01/-0.18/-0.11$ SD/SD⁻¹, $P < 0.01$). The effect of SBP was larger on LAPEF than on LAAEF, which is in line with other findings on LAPEF. We also found robust support for a casual effect of AF, which show congruent causal effects on LA volume, LAAEF, and LATEF ([Supplementary material online, Data S23](#)). Atrial fibrillation was similarly associated with an increase in LA_{\min}/\max , (beta LA_{\max} and $LA_{\min} = 0.07$ and 0.1 SD, respectively, $P < 0.01$) and decrease in LAAEF and LATEF (beta LAAEF and LATEF = -0.11 and 0.10 SD, respectively, $P < 0.01$), but had no statistically significant effect on LAPEF.

Role of left ventricular loci in left atrial traits

Three LV loci previously associated with LV mass, LVEDV/LVESV/LVEF, and LV mass to end-diastolic volume ratio, respectively, were significantly associated with LAPEF (rs2255167, $P = 1.5 \times 10^{-6}$, *TTN*; rs2042995, $P = 3.2 \times 10^{-7}$, *TTN*; rs146170154, $P = 2.7 \times 10^{-6}$, *CDKN1A*; [Supplementary material online, Data S24](#)).

Discussion

This is, to our knowledge, the first GWAS to date investigating the genetic background of LA traits derived from CMR. We identified 18 previously unreported loci associated with LA volume and function and demonstrated a genetic link between LA traits and incident AF

Table 3 Summary of functional characteristics in loci

Chr: Pos	Sentinel rsid	Phenotype	Nearest gene	eQTL co-localization	Fine mapped missense variants (gene, missense rsID, CADD score)
1:41348272	rs12026695	LATEF	<i>CITED4</i>		
1:116310967	rs4074536	LAAEF	<i>CASQ2</i>	<i>CASQ2</i> , <i>VANGL1</i>	<i>CASQ2</i> , rs4074536, 0.027
1:116310967	rs4074536	LATEF	<i>CASQ2</i>	<i>CASQ2</i> , <i>VANGL1</i>	<i>CASQ2</i> , rs4074536, 0.027
1:206796179	rs4845119	LA _{max}	<i>EIF2D</i>		
2:179381323	rs6735077	LAPEF	<i>TTN</i>		<i>TTN</i> , rs9808377, 8.518
3:99156961	rs114825396	LA _{min}	<i>COL8A1</i>		
5:180209454	rs655437	LAPEF	<i>MGAT1</i>		
6:7573455	rs17143007	LAAEF	<i>DSP</i>		<i>DSP</i> , rs17604693, 26.7
8:97223162	rs35216833	LAAEF	<i>UQCRB</i>	<i>RP11-10A14.4</i>	
9:97477793	rs4744370	LATEF	<i>C9orf3</i>	<i>C9orf3</i>	
10:13474369	rs113306707	LATEF	<i>BEND7</i>		
10:92692047	rs9664170	LA _{max}	<i>ANKRD1</i>	<i>PCGF5</i>	
10:92692047	rs9664170	LA _{min}	<i>ANKRD1</i>	<i>PCGF5</i> , <i>ANKRD1</i>	
10:92692047	rs9664170	LAAEF	<i>ANKRD1</i>	<i>PCGF5</i> , <i>RP11-236B18.2</i>	
15:65493852	rs2073708	LAAEF	<i>CILP</i>		<i>CILP</i> , rs2073711, 17.71
19:46213416	rs62111731	LA _{min}	<i>FBXO46</i>	<i>BHMG1</i> , <i>SYMPK</i> , <i>DMWD</i>	
19:46269076	rs2014576	LAAEF	<i>SIX5</i>	<i>BHMG1</i> , <i>SYMPK</i> , <i>DMWD</i>	<i>SIX5</i> , rs2014576, 17.36
22:26159289	rs133885	LATEF	<i>MYO18B</i>		<i>MYO18B</i> , rs133885, 16.91

