Original Article

Heritability of Atrial Fibrillation

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Background—Previous reports have implicated multiple genetic loci associated with AF, but the contributions of genome-wide variation to AF susceptibility have not been quantified.

Methods and Results—We assessed the contribution of genome-wide single-nucleotide polymorphism variation to AF risk (single-nucleotide polymorphism heritability, h^2_g) using data from 120 286 unrelated individuals of European ancestry (2987 with AF) in the population-based UK Biobank. We ascertained AF based on self-report, medical record billing codes, procedure codes, and death records. We estimated h^2_g using a variance components method with variants having a minor allele frequency ≥1%. We evaluated h^2_g in age, sex, and genomic strata of interest. The h^2_g for AF was 22.1% (95% confidence interval, 15.6%–28.5%) and was similar for early- versus older-onset AF (≤65 versus >65 years of age), as well as for men and women. The proportion of AF variance explained by genetic variation was mainly accounted for by common (minor allele frequency, ≥5%) variants (20.4%; 95% confidence interval, 15.1%–25.6%). Only 6.4% (95% confidence interval, 5.1%–7.7%) of AF variance was attributed to variation within known AF susceptibility, cardiac arrhythmia, and cardiomyopathy gene regions.

Conclusions—Genetic variation contributes substantially to AF risk. The risk for AF conferred by genomic variation is similar to that observed for several other cardiovascular diseases. Established AF loci only explain a moderate proportion of disease risk, suggesting that further genetic discovery, with an emphasis on common variation, is warranted to understand the causal genetic basis of AF. **(*Circ Cardiovasc Genet*. 2017;10:e001838. DOI: 10.1161/CIRCGENETICS.117.001838.)**

Key Words: atrial fibrillation ■ epidemiology ■ genome-wide association study ■ genomics ■ medical records

AF affects ≈34 million individuals worldwide[1] and imposes a major public health burden.[2] Epidemiological data underscore the heritable nature of AF.[3–8] Both traditional genetic mapping approaches and genome-wide association studies (GWAS) have identified numerous loci implicated in the pathogenesis of AF.[9–12] Despite progress identifying susceptibility loci for AF, the aggregate contributions of genetic variation to AF susceptibility remain unclear.

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Because complex phenotypes may be caused by the aggregate effects of thousands of variants that are not associated with disease at genome-wide significance levels,[13,14] several approaches have been developed to assess the total contributions of genome-wide variation to disease susceptibility.[15–18] Such methods can provide estimates of the proportion of phenotypic variance explained by additive genetic variation, otherwise referred to as narrow-sense single-nucleotide polymorphism (SNP) heritability (h^2_g). Measurements of h^2_g estimated in unrelated subjects can minimize the potential inflation of traditional heritability estimates caused by shared familial environmental factors. Moreover, such methods enable partitioning of the contributions of variation in specific genomic regions, and according to variant characteristics and annotations, to disease susceptibility.[17,18] Currently, the contribution of genome-wide variation to AF susceptibility is not well understood.

We, therefore, sought to assess the genetic architecture of AF by using genome-wide imputation data from the population-based UK Biobank, leveraging recently released data for >120 000 individuals of predominantly European ancestry. We performed a GWAS of AF in the participants and specifically

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estimated h^2_g stratified by age of AF onset, sex, allele frequency, and variation within previously implicated AF susceptibility gene regions.

Methods

Study Participants

The UK Biobank is a prospective, population-based cohort study, which enrolled >500 000 individuals aged 40 to 69 from the United Kingdom. Participants were invited to 1 of the 22 centers in the United Kingdom between 2006 and 2010 (the initial assessment visit). Blood, urine, and saliva samples, information from questionnaires on health and lifestyle, and physical measurements were collected. In the current study, we assessed 152 249 participants with available genome-wide genotyping information from an interim data release. Individuals of non-European ancestry and close relatives were removed. Unrelated individuals were chosen by omitting first-, second-, and third-degree relatives with an empirical kinship coefficient >0.044 as estimated and defined by the KING software performed by UK Biobank.[19] Individuals of European ancestry were identified from a principal component analysis of 40 538 common variants with low level of linkage disequilibrium followed by the application of an outlier detection algorithm[20] to the 2 leading principal components performed by UK Biobank as well. All participants provided written informed consent to participate in research as described previously,[21] and the UK Biobank was approved by the UK Biobank Research Ethics Committee (reference number 11/NW/0382). Use of UK Biobank data was approved by the local Partners Healthcare Institutional Review Board.

