Characterization of Different Patient Populations with Atrial Fibrillation

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5 Genetic risk and atrial fibrillation in patients with heart failure

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ABSTRACT

Aims: Study the association between an atrial fibrillation (AF) genetic risk score (GRS) with prevalent AF and all-cause mortality in patients with heart failure.

Methods: An AF GRS was calculated in 3759 European ancestry individuals (1783 with sinus rhythm (SR), 1976 with AF) from the BIOlogy Study to TAilored Treatment in Chronic Heart Failure study by summing 97 single nucleotide polymorphisms (SNPs) alleles (ranging from 0-2) weighted by the natural logarithm of the relative SNP risk from the latest AF genome-wide association study. Further, we assessed AF risk variance explained by additive SNP variation, and performance of clinical- or genetic risk factors, and the combination in classifying AF prevalence. AF was classified as AF or atrial flutter (AFL) at baseline ECG and/or a history of AF or AFL.

Results: The GRS was associated with AF after multivariable adjustment. Odds ratio for AF prevalence per 1-unit increase GRS was 2.12 (95% confidence interval 1.84-2.45, $P=2.15*10^{-24}$) in the total cohort, 2.08 (1.72-2.50, $P=1.30*10^{-14}$) in heart failure with reduced ejection fraction (HFrEF) and 2.02 (1.37-2.99, $P=4.37*10^{-4}$) in heart failure with preserved ejection fraction (HFpEF). AF-associated loci explained 22.9% of overall AF SNP-heritability. Addition of the GRS to clinical risk factors increased the C-index by 2.2% to 0.721.

Conclusion: The AF GRS was associated with increased AF prevalence in HFrEF and HFpEF. Genetic variation accounted for 22.9% overall AF SNP-heritability. Addition of genetic risk to clinical risk improved model performance in classifying AF prevalence.

INTRODUCTION

Atrial fibrillation (AF) is the most common cardiac arrhythmia and is highly prevalent in patients with heart failure.[1-3] The co-existence of these conditions can be expected by virtue of their prevalence alone: the lifetime risk of developing AF is about one in three in individuals of European ancestry and one in five in individuals of African ancestry[4-6], and after age 45 the lifetime risk of heart failure ranges between 20-45%.[7]

Furthermore, both conditions have many shared risk factors which makes their co-existence more likely.[8,9] Additionally, a reciprocal relation between both conditions seems to exist, but regardless of which conditions occurs first, the concomitant presence of both AF and heart failure is associated with substantially increased risks of mortality.[2,3]

AF is common in heart failure and prevalence of the arrhythmia increases with heart failure severity, but little is known about the mechanisms that underlie AF onset in heart failure patients.[10,11] Genetic factors could theoretically explain, at least partly, the increased risk of AF in patients with heart failure.[12] But heritability of AF is complex; in a recent study 97 genome-wide susceptibility loci for AF were identified, and the proportion of heritability explained by the loci in individuals of European ancestry was 42%.[13] Prevalence estimates of heart failure in population-based biobanks and case-referent studies used for AF-GWAS studies is limited, and it remains unclear whether individuals with AF in the context of heart failure share a similar genetic susceptibility to the arrhythmia.

We aimed to study the association between a genetic risk score based on 97 lead SNPs[13] with prevalent AF and all-cause mortality in a large sample of patients with heart failure included in The BIOlogy Study to Tailored Treatment in Chronic Heart Failure (BIOSTAT-CHF) study. Further, we assessed the variance in AF prevalence explained by additive SNP variation (SNP-heritability), and determined the discriminatory accuracy of clinical risk factors, genetic risk factors, and the combination in classifying AF prevalence.

METHODS

Study population

The prospective, observational, international BIOSTAT-CHF study included 2516 patients with heart failure from 11 European countries between December 2010 and December 2012. Another 1738 patients from Scotland were included in a validation cohort between October 2010 and April 2014. The rationale, design, and primary results

have been previously published.[14] Briefly, the majority of patients were hospitalized for acute heart failure, and the remainder presented with worsening signs/and or symptoms of heart failure at outpatient clinics. Patients had to have objective evidence of cardiac dysfunction documented either by left ventricular ejection fraction (LVEF) of ≤40%, previous heart failure hospitalization, or plasma concentrations of BNP and/or NT-proBNP >400 pg/ml or >2,000 pg/ml, respectively. According to study design all patients used diuretics but were not on optimal, evidence-based medical therapy of angiotensin-converting enzyme inhibitors / angiotensin receptor antagonists and, or beta-blockers. After inclusion patients were extensively phenotyped and genotyped, underwent physical examination and quality of life measurements, and plasma, serum, and urine samples were collected for analysis. During the first three months of follow-up medication was optimized. The study complies with the Declaration of Helsinki, medical ethics committee of participating centres approved the study, and all patients provided written informed consent before inclusion.