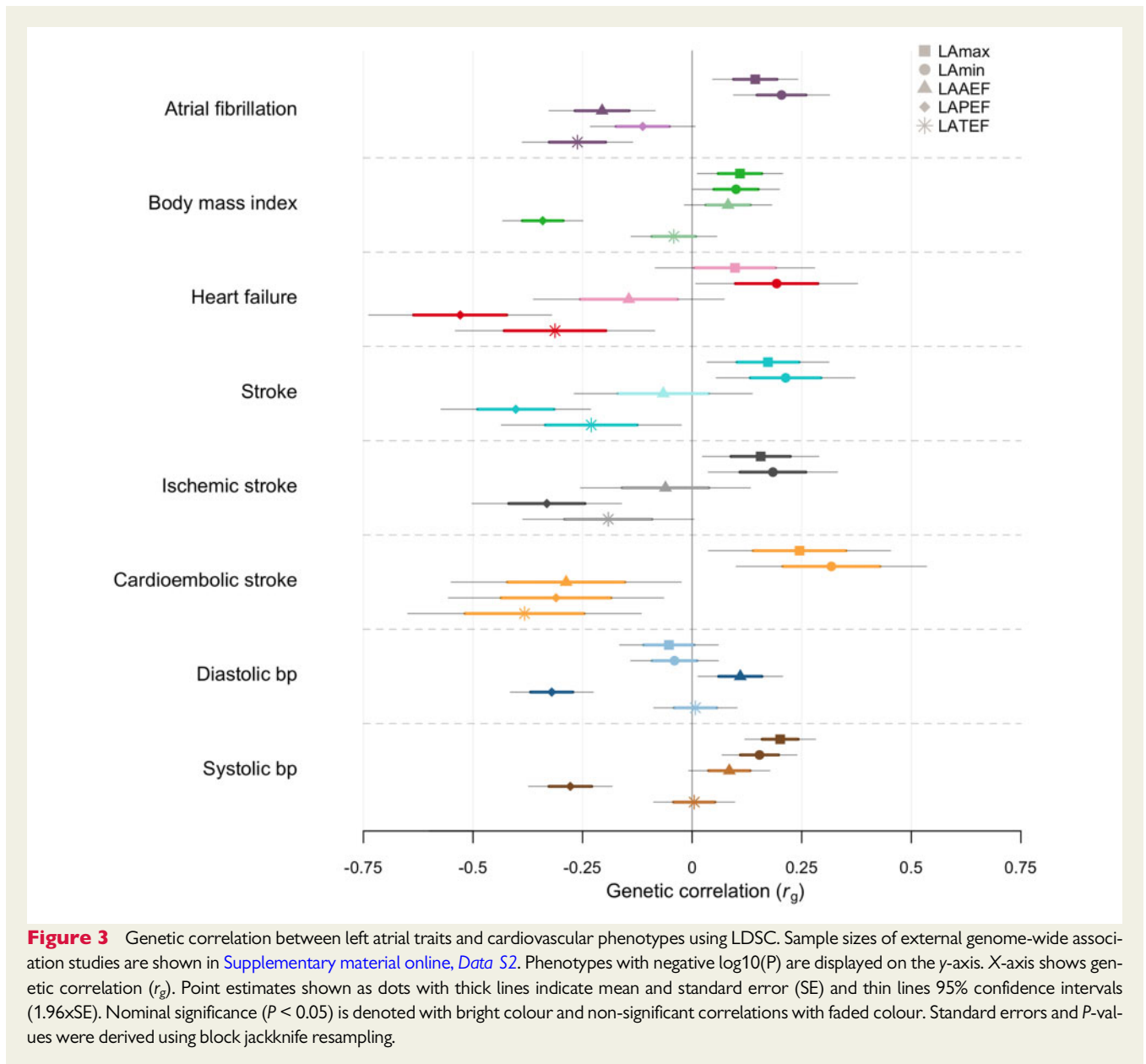
(Graphical abstract). Furthermore, we established a set of credible causal genes, of which many have a previously established relation to AF or cardiomyopathy. We evaluated the casual relationship between AF and LA traits with MR analyses and found supporting evidence of AF being causal for increased LA volume and decreased active and total emptying function. We show that polygenetic risk for AF is associated with increased LA volume and decreased LA function, in participants without prior diagnosis of AF. This suggests that structural and functional dysfunction may appear before clinically diagnosed AF.

Many of the genes identified in this study have previously been linked to cardiomyopathy or muscular dystrophy. Using fine-mapping, seven plausibly causal missense variants associated with LA function in the genes *DSP*, *SIX5*, *MYO18B*, *CILP*, *TTN*, and *CASQ2*. *SIX5* resides in a gene dense region on chromosome 19, with neighbouring genes such as *DMKP* and *DMWD*. This region is known to cause myotonic dystrophy type 1 through expansion of CTG repeats.³⁸ *MYO18B* has recently been associated with AF.³¹ Zebrafish mutants with loss of function of the *myo18b* gene show compromised sarcomere assembly and atrial enlargement,³⁹ a hallmark of atrial cardiomyopathies. Similar findings have been reported in zebrafish with titin-truncating variants.⁴⁰ *CILP*, encoding cartilage intermediate layer protein 1, has been shown as a marker for fibrosis, which can lead to HF.⁴¹ Mutations in *DSP*, encoding the protein desmoplakin, are known causes of several types of cardiomyopathy.^{42,43} The *TTN* gene encodes the protein titin, which is responsible for the passive elasticity of the cardiomyocytes and has been associated with dilated cardiomyopathy and AF.^{40,44,45} rs9664170, in proximity to *ANKRD1* encoding the cardiac ankyrin repeat protein, was associated with LAAEF and LA_{max/min} and showed evidence of co-localization with eQTLs affecting expression of *ANKRD1* and *RP11-236B18.2*. *RP11-*