AF Ascertainment

We defined AF based on data ascertained from baseline interviews, procedure codes, billing codes, and death records. A detailed description of the AF definition we derived and used is provided in Table I in the Data Supplement. Briefly, we classified participants as having AF if they reported a history of AF, atrial flutter, or had undergone cardioversion, atrioventricular node ablation, pulmonary vein isolation procedure, atrial flutter ablation, or had received an *International Classification of Diseases* (*ICD*) billing code of 427.3 (*ICD-9*) or I48 (*ICD-10*).

Genotyping, Imputation, and Variant Selection

Two thirds of the samples in the interim UK Biobank data release were genotyped on the UK Biobank Axiom array. About 50 000 samples were genotyped by the UK BiLEVE array. Both the UK Biobank Axiom array and the UK BiLEVE array include ≈800 000 SNPs, with >95% marker overlap. All genotyping was performed at Affymetrix Research Services laboratory in Santa Clara, CA.

For different arrays, different quality control filters were applied to select variants for imputation. Genotypes were prephased to estimate the underlying haplotype of each individual by SHAPEIT-3, and the haplotype estimates were then used for imputation to infer unobserved genotypes using IMPUTE2, with the 1000 Genomes Phase 3 and UK10K reference panels. Phasing and imputation were centrally performed at the Wellcome Trust Center for Human Genetics in Oxford. Additionally, the first 15 principal components of ancestry were estimated by flashPCA.[22] Detailed descriptions of the genotyping, imputation procedures, and principal component calculation can be found on the UK Biobank website (http://www.ukbiobank.ac.uk/).[23,24]

All analyses were performed using the imputed data set restricted to variants of high imputation quality (info ≥0.4) with low missingness rates (<5%), minor allele frequency (MAF) of at least 1%, and high genotype imputation probability (≥0.9) across at least 90% of samples. Variants that passed these quality control criteria were transformed to hard-called genotypes in PLINK, version 1.90b,[25] using a probability threshold ≥0.9. In total, 8 325 606 genetic variants (SNPs and indels) with MAF ≥1% and low missingness rate (<5% in hard-called genotypes) were retained for association analysis.

To estimate h^2_g, additional quality control was applied as described previously.[26] Specifically, we removed variants with a missingness rate of ≥0.5%, MAF <1%, and variants with differential missingness between cases and referents (P value, <0.05). To reduce suspicious linkage disequilibrium bias from REML estimation[15,27–29] and because of computational capacity of the software, we further performed 2 rounds of linkage disequilibrium pruning at r^2=0.9 (PLINK 1.90b;[25] ie, using a –indep-pairwise 50 5 0.9 flag) and decreased the total number of variants to 811 488 (with a small fraction of biallelic indels).

Statistical Analysis

To validate the definition of AF we developed, we performed a GWAS of AF in the UK Biobank and then compared the associations of top variants to a prior independent analysis from the AFGen Consortium.[11] The prior analysis included a GWAS in ≈18 000 individuals with AF and >100 000 without, as well as an exome-chip association analysis comprised ≈22 000 individuals with AF and 150 000 without. In total, 25 independent loci were identified in this analysis (23 in the GWAS and an additional 2 in the exome-chip analysis). To perform the GWAS in the UK Biobank, each variant was tested for association with AF using logistic regression assuming an additive genetic model. We fit models adjusted for baseline age, sex, and 1 principal component of ancestry that was associated with AF. To account for array differences, we also adjusted for array type in all analyses.

We then examined associations between the top variants at the 25 established AF susceptibility loci from the AFGen analysis with those of the present analysis in the UK Biobank for concordance. Correlation between the log-odds ratios for the top variants tagging the 25 loci in the prior analysis and the present analysis in the UK Biobank was assessed using Spearman ρ. We created a weighted linear genetic risk score for each individual in the UK Biobank using the 25 top AF variants. To create the score, we summed the product of the AF risk allele count (ie, 0, 1, or 2) for the variant multiplied by the log-relative risk for the respective variant, which we derived from the prior analysis as implemented in PLINK 1.90b using the score flag (Table II in the Data Supplement). If the hard-called variant was missing, PLINK used the mean genotype frequency in the sample instead. Thus, for each individual, we obtained a single continuous variable representing AF genetic risk, which we used in subsequent analyses.[25] Scores were treated as continuous variables and tested for association with AF using multivariable logistic regression based on a 2-sided P value <0.05, adjusted for age, sex, array, and 1 principal component of ancestry as above. For the remainder of the GWAS, we considered loci to be significantly associated with AF if a variant was associated with AF with a P value $<5\times10^{-8}$.