Patient selection

For the current analysis the BIOSTAT-CHF index cohort (N=2516) and validation cohort (N=1738) were combined to achieve a larger set of patients (N=4254). Patients with no blood samples available for genotyping (N=166), self-reported non-European ancestry (N=37), and pacemaker rhythm or missing variables that prohibited rhythm classification (N=292) were excluded (see Figure 1, flowchart).

AF prevalence and all-cause mortality

AF prevalence was defined as clinical history of AF or atrial flutter (AFL) and / or AF(L) on baseline electrocardiogram (ECG). Patients were regarded as having sinus rhythm if they had no history of AF and sinus rhythm on baseline ECG. Incident AF was not captured during follow-up.

After the optimization- (3 months) and maintenance phase (6 months)[14], patients were followed by standard clinical follow-up or telephone contact with 6-month intervals. Follow-up ended on April 1, 2015. Median follow up duration was approximately 21 months. During follow up all deaths and hospitalizations were recorded. For the current analysis we assessed all-cause mortality.

Genotyping in BIOSTAT-CHF

The two cohorts were processed, genotyped, QC'd and imputed independently, using the same protocols. Genotyping of all patients from both BIOSTAT-CHF cohorts was performed using the Affymetrix Axiom Genome-Wide UKB WCSG genotyping array. Sample level QC was performed for X chromosome homozygosity (sex mismatch) and

identity by descent (IBD) estimates (relatedness and duplicates). Prior to imputation, variants were removed if their call rate was <95% for variants with minor allele frequency (MAF) ≥5%, or <99% for variants with MAF <5%, or had a Hardy-Weinberg equilibrium $P<1x10^{-6}$. Imputation was performed using SHAPEIT2[15] and IMPUTE2[16] with the phase 3 release 1000G reference panel.[17]

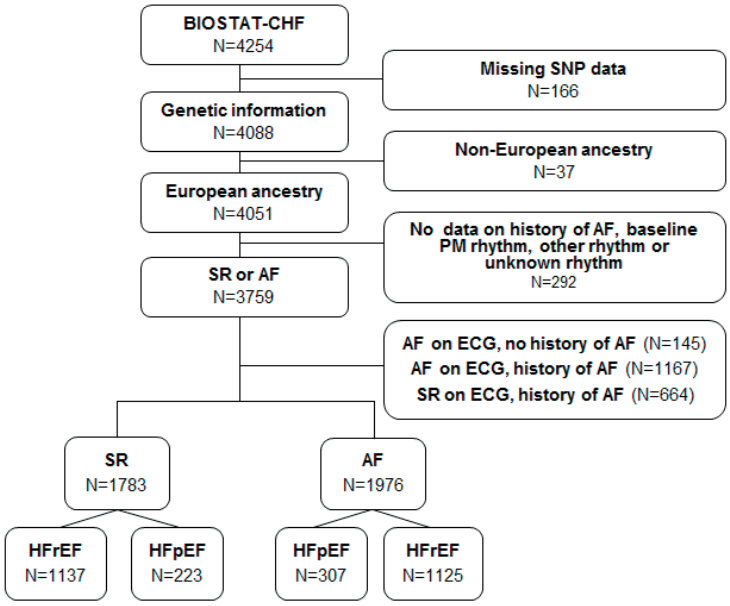

Figure 1. Flowchart
Flowchart of the BIOSTAT-CHF study population.
HFpEF denotes heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; PM, pacemaker; SNP, single nucleotide polymorphism; SR, sinus rhythm.

Genetic analysis

AF genetic risk score

Genotypes of 97 single nucleotide polymorphisms (SNPs) associated with AF risk in the latest published GWAS[13] with significance thresholds of $P<1x10^{-8}$ were used to calculate an individual patient AF genetic risk score by summing the dosage of each AF risk allele in BIOSTAT-CHF (ranging from 0-2) weighted by the natural logarithm of the relative risk for each SNP. Weights were determined by the latest AF GWAS study[13] (Supplementary Table 1). The SNP rs465276 was not available in BIOSTAT-CHF and was substituted with a proxy (rs361834, $r^2 = 0.91$, based on pairwise linkage disequilibrium from European ancestry samples in the Broad AF Study[13]). All SNPs had an INFO score >0.4 and a Hardy-Weinberg equilibrium $P>1x10^{-6}$. AF genetic risk scores were calculated using PLINK v2.00.[18]