236B18.2 is a long non-coding RNA (lncRNA), residing adjacent to *ANKRD1*. Long non-coding RNA has been shown to regulate gene expression.⁴⁶ Cardiac ankyrin repeat protein functions as a transcription co-factor and is a member of the titin-N2A mechanosensory complex that translocate to the cell nucleus in response to stretch, and has previously been associated with dilated cardiomyopathy.⁴⁷ Cardiac ankyrin repeat protein expression is up-regulated in HF, where it has been shown to suppress the expression of sarcomere proteins.⁴⁷ The SNP rs4074536 was associated with LAAEF and LATEF and showed evidence of co-localization with *CASQ2* eQTLs, also supported by TWAS. *CASQ2*, encoding the gene calsequestrin 2, is an important gene in calcium handling and cardiac contraction, which is functionally in line with its association with LAAEF. *CASQ2* has previously been associated with AF⁴⁸ and catecholaminergic polymorphic ventricular tachycardia.⁴⁹ Although some of the identified loci have previously been associated with AF and HF, adjusting our analyses for AF or HF genetics with conditional analyses did not show any mediating effects.

Due to a growing interest in atrial cardiomyopathy, a number of studies focusing on LA volume and function have previously been conducted. These studies indicate that LA traits are independent predictors of a number of clinical outcomes such as stroke, HF, and AF.⁵⁰ In this study, we estimate that these LA traits have a SNP heritability of ~16–26%. Left atrial volume is more heritable than LA function. Left atrial volume traits are highly genetically correlated but LAAEF and LAPEF are different genetic entities based on genetic correlation.

Recent GWAS on AF have implicated numerous genes associated with cardiomyocyte function.³¹ Moreover, a growing body of evidence suggests that rare variants in structural genes cause AF.^{40,51} Previous reports have shown that decreased LAAEF is associated



with an increased risk of AF.⁵² To evaluate the influence of predisposition to AF on the volumetric and functional properties of the atria, we produced an AF-PRS. We found that our AF-PRS was associated with lower LAAEF in individuals without known AF. This suggests that the increased risk of AF in part is mediated by predisposition to alterations in atrial function that precede the development of AF. In fact, LAAEF was associated with genes previously associated with cardiomyopathy. Whether the same genes expressed in the atria give rise to an atrial cardiomyopathy that precedes overt AF remains to be established.

By separating the passive and active emptying function in our genetic analyses of the LA, we discern that these traits have vastly different genetic bases. We observed a higher genetic correlation between LAAEF, AF, and CES compared with LAPEF. With regards to LAPEF, we found no significant association between the genetically

predictive risk of AF and LAPEF. However, we found larger genetic correlations for LAPEF with traditional cardiovascular risk factors and diseases (e.g. BMI, all-cause stroke, blood pressure, and HF) compared with LAAEF. This could be of importance since the mechanisms giving rise to stroke may differ and the link between the atria and CES needs further refinement. When looking at the overlap between LV and LA genetics, we found LV loci close to the *TTN* gene associated significantly with LAPEF. It is possible that this overlap could be mediated by the gene *TTN*, which plays an important role in ventricular cardiomyocyte sarcomere elasticity. Impaired ventricular elasticity could in theory influence the passive emptying of the atria.

Traditionally, LA_{max} is used to assess LA volume. However, LA_{min} has previously been found to have a stronger predictive value of AF, as well as subclinical AF, than LA_{max} .^{2,52} In line with these reports, our genetic analyses show that LA_{min} has a larger genetic overlap with AF

and CES than LA_{max} . Using MR analyses, we measured that AF had a larger effect on LA_{min} than LA_{max} , altogether reiterating the role of LA_{min} as important when evaluating cardiac disease.

Our findings should be interpreted within the context of their limitations. To avoid population stratification bias, we excluded persons with non-European ancestry from analyses. Our findings are therefore limited to European ancestry only and cannot be directly extrapolated to other populations. However, they do inform us of fundamental important biological processes in the LA. Annotation of LA traits is based on machine learning algorithms with the resulting strengths and weaknesses. To evaluate the accuracy of the deep learning model, we performed manual segmentation on a subset of the dataset which validated the performance of the model. Although our manual segmentation was highly correlated with the automated segmentation, an overestimation was present for the automated segmentation compared with the manual. The overestimation was systematic and should therefore not influence the genetic results. Bai et al.¹⁵ have previously validated the algorithm on UKBB CMR at a larger scale with a Dice metric of 0.93 for LA (2-ch) and 0.95 LA (4-ch). Also, they note that the automated method is trained by multiple observers which may make it less susceptible to biases by learning a consensus estimate, since manual inter-observer variability is to be expected. However, the LA is challenging to measure and even after quality control filtering, errors might remain that dilute the genetic signal. In the future, refinements addressing these issues might be able to enhance the genetic signal of these traits. Although the majority of our findings are plausible candidate genes with well-described roles in cardiac pathophysiology, future studies are needed to cement their role in LA volume and function.

In conclusion, we report on 18 genetic loci associated with LA function and volume. We show evidence for several plausible candidate genes important for atrial structure. Many of the genes associated with LA function have previously been linked with cardiomyopathy. The results found in this study suggest that compromised LA function may share genetic underpinning with cardiomyopathy. Furthermore, we show that the genetic risk of AF influences LA structure prior to diagnosis of AF, suggesting an intrinsic relationship.