We calculated h^2_g based on linkage disequilibrium-pruned markers using BOLT-REML as specified previously.[15] In brief, BOLT-REML uses an efficient implementation of restricted maximum likelihood to estimate the genetic and residual variance components for a given phenotype using genetic data. In the analysis, we adjusted for baseline age, sex, array, and 1 AF-related principal component of ancestry. We converted the estimate on the observed scale into a liability scale[26] by setting the disease prevalence as the observed proportion of AF cases in the UK Biobank sample. For all h^2_g estimates, we provided the 95% confidence intervals (CI).

Given the association between age and AF risk, we estimated h^2_g stratified by early- versus older-onset AF status to determine whether h^2_g varies by age. In the early-onset AF analyses, we included individuals in whom the last follow-up or AF onset occurred ≤65 years of age. In the older-onset AF analyses, we included individuals in whom the last follow-up or AF onset was >65 years of age. We also stratified h^2_g estimates by sex given the increased risk of AF among men, to assess whether additive genetic variation contributes to disease risk differentially in each stratum. Additionally, we computed estimates for low-frequency variants (MAF between 1% and 5%), and common variants (MAF, ≥5%), separately to investigate the contribution of variant frequency to the genetic architecture of AF.

To determine the extent to which previously identified AF susceptibility loci contributed to disease risk, we further assessed h^2_g

stratified by variation at established GWAS loci for AF alone, with additional putative AF susceptibility genes and with an expanded panel of AF, cardiac arrhythmia, and cardiomyopathy genes given the associations between such conditions and AF. We defined established AF GWAS loci as the top SNP±500 kb for each of 25 loci identified in a prior independent GWAS[11] (Table II in the Data Supplement). We defined putative AF susceptibility genes (n=37) as those implicated through prior linkage, candidate gene sequencing, or large-scale association testing reports (Table III in the Data Supplement) as described previously.[30] The expanded panel of AF, cardiac arrhythmia, and cardiomyopathy genes (n=82) included all previously implicated putative AF genes, in addition to nonoverlapping clinically established cardiac arrhythmia and cardiomyopathy genes (n=53; Table IV in the Data Supplement). For the AF and expanded cardiac arrhythmia and cardiomyopathy gene sets, we included variants within 5 kb upstream and downstream of each included gene, to capture potential regulatory variation related to each gene. We then partitioned the variance in AF risk explained by genetic variation in these regions. For all stratified analyses of h^2_g, we used the observed prevalence of AF within each stratum to convert the measurement from the observed to the liability scale.

Statistical analyses were performed using PLINK, version 1.90b,[25] R 3.2.1, and BOLT-REML.[15] Manhattan plots and regional association plots were created using R (qqman)[31] and LocusZoom,[32] respectively.

Results

In total, 120 286 unrelated individuals of European ancestry were included in our analysis, of whom 2987 fulfilled our definition for AF. Individuals with AF were older than individuals without AF, and a greater proportion were men, in keeping with well-established epidemiological observations (Table).[33]

Genetic Associations With AF

We tested ≈8.5 million variants that passed quality control filters for association with AF. No substantive genomic inflation was observed ($\hat{\lambda}$=1.026; Figure I in the Data Supplement) with additional principal components of ancestry. The results of the GWAS for AF are displayed in Figure 1. Seven loci exceeded the genome-wide significance threshold, 5 of which were known previously and 2 of which have not been reported previously (Figure 2; Table V in the Data Supplement). Of the 2 loci not reported previously, 1 was located on chromosome 5q31 (rs31209; ≈3 kb downstream of *PITX1*; *P* value, 1.7×10^{-8}). SNP rs31209 is also an expression quantitative trait loci for *PITX1* with reduced expression associated with the protective T allele in testis (*P* value, 2.7×10^{-12}).[34] The second locus was identified on chromosome 12p12 (rs117640426; upstream of *RASSF8*; *P* value, 8.7×10^{-9}; Table V in the Data Supplement). Given the relatively low frequency of the top variant at the *RASSF8* locus, we repeated the analysis stratified by the genotyping array used to minimize the possibility of confounding by an unrecognized batch effect. We observed concordant directions of allelic effect and nominally significant associations between rs117640426 and AF, although the effect sizes differed between the 2 arrays (Table VI in the Data Supplement).