Proportion of heritability explained

We assessed the proportion of AF phenotypic variance explained by additive genetic variation, otherwise referred to as SNP-heritability (h^2_g). h^2_g was calculated with the software BOLT-LMM v2.3.2.[19] The AF loci were defined as a region of 1 Mb (±500kb) around each of the 97 reported sentinel variants from the latest AF GWAS analysis.[13] We used the imputed genotype data, filtered the variants for imputation quality >0.8, as calculated by QCTOOL v2[20], hard-called the genotypes with a genotype probability threshold >0.9 with PLINK v2.00[18], and combined the overlapping variants that remained from the index and validation cohort of BIOSTAT-CHF. Additional filtering removed variants with MAF <1% and variant call rate missingness >0.5%. We then applied one round of pruning with the settings - indep-pairwise 50 5 0.9 in PLINK. The heritability calculation was performed on the remaining 806,130 variants. We included age, sex, and the first 5 principal components as covariates. The observed heritability estimates were converted to the liability scale following equation 17 from Lee et al.[21] and using the AF prevalence in the BIOSTAT-CHF cohorts (AF prevalence = 53%) as disease prevalence in a heart failure population.

Statistical analyses

Normally distributed variables are depicted as means ± standard deviation and non-normally distributed variables as median with the first and third quartile (q1-q3). Categorical variables are presented as numbers with percentages. Multivariable logistic regression models were used to examine whether a genetic risk score build of 97 AF genetic loci was associated with AF prevalence. Model one was adjusted for age, sex, and the first ten principal components of ancestry. Model 2 was adjusted for clinical AF risk factors from the CHARGE-AF risk model[22], a model aimed to predict future risk of AF. Variables include: age, height, weight, systolic- and diastolic blood pressure, current smoking, hypertension as a proxy for antihypertensive treatment, diabetes, myocardial infarction, and the first ten principal components of ancestry. The CHARGE-AF risk model variables heart failure and race were not included since our population consists of European ancestry patients with heart failure. 96 patients had missing values and were excluded. We calculated area under the receiver-operator curve (AUC) in logistic regression models for AF prevalence. All calculations included the first ten principal components and were performed in R using the package *pROC*[23] to calculate the AUC and the 95% confidence intervals with the DeLong method. Cox proportional hazard analysis was performed to determine hazard ratios with 95% confidence intervals (CI) for the genetic risk score and all-cause mortality. All hazard ratios were adjusted for covariates of the CHARGE-AF risk model. The Cox proportional hazards assumption was assessed by visually inspecting plots of Schoenfeld residuals against time, which showed no proportionality violation (i.e. the plots showed random patterns of residuals

against time). Interaction testing was performed to determine whether the effect of the genetic risk score differed between the heart failure phenotypes, with regard to AF prevalence and all-cause mortality risk.
Secondary analyses were performed in subgroups based on LVEF: LVEF<40% and LVEF≥50%, respectively heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). Patients with a mid-range ejection fraction (LVEF 40-49%) or missing LVEF data were not assessed separately. Analyses were performed using IBM SPSS Statistics version 23. The a priori significance threshold for all analyses was P<0.05 using 2-sided tests.

RESULTS

Patient characteristics

An overview of the cohort is shown in Table 1. A total of 3759 European ancestry individuals from BIOSTAT-CHF were included, of whom 1976 (53%) had prevalent AF. Mean age was 72.8±11.5, 30% were women. These patients were further stratified in 2262 HFrEF patients, of whom 1137 (50.3%) were in sinus rhythm and 1125 (49.7%) had AF; and 530 HFpEF patients, of whom 223 (42%) were in sinus rhythm and 307 (58%) had AF (Figure 1). Overall, patients with AF were older (75.0±10.2 versus 70.3±12.3), more often men (73% versus 67%), and had a higher BMI (28.7±5.9 versus 28.0±5.9). AF patients more often had renal disease (38% versus 29%), but less often had coronary artery disease (43% versus 54%) (all P<0.001).

Genetic risk score and AF prevalence

In the total cohort the AF genetic risk score ranged between 4.62 to 8.29 with a median of 6.37. After multivariable adjustment the odds ratio for AF presence was 2.12 per 1-unit increase in genetic risk score (95% CI 1.84-2.45, $P=2.15*10^{-24}$) in the total BIOSTAT-CHF cohort (Figure 2, Model 2). The odds ratio were 2.08 per 1-unit increase in genetic risk score (95% CI 1.72-2.50, $P=1.30*10^{-14}$) in HFrEF and 2.02 per 1-unit increase (95% CI 1.37-2.99, $P=4.37*10^{-4}$) in HFpEF, respectively.