URLs

FCN model: https://github.com/baiwenjia/ukbb_cardiac
 Precomputed LD scores etc.: <https://data.broadinstitute.org/alkesgroup/LDSCORE/>
 LDSC: <https://github.com/bulik/ldsc>
 GTEx v.8: <https://www.gtexportal.org/>
 MetaXcan transcriptome models: <http://predictdb.org/>

Supplementary material

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

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Conflict of interest: none declared.

Data availability

Summary statistics are available for download at <https://zenodo.org/record/5074929>. Data generated in this study have been returned to the UKBB. The UKBB will make these data available to all researchers who apply by standard access procedures. Please see the UKBB's website for access procedures (<https://www.ukbiobank.ac.uk/enable-your-research/apply-for-access>).

References

- Kaminski M, Steel K, Jerosch-Herold M, Khin M, Tsang S, Hauser T, Kwong RY. Strong cardiovascular prognostic implication of quantitative left atrial contractile function assessed by cardiac magnetic resonance imaging in patients with chronic hypertension. *J Cardiovasc Magn Reson* 2011;**13**:42.
- Olsen FJ, Møgelvang R, Jensen GB, Jensen JS, Biering-Sørensen T. Relationship between left atrial functional measures and incident atrial fibrillation in the general population: the Copenhagen City Heart Study. *JACC Cardiovasc Imaging* 2019;**12**: 981–989.
- Habibi M, Zareian M, Venkatesh BA, Samiei S, Imai M, Wu C, Launer LJ, Shea S, Gottesman RF, Heckbert SR, Bluemke DA, Lima JAC. Left atrial mechanical function and incident ischemic cerebrovascular events independent of AF: insights from the MESA study. *JACC Cardiovasc Imaging* 2019;**12**:2417–2427.
- Gupta S, Matulevicius SA, Ayers CR, Berry JD, Patel PC, Markham DW, Levine BD, Chin KM, Lemos J. D, Peshock RM, Drazner MH. Left atrial structure and function and clinical outcomes in the general population. *Eur Heart J* 2013;**34**: 278–285.
- Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt JU. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015;**16**:233–270.
- Goette A, Kalman JM, Aguinaga L, Akar J, Cabrera JA, Chen SA, Chugh SS, Corradi D, D'Avila A, Dobrev D, Fenelon G, Gonzalez M, Hatem SN, Helm R, Hindricks G, Ho SY, Hoit B, Jalife J, Kim Y-H, Lip GYH, Ma C-S, Marcus GM, Murray K, Nogami A, Sanders P, Uribe W, Van Wagoner DR, Nattel S; Document Reviewers. EHRA/HRS/APHRS/SOLAECE expert consensus on atrial cardiomyopathies: definition, characterization, and clinical implication. *Europace* 2016;**18**:1455–1490.
- Stefanadis C, Dernellis J, Toutouzas P. A clinical appraisal of left atrial function. *Eur Heart J* 2001;**22**:22–36.
- Gal P, Marrouche NF. Magnetic resonance imaging of atrial fibrosis: redefining atrial fibrillation to a syndrome. *Eur Heart J* 2017;**38**:14–19.
- Kottkamp H. Human atrial fibrillation substrate: towards a specific fibrotic atrial cardiomyopathy. *Eur Heart J* 2013;**34**:2731–2738.
- Grothues F, Smith GC, Moon JCC, Bellenger NG, Collins P, Klein HU, Pennell DJ. Comparison of interstudy reproducibility of cardiovascular magnetic resonance with two-dimensional echocardiography in normal subjects and in patients with heart failure or left ventricular hypertrophy. *Am J Cardiol* 2002;**90**:29–34.
- Vasan RS, Larson MG, Aragam J, Wang TJ, Mitchell GF, Kathiresan S, Newton-Cheh C, Vita JA, Keyes MJ, O'Donnell CJ, Levy D, Benjamin EJ. Genome-wide association of echocardiographic dimensions, brachial artery endothelial function