Twenty of 25 variants tagging established AF susceptibility loci from a prior independent analysis[11] were associated with AF in the UK Biobank with a *P* value <0.05 (Figure 1). The log-odds ratios for concordant risk alleles from the current analysis were highly correlated with the log-relative risks from the prior analysis[11] (ρ=0.904; *P* value, 5.7×10^{-10}). When the 25 established AF variants were combined using a weighted genetic risk score approach and tested for association with AF in the UK Biobank, a significant association was observed (*P* value, 1.4×10^{-112}; Table II in the Data Supplement). Based on our results, the AF definition we used to ascertain AF in the UK Biobank was considered valid for further analysis.

Table. Characteristics of the 120 286 Subjects Included in the Analysis

	AF Cases	Referents
N	2987	117299
Baseline age, y	62.3 (5.9)	56.8 (7.9)
Women, %	31	53
Systolic blood pressure, mm Hg	140 (19)	138 (19)
Diastolic blood pressure, mm Hg	82 (11)	82 (10)
Body mass index at baseline, kg/m^2	29.1 (5.5)	27.5 (4.8)
Hypertension, %	50	46
UK BiLEVE array, %	39	34

Data shown as mean (SD), unless otherwise indicated. AF indicates atrial fibrillation.

SNP Heritability of AF

We estimated the overall h^2_g of AF attributable to additive common and low-frequency genetic variation to be 22.1% (95% CI, 15.6%–28.5%; Figure 3). The h^2_g estimation was similar for early-onset AF (22.8%; 95% CI, 13.9%–31.7%) and older-onset AF (24.2%; 95% CI, 2.5%–45.9%). The h^2_g estimates were similar for both sexes (21.0%; 95% CI, 3.6%–38.4%, in women versus 22.1%; 95% CI, 11.7%–32.4%, in men). We observed that common variants (MAF, ≥5%) explained 20.4% (95% CI, 15.1%–25.6%) of AF variance, whereas low-frequency variants (MAF, 1%–5%) provided minimal contribution to AF susceptibility.

In aggregate, the 25 known AF susceptibility loci from a prior GWAS explained 5.3% (95% CI, 4.2%–6.5%) of variance in AF risk, corresponding to nearly one fourth of the AF h^2_g. Collectively, the 23 known loci and additional 37 putative AF susceptibility genes explained only 5.4% (95% CI, 4.3%–6.6%) of total AF variance, whereas the additional inclusion of cardiac arrhythmia and cardiomyopathy gene regions (total n, 82 genes) explained a total of 6.4% (95% CI, 5.1%–7.7%) of AF variance. In aggregate, ≈29% (=6.4%/22.1%) of AF h^2_g was explained by accounting for all known AF loci, putative cardiac arrhythmia, and cardiomyopathy gene regions.

Discussion

In our analysis of 120 286 individuals of European ancestry from the population-based UK Biobank, common and low-frequency genetic variation accounted for 22.1% of variance in AF risk. The proportion of variance in AF risk was similar for early-onset and for older-onset AF and between men and women. Nearly all the observed variance in AF risk explained by genetic variation was attributed to common variants. Established AF susceptibility loci accounted for only one fourth of the AF h^2_g, indicating that a substantial proportion of AF risk is driven by variation in regions that have not exceeded genome-wide significance in analyses to date.