There was no interaction between genetic risk score and heart failure type on AF prevalence (P=0.99). We estimated odds ratios comparing individuals in genetic risk score tertiles (Figure 3). The odds ratio for AF prevalence increased with higher genetic risk score categories. For the total BIOSTAT-CHF population, those in the highest tertile had 2.23 fold increased risk of AF compared to those in the lowest tertile (95% CI 1.87-2.65, $P=1.26x10^{-19}$).

Table 1. Baseline characteristics

	Overall (N=3759)	AF (N=1976 [53%])	SR (N=1783 [47%])	P-value
Demographics				
Age - years	72.8±11.5	75.0±10.2	70.3±12.3	<0.001
Women	1128 (30)	537 (27)	591 (33)	<0.001
NYHA I/II/III/IV (%)	6/43/36/7	5/46/41/8	8/47/37/8	0.001
Clinical variables				
BMI - kg/m^2	28.3±5.9	28.7±5.9	28.0±5.9	<0.001
Blood pressure - mmHg				
Systolic	125±22	124±21	127±23	0.002
Diastolic	73±14	73±14	72±13	0.01
Heart rate - bpm	78±19	80±21	75±16	<0.001
Medical history				
Coronary artery disease*	1814 (48)	856 (43)	958 (54)	<0.001
Hypertension	2295 (61)	1221 (62)	1074 (60)	0.32
Diabetes mellitus	1218 (32)	657 (33)	561 (31)	0.25
Renal disease*	1276 (34)	757 (38)	519 (29)	<0.001
Echocardiographic data				
LVEF - %	35±13	36±13	34±13	<0.001
HFrEF†	2262 (60)	1125 (57)	1137 (64)	<0.001
HFpEF‡	530 (14)	307 (16)	223 (13)	<0.001
Laboratory data				
NT-proBNP - ng/L – median (IQR)	2096 (825-4861)	2537 (1128-5122)	1588 (515-4510)	<0.001
Medications				
ACE-i/ARB	2681 (71)	1370 (69)	1311 (74)	0.005
Beta-blocker	2410 (64)	1307 (66)	1103 (62)	0.18
MRA	1670 (44)	872 (44)	798 (45)	0.37
Diuretics	3735 (99)	1960 (99)	1775 (99)	0.01

Data are depicted as number (%) or mean±SD unless stated otherwise.
* Coronary artery disease defined as: previous myocardial infarction, percutaneous coronary intervention and/or coronary artery bypass graft. Renal disease defined as estimated glomerular filtration rate <60 ml/min/1.73$m^{2.}$
† HFrEF defined as: LVEF <40%. ‡ HFpEF defined as: LVEF ≥50%.
ACE-i denotes angiotensin converting enzyme inhibitor; AF, atrial fibrillation; ARB, angiotensin receptor blocker; bpm, beats per minute; BMI, body mass index; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; IQR, interquartile range; LAD, left atrial diameter; LVEDD, left ventricular end diastolic diameter; LVESD, left ventricular end systolic diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association; SD, standard deviation; SR, sinus rhythm.

Heritability and AF prevalence classification models

AF-associated loci explain 22.9% of the overall AF SNP-heritability (h_2g) in our heart failure sample (Table 2.)

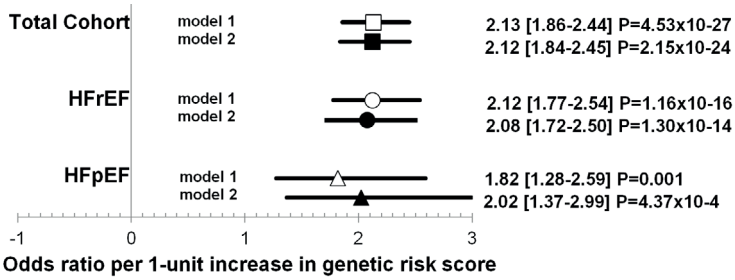

Figure 2. Genetic risk score and risk of AF prevalence
The bars signify the 95% confidence interval, the clear symbols represent results of model 1 and the solid symbols results of model 2. Squares indicate the total cohort, circles patients with HFrEF, and triangles patients with HFpEF.
Model 1: adjusted for age, sex, and first 10 principal components of ancestry.
Model 2: adjusted for age, height, weight, systolic and diastolic blood pressure, current smoking, hypertension, diabetes, myocardial infarction, and first 10 principal components of ancestry.
HFpEF denotes heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction.