- and treadmill exercise responses in the Framingham Heart Study. *BMC Med Genet* 2007;**8**:S2.
12. Aung N, Vargas JD, Yang C, Cabrera CP, Warren HR, Fung K, Tzani E, Barnes MR, Rotter JI, Taylor KD, Manichaikul AW, Lima JAC, Bluemke DA, Piechnik SK, Neubauer S, Munroe PB, Petersen SE. Genome-wide analysis of left ventricular image-derived phenotypes identifies fourteen loci associated with cardiac morphogenesis and heart failure development. *Circulation* 2019;**140**:1318–1330.
 13. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, Cortes A, Welsh S, Young A, Effingham M, McVean G, Leslie S, Allen N, Donnelly P, Marchini J. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018;**562**:203–209.
 14. Petersen SE, Matthews PM, Francis JM, Robson MD, Zemrak F, Boubertakh R, Young AA, Hudson S, Weale P, Garratt S, Collins R, Piechnik S, Neubauer S. UK Biobank's cardiovascular magnetic resonance protocol. *J Cardiovasc Magn Reson* 2016;**18**:8–7.
 15. Bai W, Sinclair M, Tarroni G, Oktay O, Rajchl M, Vaillant G, Lee AM, Aung N, Lukaschuk E, Sanghi MM, Zemrak F, Fung K, Paiva JM, Carapella V, Kim YJ, Suzuki H, Kainz B, Matthews PM, Petersen SE, Piechnik SK, Neubauer S, Glocker B, Rueckert D. Automated cardiovascular magnetic resonance image analysis with fully convolutional networks. *J Cardiovasc Magn Reson* 2018;**20**:65.
 16. Du Bois D, Du Bois EF. A formula to estimate the approximate surface area if height and weight be known. 1916. *Nutrition* 1989;**5**:303–311; discussion 312–313.
 17. Abraham G, Qiu Y, Inouye M. FlashPCA2: principal component analysis of Biobank-scale genotype datasets. *Bioinformatics* 2017;**33**:2776–2778.
 18. Loh P-R, Kichaev G, Gazal S, Schoech AP, Price AL. Mixed-model association for biobank-scale datasets. *Nat Genet* 2018;**50**:906–908.
 19. Tobin MD, Sheehan NA, Scurrah KJ, Burton PR. Adjusting for treatment effects in studies of quantitative traits: antihypertensive therapy and systolic blood pressure. *Stat Med* 2005;**24**:2911–2935.
 20. Loh P-R, Bhatia G, Gusev A, Finucane HK, Bulik-Sullivan BK, Pollack SJ, de Candia TR, Lee SH, Wray NR, Kendler KS, O'Donovan MC, Neale BM, Patterson N, Price AL; Schizophrenia Working Group of Psychiatric Genomics Consortium. Contrasting genetic architectures of schizophrenia and other complex diseases using fast variance components analysis. *Nat Genet* 2015;**47**:1385–1392.
 21. Burgess S, Dudbridge F, Thompson SG. Combining information on multiple instrumental variables in Mendelian randomization: comparison of allele score and summarized data methods. *Stat Med* 2016;**35**:1880–1906.
 22. Bulik-Sullivan BK, Loh P-R, Finucane HK, Ripke S, Yang J, Patterson N, Daly MJ, Price AL, Neale BM; Schizophrenia Working Group of the Psychiatric Genomics Consortium. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* 2015;**47**:291–295.
 23. Yang J, Ferreira T, Morris AP, Medland SE, Madden PAF, Heath AC, Martin NG, Montgomery GW, Weedon MN, Loos RJ, Frayling TM, McCarthy MI, Hirschhorn JN, Goddard ME, Visscher PM; DIABetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Conditional and joint multiple-SNP analysis of GWAS summary statistics identifies additional variants influencing complex traits. *Nat Genet* 2012;**44**:369–375.
 24. Giambartolomei C, Vukcevic D, Schadt EE, Franke L, Hingorani AD, Wallace C, Plagnol V. Bayesian test for colocalisation between pairs of genetic association studies using summary statistics. *PLoS Genet* 2014;**10**:e1004383.