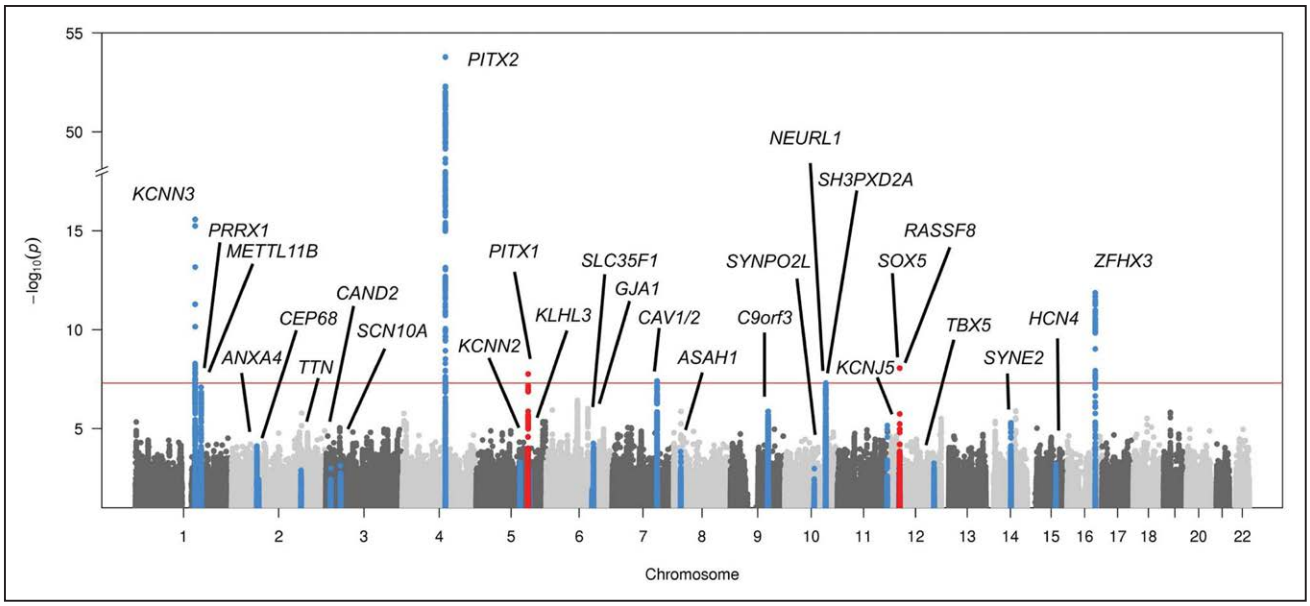

Figure 1. Manhattan plot demonstrating associations between tested variants and atrial fibrillation (AF). Known AF loci are shown in blue, and loci uniquely associated with AF in the UK Biobank are shown in red.

Our population-based study supports and extends prior observations of AF heritability by providing quantitative and genetically determined estimates. In the Framingham Heart Study, individuals with an affected first-degree relative had a ≈40% increased hazard after accounting for clinical risk factors for disease.[6] Similarly, an Icelandic study indicated that the relative risk of AF declined from 1.77 with an affected first-degree relative to 1.05 with an affected fifth-degree relative.[3] A Danish twin study estimated the heritability of AF to be as high as 62%,[4] although overestimation of heritability can occur in family-based studies.[35] Our approach limits the potential confounding by shared environmental factors and provides an unbiased estimate of additive genetic contribution to AF variance from common genetic variants.

Our findings have 3 major implications. First, the fact that about one fifth of variance in AF risk was explained by additive genetic variation underscores the substantial contribution of genome-wide variation to AF susceptibility. Common genetic variation accounted for most AF genetic risk, highlighting the complex polygenic architecture of AF. The complex genetic architecture of AF contrasts with some other inherited arrhythmia syndromes, such as long QT syndrome and catecholaminergic polymorphic ventricular tachycardia, which are often driven by rare and penetrant mutations.[36] Our findings also indicate that variation at known AF susceptibility loci, as well as at putative arrhythmia and cardiomyopathy gene regions, accounts for only a modest proportion of AF risk observed in the community, thereby justifying future genetic discovery efforts in larger samples.

Second, the h^2_g of AF we observed is similar to that of other complex cardiovascular traits, including hypertension, dyslipidemia, and type 2 diabetes mellitus, which range from