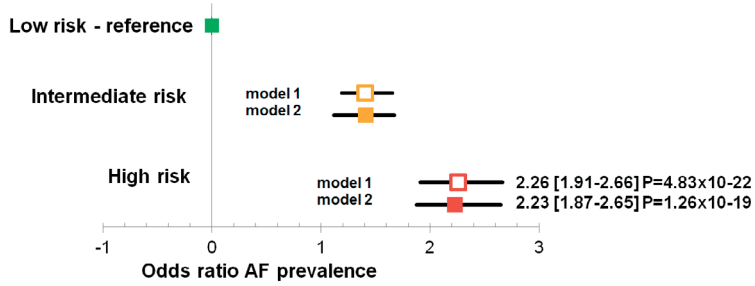

Figure 3. Increasing AF risk according to genetic risk score tertiles in the total cohort
The bars signify the 95% confidence interval, the clear symbols represent results of model 1 and the solid symbols results of model 2. Squares indicate the total cohort.
Model 1: adjusted for age, sex, and first 10 principal components of ancestry.
Model 2: adjusted for age, height, weight, systolic- and diastolic blood pressure, current smoking, hypertension, diabetes, myocardial infarction, and first 10 principal components of ancestry.

Table 2. Proportion of heritability explained by AF loci

Study	AF-loci h^2_g observed (SE)	AF-loci h^2_g liability scale (SE)	Remaining genome h^2_g observed (SE)	Remaining genome h^2_g liability scale (SE)	Overall h^2_g liability scale	Proportion explained
97 AF loci	0.0557 (0.0297)	0.0876 (0.0468)	0.1873 (0.1135)	0.2947 (0.1786)	0.3823	22.92 %

Proportion of AF-SNP heritability explained by AF loci, defined as a 1 Mb region around sentinel variants.
AF denotes atrial fibrillation; h^2_g, SNP-heritability; SE, standard error.

The CHARGE-AF risk model had an AUC of 0.699 (95% CI 0.682-0.716) for accurately classifying AF prevalence, and was better than the genetic risk score alone (AUC 0.606; 95% CI 0.588-0.624). Combining the AF genetic risk score with the CHARGE-AF risk variables led to a model with an AUC of 0.721 (95% CI 0.704-0.737), a 2.2 % increase over the CHARGE-AF risk model alone (Table 3).

Table 3. Area under the receiver-operator curves for AF risk models

Risk model	AUC (95% CI)	P-value
CHARGE-AF clinical risk score	0.699 (0.682 - 0.716)	<0.001
AF genetic risk score	0.606 (0.588 - 0.624)	<0.001
CHARGE-AF clinical risk score + AF genetic risk score	0.721 (0.704 - 0.737)	<0.001

AF denotes atrial fibrillation; AUC, area under the receiver-operator curve; CI, confidence interval; GRS, genetic risk score.

Genetic risk score and all-cause mortality

During follow-up, with a median of 656 days [inter quartile range 448-872 days], 1062 patients died (28%). In the total cohort, the genetic risk score was not associated with an increased risk for all-cause mortality after multivariable adjustment (hazard ratio (HR) 0.93, 95% CI 0.82-1.05, P=0.22). Similar results were observed for the HFrEF (HR 0.92, 95% CI 0.78-1.08, P=0.31) and HFpEF (HR 1.12, 95% CI 0.85-1.48, P=0.44) subgroups. There was no interaction between heart failure subgroup and the genetic risk score on outcome (P=0.63)

DISCUSSION

In 3759 heart failure patients of European ancestry, an AF genetic risk score, based on lead SNPs at 97 AF loci, was associated with a higher prevalence of AF after adjustment for clinical AF risk variables from the CHARGE-AF risk model. We observed that 22.9% of variance in AF risk was attributable to additive genetic variation. Furthermore, addition of the AF genetic risk score to clinical risk factors improved risk model performance in classifying AF prevalence. The AF genetic risk score was not associated with all-cause mortality. Our findings support and extend the prior observation that there is, at least, a partial genetic basis for AF in patients with HFrEF and HFpEF.[12]

Genetic basis for AF in heart failure patients

AF and heart failure frequently co-exist, but direct causality has not been unequivocally proven. Additionally, the underlying mechanisms that lead to the development of AF in heart failure with reduced and preserved ejection fraction and vice versa remain

complex and not completely understood. Previously the *ZFHX3* gene was found to be associated with AF presence in a heart failure population.[12] Our comprehensive AF genetic risk score of 97 SNPs, together with the estimation that 22.9% of the phenotypic variance is explained by additive genetic variation, provide evidence of a substantial contribution of genome-wide variation to AF susceptibility in heart failure patients.