 25. Barbeira AN, Dickinson SP, Bonazzola R, Zheng J, Wheeler HE, Torres JM, Torstenson ES, Shah KP, Garcia T, Edwards TL, Stahl EA, Huckins LM, Nicolae DL, Cox NJ, Im HK; GTEx Consortium. Exploring the phenotypic consequences of tissue specific gene expression variation inferred from GWAS summary statistics. *Nat Commun* 2018;**9**:1825.
 26. Benner C, Spencer CCA, Havulinna AS, Salomaa V, Ripatti S, Pirinen M. FINEMAP: efficient variable selection using summary data from genome-wide association studies. *Bioinformatics* 2016;**32**:1493–1501.
 27. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, Land SJ, Lu X, Ruden DM. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of *Drosophila melanogaster* strain w1118; iso-2; iso-3. *Fly* 2012;**6**:80–92.
 28. Liu X, Jian X, Boerwinkle E. dbNSFP v2.0: a database of human non-synonymous SNVs and their functional predictions and annotations. *Hum Mutat* 2013;**34**:E2393–E2402.
 29. Zhu Z, Zheng Z, Zhang F, Wu Y, Trzaskowski M, Maier R, Robinson MR, McGrath JJ, Visscher PM, Wray NR, Yang J. Causal associations between risk factors and common diseases inferred from GWAS summary data. *Nat Commun* 2018;**9**:224.
 30. Aschard H, Vilhjalmsdottir BJ, Joshi AD, Price AL, Kraft P. Adjusting for heritable covariates can bias effect estimates in genome-wide association studies. *Am J Hum Genet* 2015;**96**:329–339.
 31. Nielsen JB, Thorolfsdottir RB, Fritsche LG, Zhou W, Skov MW, Graham SE, Herron TJ, McCarthy S, Schmidt EM, Sveinbjornsson G, Surakka I, Mathis MR, Yamazaki M, Crawford RD, Gabrielsen ME, Skogholt AH, Holmen OL, Lin M, Wolford BN, Dey R, Dalen H, Sulem P, Chung JH, Backman JD, Arnar DO, Thorsteinsdottir U, Baras A, O'Dushlaine C, Holst AG, Wen X, Hornsby W, Dewey FE, Boehnke M, Khetarpal S, Mukherjee B, Lee S, Kang HM, Holm H, Kitzman J, Shavit JA, Jalife J, Brummett CM, Teslovich TM, Carey DJ, Gudbjartsson DF, Stefansson K, Abecasis GR, Hveem K, Willer CJ. Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. *Nat Genet* 2018;**50**:1234–1239.
 32. Shah S, Henry A, Roselli C, Lin H, Sveinbjornsson G, Fatemifar G, Hedman ÅK, Wilk JB, Morley MP, Chaffin MD, Helgadóttir A, Verweij N, Dehghan A, Almgren P, Andersson C, Aragam KG, Ärnlöv J, Backman JD, Biggs ML, Bloom HL, Brandimarto J, Brown MR, Buckbinder L, Carey DJ, Chasman DI, Chen X, Chen X, Chung J, Chutkow W, Cook JP, Delgado GE, Denaxas S, Doney AS, Dörr M, Dudley SC, Dunn ME, Engström G, Esko T, Felix SB, Finan C, Ford I, Ghanbari M, Ghasemi S, Giedraitis V, Giulianini F, Gottdiener JS, Gross S, Guðbjartsson DF, Gutmann R, Haggerty CM, van der Harst P, Hyde CL, Ingelsson E, Jukema JW, Kavousi M, Khaw K-T, Kleber ME, Køber L, Koekemoer A, Langenberg C, Lind L, Lindgren CM, London B, Lotta LA, Lovering RC, Luan J, Magnusson P, Mahajan A, Margulies KB, März W, Melander O, Mordi IR, Morgan T, Morris AD, Morris AP, Morrison AC, Nagle MW, Nelson CP, Niessner A, Niiranen T, O'Donoghue ML, Owens AT, Palmer CNA, Parry HM, Perola M, Portilla-Fernandez E, Psaty BM, Rice KM, Ridker PM, Romaine SPR, Rotter JI, Salo P, Salomaa V, van Setten J, Shalaby AA, Smelser DT, Smith NL, Stender S, Stott DJ, Svensson P, Tammesoo M-L, Taylor KD, Teder-Laving M, Teumer A, Thorgerisson G, Thorsteinsdottir U, Torp-Pedersen C, Trompet S, Tyl B, Uitterlinden AG, Veluchamy A, Völker U, Voors AA, Wang X, Wareham NJ, Waterworth D, Weeke PE, Weiss R, Wiggins KL, Xing H, Yerges-Armstrong LM, Yu B, Zannad F, Zhao JH, Hemingway H, Samani NJ, McMurray JJV, Yang J, Visscher PM, Newton-Cheh C, Malarstig A, Holm H, Lubitz SA, Sattar N, Holmes MV, Cappola TP, Asselbergs FW, Hingorani AD, Kuchenbaecker K, Ellinor PT, Lang CC, Stefansson K, Smith JG, Vasari RS, Swerdlow DL, Lumbers RT; Regeneron Genetics Center. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* 2020;**11**:163.
 33. Privé F, Vilhjalmsdottir BJ, Aschard H, Blum MGB. Making the most of clumping and thresholding for polygenic scores. *Am J Hum Genet* 2019;**105**:1213–1221.
 34. Christophersen IE, Rienstra M, Roselli C, Yin X, Geelhoed B, Barnard J, Lin H, Arking DE, Smith AV, Albert CM, Chaffin M, Tucker NR, Li M, Klarin D, Bihlmeyer NA, Low S-K, Weeke PE, Müller-Nurasyid M, Smith JG, Brody JA, Niemeijer MN, Dörr M, Trompet S, Huffman J, Gustafsson S, Schurmann C, Kleber ME, Lyytikäinen L-P, Seppälä I, Malik R, Horimoto ARVR, Perez M, Sinisalo J, Aeschbacher S, Thériault S, Yao J, Radmanesh F, Weiss S, Teumer A, Choi SH, Weng L-C, Clauss S, Deo R, Rader DJ, Shah SH, Sun A, Hopewell JC, Dobbie S, Chauhan G, Yang Q, Worrall BB, Paré G, Kamatani Y, Hagemeijer YP, Verweij N, Siland JE, Kubo M, Smith JD, Van Wagoner DR, Bis JC, Perz S, Psaty BM, Ridker PM, Magnani JW, Harris TB, Launer LJ, Shoemaker MB, Padmanabhan S, Haessler J, Bartz TM, Müller-Berger M, Lichtner P, Arendt M, Krieger JE, Kähönen M, Risch L, Mansur AJ, Peters A, Smith BH, Lind L, Scott SA, Lu Y, Bottinger EB, Hernessniemi J, Lindgren CM, Wong JA, Huang J, Eskola M, Morris AP, Ford I, Reiner AP, Delgado G, Chen LY, Chen Y-DI, Sandhu RK, Li M, Boerwinkle E, Eisele L, Lannfelt L, Rost N, Anderson CD, Taylor KD, Campbell A, Magnusson PK, Porteous D, Hocking LJ, Vlachopoulou E, Pedersen NL, Nikus K, Orho-Melander M, Hamsten A, Heeringa J, Denny JC, Kriebel J, Darbar D, Newton-Cheh C, Shaffer C, Macfarlane PW, Heilmann-Heimbach S, Almgren P, Huang PL, Sotoodehnia N, Soliman EZ, Uitterlinden AG, Hofman AD, Franco OH, Völker U, Jöckel K-H, Sinner MF, Lin HJ, Guo X, Dichgans M, Ingelsson E, Kooperberg C, Melander O, Loos RJF, Laurikka J, Conen D, Rosand J, van der Harst P, Lokki M-L, Kathiresan S, Pereira A, Jukema JW, Hayward C, Rotter JI, März W, Lehtimäki T, Stricker BH, Chung MK, Felix SB, Gudnason V, Alonso A, Roden DM, Kääb S, Chasman DI, Heckbert SR, Benjamin EJ, Tanaka T, Lunetta KL, Lubitz SA, Ellinor PT; AFGEN Consortium; METASTROKE Consortium. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. *Nat Genet* 2017;**49**:946–952.
 35. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;**37**:658–665.
 36. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, Laurin C, Burgess S, Bowden J, Langdon R, Tan VY, Yarmolinsky J, Shihab HA, Timpson NJ, Evans DM, Relton C, Martin RM, Davey Smith G, Gaunt TR, Haycock PC. The MR-Base platform supports systematic causal inference across the human phenome. *eLife* 2018;**7**:e34408.
 37. Petersen SE, Aung N, Sanghi MM, Zemrak F, Fung K, Paiva JM, Francis JM, Khanji MY, Lukaschuk E, Lee AM, Carapella V, Kim YJ, Leeson P, Piechnik SK, Neubauer S. Reference ranges for cardiac structure and function using cardiovascular magnetic resonance (CMR) in Caucasians from the UK Biobank population cohort. *J Cardiovasc Magn Reson* 2017;**19**:18.