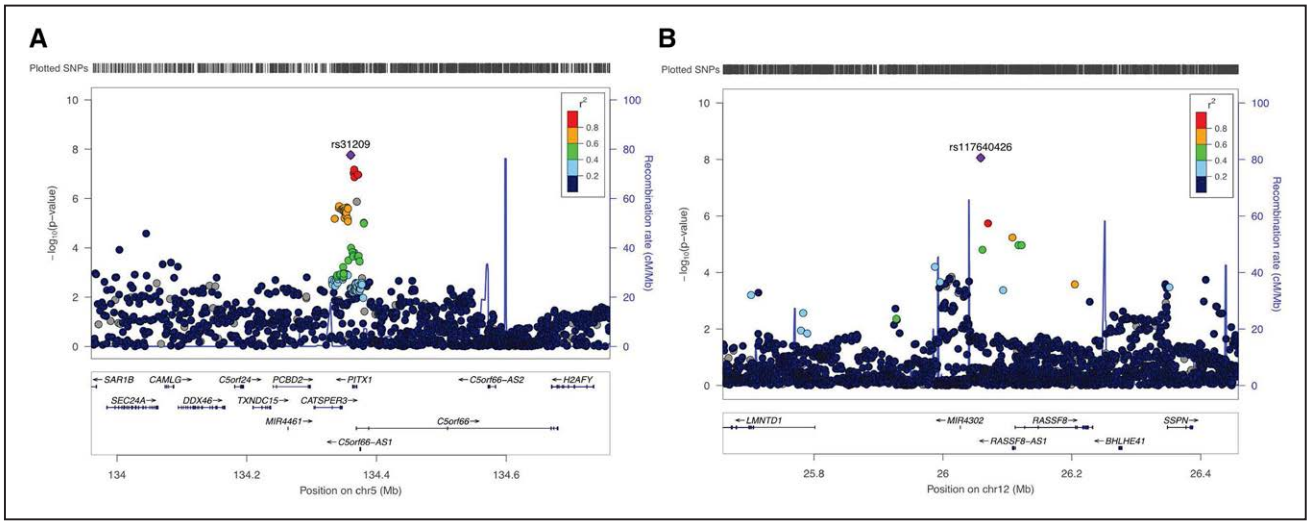

Figure 2. Regional association plots for loci uniquely associated with atrial fibrillation in the UK Biobank. **A**, associations at chromosome 5q31 and (**B**) at 12p12. SNP indicates single-nucleotide polymorphism.

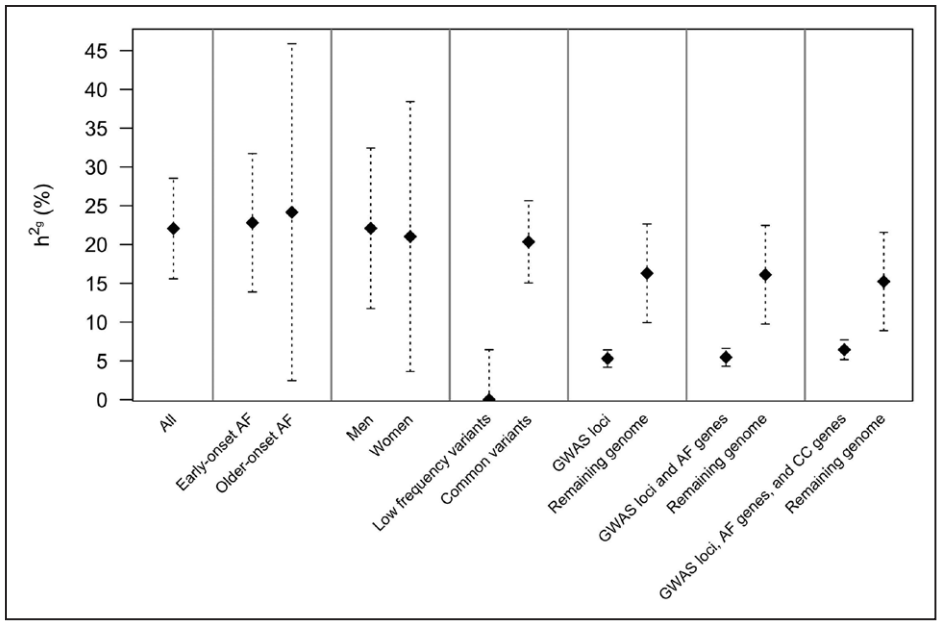

Figure 3. SNP heritability of atrial fibrillation (AF) in clinical and genomic strata. Error bars represent the 95% confidence intervals for the h^2_g estimates. Early-onset AF was defined as that occurring on or before age 65, and older-onset AF as occurring on or after age 65. Low-frequency variants were SNPs with minor allele frequencies between 1% and 5% and common variants with frequencies ≥5%. Genome-wide association studies (GWAS) loci were comprised of the 23 top SNPs from a prior independent association study±500 kb. AF genes included 37 putative AF susceptibility genes±5 kb. AF, cardiac arrhythmia, and cardiomyopathy genes included 82 putative susceptibility genes±5 kb. The numbers of individuals and variants included in each stratum are provided in Table VII in the Data Supplement.