The genetic contribution to AF in our heart failure sample is less than what was previously observed in population based- and case-referent AF-GWAS studies which also included a proportion of patients with heart failure (approximately 23% versus 42%). Part of this portion of missing heritability may be caused by unidentified common genetic variants. Gene-environment interactions may also play a role, as genetic variants can also have associations with risk factors [pleiotropic effects]. Heart failure patients have many risk factors including age, hypertension, diabetes, obesity, as well as valvular, ischemic and non-ischemic structural heart disease.[10,11] On the other hand increased cardiac filling pressures and consequently atrial stretch, cardiac fibrosis, dysregulation of intracellular calcium, and autonomic and neuroendocrine dysfunction in the setting of heart failure may evoke atrial fibrillation. It is possible that in the context of heart failure, with several concomitant risk factors, genetics may play a smaller role than in the general population.

It is hypothesized that AF in the presence of HFrEF is a marker of more advanced cardiac disease, with ventricular function deterioration and increased neurohormonal activation, while patients with AF and HFpEF share a more underlying substrate, albeit heterogeneous, with many shared risk factors.[10,11] A difference in the genetic contribution to AF in HFrEF or HFpEF is not evident from current results as no interaction between genetic risk score and heart failure type was observed.

AF genetic risk score and all-cause mortality

Previous analyses in BIOSTAT have shown that worse cardiovascular outcomes were seen in heart failure patients with AF compared to SR.[24] Nevertheless, after multivariable adjustment, the AF genetic risk score was not associated with all-cause mortality. But a genetic risk score alone does not capture the clinical significance of AF presence in patients with an extensive cardiac substrate and other underlying risk factors. Additionally, current observations may be affected by survival bias.

Implications

The clinical risk factor model alone outperformed the genetic risk score, this is to be expected since compared to clinical risk factors the effect size of genetic variants is small, even when combined in a polygenic risk score. Although the genetic risk score

had moderate discriminatory accuracy, we demonstrated that a combined risk model, consisting of the AF genetic risk score with clinical AF risk factors as present in the CHARGE-AF risk model, performed better than either risk model alone. But statistical significance does not automatically translate into clinical significance, and currently translation of genetics into clinical practice remains unresolved.

In the future genetic profiling may provide insight into the mechanisms that underlie why some patients develop AF and others do not. The individual SNPs implicate genes that may reveal some of the mechanisms underlying AF (Supplementary Figure 1).[13] Currently most genes represent gene candidates at the loci, while the causal gene remains unknown. Experimental observations illustrate the pleiotropic nature of genes that are associated with this challenging arrhythmia and underscore the complexity of AF: so does *PITX2* encodes a transcription factor that plays a role in the formation of the pulmonary vein myocardium[25], does *TBX5* encodes transcription factors that are required for patterning and maturing of the cardiac conduction system in mice[26] and have *KCNN3* and *SCN5A*, which both encode subunits of the potassium channel complex, been previously been linked to AF through candidate gene analyses and family-based studies.[27] More insights into the functional consequences of SNPS and genes is critical to identify potential therapeutic targets for this major health burden.[28] However, whether the genetic proportion to AF risk has a meaningful contribution to clinical risk assessment warrants further investigation.

Limitations

Current results, based on genetic data of 97 SNPS in 3759 patients from a well-defined heart failure cohort, point towards a genetic basis for AF in the context of heart failure. Analyses were limited to European ancestry individuals, and the current heart failure sample had a higher percentage of men with only 30% women, and a higher percentage of HFrEF than is typical in the community; the findings may not be completely generalizable to individuals of different ancestral backgrounds, regions, or the general heart failure population. Additionally, women and men generally have a different risk factor burden, which next to genetics and the underlying heart failure substrate, may be of different importance in the presence of concomitant heart failure and prevalent AF. Second, the genetic risk models were linear in nature with a single predictor variable and did not account for potential non-additive genetic effects, interactions between genetic variants, or interactions between genetic variants and environmental factors. Therefore, all observations are vulnerable to the risk of residual confounding that may bias mentioned estimates. Thirdly, AF ascertainment was partially based on physician reported AF. This means that the percentage of AF is likely an underestimation since subclinical AF may have gone undetected. Fourthly, whether heart failure developed before the onset of AF,