38. Thornton CA. Myotonic dystrophy. *Neural Clin* 2014;**32**:705–719.
39. Gurlung R, Ono Y, Baxendale S, Lee SLC, Moore S, Calvert M, Ingham PW. A zebrafish model for a human myopathy associated with mutation of the unconventional myosin MYO18B. *Genetics* 2017;**205**:725–735.
40. Ahlberg G, Refsgaard L, Lundegaard PR, Andreasen L, Ranthe MF, Linscheid N, Nielsen JB, Melbye M, Haunsø S, Sajadieh A, Camp L, Olesen S-P, Rasmussen S, Lundby A, Ellinor PT, Holst AG, Svendsen JH, Olesen MS. Rare truncating variants in the sarcomeric protein titin associate with familial and early-onset atrial fibrillation. *Nat Commun* 2018;**9**:4316.
41. van Nieuwenhoven FA, Munts C, op't Veld RC, González A, Díez J, Heymans S, Schroen B, van Bilsen M. Cartilage intermediate layer protein 1 (CILP1): a novel mediator of cardiac extracellular matrix remodelling. *Sci Rep* 2017;**7**:16042.
42. Norgett EE, Hatsell SJ, Carvajal-Huerta L, Cabezas JC, Common J, Purkis PE, Whittock N, Leigh IM, Stevens HP, Kelsell DP. Recessive mutation in desmoplakin disrupts desmoplakin-intermediate filament interactions and causes dilated cardiomyopathy, woolly hair and keratoderma. *Hum Mol Genet* 2000;**9**:2761–2766.
43. Norman M, Simpson M, Mogensen J, Shaw A, Hughes S, Syrris P, Sen-Chowdhry S, Rowland E, Crosby A, McKenna WJ. Novel mutation in desmoplakin causes arrhythmogenic left ventricular cardiomyopathy. *Circulation* 2005;**112**:636–642.
44. Herman DS, Lam L, Taylor MRG, Wang L, Teekakirikul P, Christodoulou D, Conner L, DePalma SR, McDonough B, Sparks E, Teodorescu DL, Cirino AL, Banner NR, Pennell DJ, Graw S, Merlo M, Di Lenarda A, Sinagra G, Bos JM, Ackerman MJ, Mitchell RN, Murry CE, Lakdawala NK, Ho CY, Barton PJR, Cook SA, Mestroni L, Seidman JG, Seidman CE. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med* 2012;**366**:619–628.
45. Choi SH, Weng L-C, Roselli C, Lin H, Haggerty CM, Shoemaker MB, Barnard J, Arking DE, Chasman DI, Albert CM, Chaffin M, Tucker NR, Smith JD, Gupta N, Gabriel S, Margolin L, Shea MA, Shaffer CM, Yoneda ZT, Boerwinkle E, Smith NL, Silverman EK, Redline S, Vasani RS, Burchard EG, Gogarten SM, Laurie C, Blackwell TW, Abecasis G, Carey DJ, Fornwalt BK, Smelser DT, Baras A, Dewey FE, Jaquish CE, Papanicolaou GJ, Sotoodehnia N, Wagoner DRV, Psaty BM, Kathiresan S, Darbar D, Alonso A, Heckbert SR, Chung MK, Roden DM, Benjamin EJ, Murray MF, Lunetta KL, Lubitz SA, Ellinor PT; For the DiscovEHR study and the NHLBI Trans-Omics for Precision Medicine (TOPMed) Consortium. Association between titin loss-of-function variants and early-onset atrial fibrillation. *JAMA* 2018;**320**:2354–2364.
46. Luo S, Lu JY, Liu L, Yin Y, Chen C, Han X, Wu B, Xu R, Liu W, Yan P, Shao W, Lu Z, Li H, Na J, Tang F, Wang J, Zhang YE, Shen X. Divergent lncRNAs regulate gene expression and lineage differentiation in pluripotent cells. *Cell Stem Cell* 2016;**18**:637–652.
47. Moulik M, Vatta M, Witt SH, Arola AM, Murphy RT, McKenna WJ, Boriek AM, Oka K, Labeit S, Bowles NE, Arimura T, Kimura A, Towbin JA. ANKRD1, the gene encoding cardiac ankyrin repeat protein, is a novel dilated cardiomyopathy gene. *J Am Coll Cardiol* 2009;**54**:325–333.
48. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, Arking DE, Müller-Nurasyid M, Krijthe BP, Lubitz SA, Bis JC, Chung MK, Dörr M, Ozaki K, Roberts JD, Smith JG, Pfeufer A, Sinner MF, Lohman K, Ding J, Smith NL, Smith JD, Rienstra M, Rice KM, Van Wagoner DR, Magnani JW, Wakili R, Clauss S, Rotter JI, Steinbeck G, Launer LJ, Davies RW, Borkovich M, Harris TB, Lin H, Völker U, Völzke H, Milan DJ, Hofman A, Boerwinkle E, Chen LY, Soliman EZ, Voight BF, Li G, Chakravarti A, Kubo M, Tedrow UB, Rose LM, Ridker PM, Conen D, Tsunoda T, Furukawa T, Sotoodehnia N, Xu S, Kamatani N, Levy D, Nakamura Y, Parvez B, Mahida S, Furie KL, Rosand J, Muhammad R, Psaty BM, Meitinger T, Perz S, Wichmann HE, Witteman JCM, Kao WHL, Kathiresan S, Roden DM, Uitterlinden AG, Rivadeneira F, McKnight B, Sjögren M, Newman AB, Liu Y, Gollob MH, Melander O, Tanaka T, Stricker BHC, Felix SB, Alonso A, Darbar D, Barnard J, Chasman DI, Heckbert SR, Benjamin EJ, Gudnason V, Kääb S. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet* 2012;**44**:670–675.
49. Roston TM, Yuchi Z, Kannankeril PJ, Hathaway J, Vinocur JM, Etheridge SP, Potts JE, Maginot KR, Salerno JC, Cohen MI, Hamilton RM, Pflaumer A, Mohammed S, Kimlicka L, Kanter RJ, LaPage MJ, Collins KK, Gebauer RA, Temple JD, Batra AS, Erickson C, Miszczak-Knecht M, Kubuš P, Bar-Cohen Y, Kantoch M, Thomas VC, Hessling G, Anderson C, Young M-L, Choi SHJ, Cabrera Ortega M, Lau YR, Johnsrude CL, Fournier A, Van Petegem F, Sanatani S. The clinical and genetic spectrum of catecholaminergic polymorphic ventricular tachycardia: findings from an international multicentre registry. *Europace* 2018;**20**:541–547.
50. Gottdiener JS, Kitzman DW, Aurigemma GP, Arnold AM, Manolio TA. Left atrial volume, geometry, and function in systolic and diastolic heart failure of persons ≥65 years of age (The Cardiovascular Health Study). *Am J Cardiol* 2006;**97**:83–89.
51. Orr N, Arnaout R, Gula LJ, Spears DA, Leong-Sit P, Li Q, Tarhuni W, Reischauer S, Chauhan VS, Borkovich M, Uppal S, Adler A, Coughlin SR, Stainer DYR, Gollob MH. A mutation in the atrial-specific myosin light chain gene (MYL4) causes familial atrial fibrillation. *Nat Commun* 2016;**7**:11303.
52. Bertelsen L, Diederichsen SZ, Haugan KJ, Brandes A, Graff C, Krieger D, Kronborg C, Køber L, Højberg S, Vejstrup N, Svendsen JH. Left atrial volume and function assessed by cardiac magnetic resonance imaging are markers of sub-clinical atrial fibrillation as detected by continuous monitoring. *Europace* 2020;**22**:724–731.