26% to 30%.[15] A prior effort in a smaller subset of the UK Biobank demonstrated a heritability of AF of 11.3% using a definition of AF based on a single *ICD-10* code.[37] In contrast, our comprehensive AF definition included self-report, *ICD-9* and *ICD-10* codes, and AF-related procedures, such as catheter ablation, which likely minimized misclassification of AF cases as referents. Genetic risk for AF has previously been associated with a variety of clinical outcomes, including incident AF,[38,39] ischemic stroke,[39,40] and variably with catheter ablation success,[41] although any potential clinical application of genetic risk information may be highly context dependent. Whether the magnitude of AF risk explained by genome-wide variation, or perhaps family history of AF, will contribute meaningfully to clinical risk assessment warrants further evaluation.

Third, we identified 2 loci associated with AF risk, although neither was associated with AF in the prior independent meta-analysis of ≈18 000 individuals with and >100 000 without AF (Table V in the Data Supplement),[11] which may warrant further evaluation. The first locus was ≈3 kb downstream of *PITX1* at chromosome 5q31. *PITX1* belongs to the same *RIEG/PITX* homeobox family as a known AF susceptibility gene, *PITX2*. *PITX2* is involved in specifying pulmonary venous myocardial sleeves, suppression of a default left-atrial sinus node, and conditional knockout of the *Pitx2c* transcript has been linked to increased AF susceptibility in mice.[42–44] Activation of *PITX1* was suggested to be associated with tumor suppression in various cancers,[45,46] but the function of *PITX1* in cardiac function has not been well-understood. The second novel locus is at 53 kb upstream of *RASSF8,* encoding a candidate tumor suppressor protein,[47] but the association of *RASSF8* and cardiac physiology remains unclear. These variants may represent spurious associations or, alternatively, may be sample-specific associations. Future examination with the upcoming release of the full 500 000-person UK Biobank data set will help clarify the validity of these loci.

Our study should be interpreted in the context of the study design. First, our study is comprised of individuals of European ancestry, so the findings may not be generalizable to other ancestral groups. Genetic association studies in diverse populations are warranted. Second, we did not observe a difference in h^2_g according to age, in contrast to epidemiological evidence supporting greater heritability for early-onset AF.[6] It is likely that older onset forms of AF are less heritable than earlier onset forms,[6] although our analysis was underpowered to precisely quantify h^2_g values in the older age stratum. Larger samples will be necessary to fully address the contributions of genetic variation across a broad spectrum of ages. Our power calculations[48,49] indicated that the minimum h^2_g that could be detected with 80% power in the older-onset group was 18.9%, in contrast to 5.3% in the overall sample (Table VIII in the Data Supplement). Larger analyses are warranted to quantify the relative contributions of genetic variation to AF risk in different age and important clinical risk factor strata. Third, potential misclassification of some referent individuals may have created a negative bias in the h^2_g estimates we observed, particularly because AF may be subclinical. Fourth, we assessed variants with MAF ≥1%, and, therefore, the contribution of rare or loss of function variants to total AF variance was not assessed. Whole genome-sequencing results may provide insights into the contribution of rare variation to AF susceptibility. We acknowledge that our approach of using hard-called genotypes may have reduced power for discovery as compared with an approach using dosages and estimated

genotype probabilities. However, we submit that our computationally efficient approach was balanced by the use of robust high-confidence genotype information.

Conclusion

In a population-based sample of European ancestry, we observed that ≈22% of AF susceptibility was attributable to additive genetic variation, with a predominant enrichment for common genetic variants. Genetic variants located at known AF loci contributed modestly to AF variance, indicating that substantial additional variation exists that is not associated at genome-wide significance thresholds in current studies. Further analyses to explore AF genetic architecture in larger datasets, including partitioning by genomic annotation and by variation underlying clinical AF risk factors, is warranted.