or AF before the onset of heart failure may be associated with a different genetic risk. Also the sequence in which the diseases develop can impact outcome. Unfortunately, we did not have information on the onset of AF and heart failure; therefore a temporal sequence of diagnoses was unknown, prohibiting time-dependent analyses. AF occurrence during follow-up was not systematically collected and therefore current analyses focus on baseline AF prevalence. Additionally, there was a lack of data on type and duration of AF, as well as applied therapies for AF. Fifthly, electro- and echocardiographic variables such as left atrial volume were omitted from the models since they were not available in a large proportion of patients. Additionally, these biomarkers will be influenced by both the underlying heart failure substrate as well as AF presence, duration, and severity. Covariates including LVEF, NYHA class and NT-proBNP will be confounded by AF itself as it inhibits adequate echocardiographic determination of the ejection fraction, is associated with symptoms of dyspnoea, and will lead to an increase in NT-proBNP levels. In line with the previous limitation we did not adjust for HF severity in the multivariable models. We acknowledge that the CHARGE-AF model application in heart failure was not ideal, albeit the best validated AF risk score. Sixthly, in determining SNP-heritability we assessed variants with MAF ≥1%, and, therefore, the contribution of rare or loss of function variants to total AF variance was not assessed. Furthermore, the estimates for SNP-heritability have large standard errors bringing a level of uncertainty to these estimates. Seventhly, we cannot attribute the AF risk variance to functional categories. It remains challenging to identify the causal gene at each locus since the AF associated SNPs predominantly fall within noncoding portions of the genome. Additionally, the association of genes to functional groups is based on their affiliation to enriched gene sets that were identified in an *in silico* analysis. Lastly, establishing a heart failure cohort of sufficient size is complex, and the current study is underpowered to study individual SNPs or perform extensive sub-group analyses. Larger studies, powered for outcomes, are warranted to investigate the genetic contribution to incident AF in heart failure populations, both HFrEF and HFpEF. Further efforts are needed to uncover the functional consequence of SNPs and genes at each locus on AF risk in patients with incident heart failure.

CONCLUSION

The AF genetic risk score was associated with increased AF prevalence in heart failure patients with reduced and preserved ejection fraction. Genetic variation accounted for 22.9% overall AF SNP-heritability. Addition of the AF genetic risk score to clinical risk factors improved risk model performance in classifying AF prevalence. Efforts are warranted to consider the role and mechanisms of genetic susceptibility of AF risk in heart failure patients.

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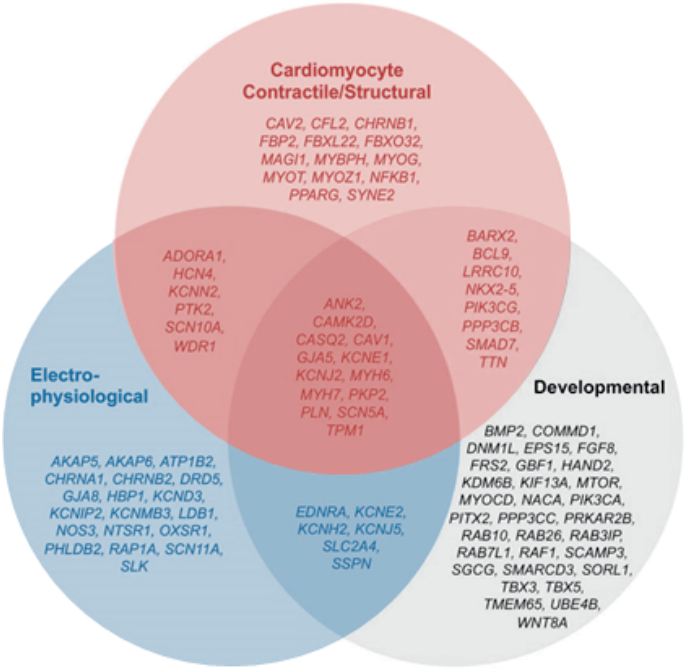
SUPPLEMENTARY MATERIAL



Supplementary Figure 1. Venn diagram
The Venn diagram shows overlap between the gene(s) closest to the 97 SNPs and functional categories based on their corresponding gene sets. Genes were manually assigned to one or more functional groups based on their affiliation to gene sets. Adapted from Roselli et al. *Nat Genetics* 2018 with permission.

Supplementary Table 1. SNPs and weights used in the AF genetic risk score

Rsid	Chr	BP (hg19)	Risk allele	Weight
rs187585530	1	10167425	A	0.4390
rs880315	1	10796866	C	0.0437
rs146518726	1	51535039	A	0.1617
rs12044963	1	112392360	T	0.0795
rs4484922	1	116310818	G	0.0630
rs79187193	1	147255831	G	0.1116
rs11264280	1	154862952	T	0.1270
rs72700114	1	170193825	C	0.2026
rs608930	1	170617306	G	0.0968
rs10753933	1	203026214	T	0.0743
rs4951261	1	205717823	C	0.0441
rs6546620	2	26159940	C	0.0708
rs6742276	2	61768745	A	0.0485
rs2540949	2	65284231	A	0.0752
rs10165883	2	70117015	C	0.0642
rs72926475	2	86594487	G	0.0708
rs56181519	2	175555714	C	0.0778
rs35504893	2	179421294	T	0.0900
rs295114	2	201195602	C	0.0676
rs6810325	3	12840934	C	0.0747
rs6790396	3	38771925	G	0.0636
rs2306272	3	66434643	C	0.0512
rs7632427	3	89534377	T	0.0460
rs17490701	3	111587879	G	0.0700
rs4855075	3	179170494	T	0.0604
rs3822259	4	10118745	T	0.0463
rs3960788	4	103915618	C	0.0507
rs2129977	4	111712432	A	0.4016
rs55754224	4	114428714	T	0.0477
rs10213171	4	148937537	G	0.1041
rs10520260	4	174447349	A	0.0539
rs716845	5	113736416	A	0.0594
rs34750263	5	137434172	T	0.0873
rs174048	5	142650404	C	0.0665
rs6882776	5	172664163	G	0.0600
rs73366713	6	16415751	G	0.1052
rs34969716	6	18210109	A	0.0875
rs3176326	6	36647289	G	0.0599
rs17079881	6	118566187	G	0.0851
rs13191450	6	122392136	A	0.0704

Supplementary Table 1. SNPs and weights used in the AF genetic risk score (continued)

Rsid	Chr	BP (hg19)	Risk Allele	Weight
rs117984853	6	149399100	T	0.1132
rs55734480	7	14372009	A	0.0504
rs6462078	7	28413187	A	0.0580
rs74910854	7	74110705	G	0.0942
rs11773884	7	92285123	A	0.0486
rs62483627	7	106856002	A	0.0489
rs11773845	7	116191301	A	0.1162
rs7789146	7	150661409	G	0.0571
rs7508	8	17913970	A	0.0720
rs7846485	8	21803735	C	0.0872
rs62521286	8	124551975	G	0.1224
rs35006907	8	125859817	A	0.0454
rs6993266	8	141762659	A	0.0443
rs4977397	9	20235004	A	0.0432
rs4385527	9	97648587	A	0.0920
rs4743034	9	109632353	A	0.0490
rs10760361	9	127178266	G	0.0434
rs7919685	10	65315800	G	0.0579
rs60212594	10	75414344	G	0.1097
rs11001667	10	77935345	G	0.0619
rs1044258	10	103605714	T	0.0463
rs11598047	10	105342672	G	0.1533
rs1822273	11	20010513	G	0.0683
rs949078	11	121629007	C	0.0534
rs76097649	11	128764570	A	0.1264
rs10842383	12	24771967	C	0.1088
rs113819537	12	26348429	C	0.0490
rs12809354	12	32978437	C	0.0810
rs7978685	12	57103154	T	0.0547
rs35349325	12	70097464	T	0.0524
rs11180703	12	76223817	G	0.0457
rs883079	12	114793240	T	0.1196
rs12810346	12	115091017	T	0.0658
rs12298484	12	124418674	C	0.0455
rs9580438	13	23373406	C	0.0568
rs28631169	14	23888183	T	0.0700
rs2145587	14	32981484	A	0.0754
rs73241997	14	35173775	T	0.0720
rs2738413	14	64679960	A	0.0807
rs10873299	14	77426711	A	0.0483

Supplementary Table 1. SNPs and weights used in the AF genetic risk score (continued)

Rsid	Chr	BP (hg19)	Risk Allele	Weight
rs62011291	15	63800013	G	0.0519
rs12591736	15	70454139	G	0.0606
rs74022964	15	73677264	T	0.1059
rs12908004	15	80676925	G	0.0753
rs12908437	15	99287375	T	0.0468
rs2286466	16	2014283	G	0.0718
rs2359171	16	73053022	A	0.1884
rs8073937	17	7435040	G	0.0504
rs72811294	17	12618680	G	0.0667
rs242557	17	44019712	G	0.0439
rs76774446	17	45046368	A	0.0654
rs7219869	17	68337185	G	0.0460
rs9953366	18	46474192	C	0.0504
rs2145274	20	6572014	A	0.1015
rs7269123	20	61157939	C	0.0443
rs2834618	21	36119111	T	0.1096
rs361834*	22	18597404	G	0.0470

* proxy for rs465276 (r^2=0.91)