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Disclosures

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References

1. Chugh SS, Havmoeller R, Narayanan K, Singh D, Rienstra M, Benjamin EJ, et al. Worldwide epidemiology of atrial fibrillation: a Global Burden of Disease 2010 Study. *Circulation*. 2014;129:837–847. doi: 10.1161/CIRCULATIONAHA.113.005119.
2. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Executive summary: heart disease and stroke statistics-2016 update: a report from the American Heart Association. *Circulation*. 2016;133:447–454.
3. Arnar DO, Thorvaldsson S, Manolio TA, Thorgeirsson G, Kristjansson K, Hakonarson H, et al. Familial aggregation of atrial fibrillation in Iceland. *Eur Heart J*. 2006;27:708–712. doi: 10.1093/eurheartj/ehi727.
4. Christophersen IE, Ravn LS, Budtz-Joergensen E, Skytthe A, Haunsoe S, Svendsen JH, et al. Familial aggregation of atrial fibrillation: a study in Danish twins. *Circ Arrhythm Electrophysiol*. 2009;2:378–383. doi: 10.1161/CIRCEP.108.786665.
5. Ellinor PT, Yoerger DM, Ruskin JN, MacRae CA. Familial aggregation in lone atrial fibrillation. *Hum Genet*. 2005;118:179–184. doi: 10.1007/s00439-005-0034-8.
6. Lubitz SA, Yin X, Fontes JD, Magnani JW, Rienstra M, Pai M, et al. Association between familial atrial fibrillation and risk of new-onset atrial fibrillation. *JAMA*. 2010;304:2263–2269. doi: 10.1001/jama.2010.1690.
7. Oyen N, Ranthe MF, Carstensen L, Boyd HA, Olesen MS, Olesen SP, et al. Familial aggregation of lone atrial fibrillation in young persons. *J Am Coll Cardiol*. 2012;60:917–921. doi: 10.1016/j.jacc.2012.03.046.
8. Yang YQ, Zhang XL, Wang XH, Tan HW, Shi HF, Fang WY, et al. Familial aggregation of lone atrial fibrillation in the Chinese population. *Intern Med*. 2010;49:2385–2391.
9. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nat Genet*. 2012;44:670–675. doi: 10.1038/ng.2261.
10. Sinner MF, Tucker NR, Lunetta KL, Ozaki K, Smith JG, Trompet S, et al; METASTROKE Consortium; AFGen Consortium. Integrating genetic, transcriptional, and functional analyses to identify 5 novel genes for atrial fibrillation. *Circulation*. 2014;130:1225–1235. doi: 10.1161/CIRCULATIONAHA.114.009892.
11. Christophersen IE, Rienstra M, Roselli C, Yin X, Geelhoed B, Barnard J, et al; METASTROKE Consortium of the ISGC; Neurology Working Group of the CHARGE Consortium; AFGen Consortium. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. *Nat Genet*. 2017;49:946–952. doi: 10.1038/ng.3843.
12. Low SK, Takahashi A, Ebana Y, Ozaki K, Christophersen IE, Ellinor PT, et al; AFGen Consortium. Identification of six new genetic loci associated with atrial fibrillation in the Japanese population. *Nat Genet*. 2017;49:953–958. doi: 10.1038/ng.3842.
13. Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, Sullivan PF, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature*. 2009;460:748–752.
14. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F, et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. *Nature*. 2010;467:832–838. doi: 10.1038/nature09410.
15. Loh PR, Bhatia G, Gusev A, Finucane HK, Bulik-Sullivan BK, Pollack SJ, et 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. doi: 10.1038/ng.3431.
16. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet*. 2011;88:76–82. doi: 10.1016/j.ajhg.2010.11.011.
17. Yang J, Manolio TA, Pasquale LR, Boerwinkle E, Caporaso N, Cunningham JM, et al. Genome partitioning of genetic variation for complex traits using common SNPs. *Nat Genet*. 2011;43:519–525. doi: 10.1038/ng.823.
18. Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh PR, et al; ReproGen Consortium; Schizophrenia Working Group of the Psychiatric Genomics Consortium; RACI Consortium. Partitioning heritability by functional annotation using genome-wide association summary statistics. *Nat Genet*. 2015;47:1228–1235. doi: 10.1038/ng.3404.
19. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. Robust relationship inference in genome-wide association studies. *Bioinformatics*. 2010;26:2867–2873. doi: 10.1093/bioinformatics/btq559.
20. Bellenguez C, Strange A, Freeman C, Donnelly P, Spencer CC; Wellcome Trust Case Control Consortium. A robust clustering algorithm for identifying problematic samples in genome-wide association studies. *Bioinformatics*. 2012;28:134–135. doi: 10.1093/bioinformatics/btr599.
21. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med*. 2015;12:e1001779. doi: 10.1371/journal.pmed.1001779.
22. Abraham G, Inouye M. Fast principal component analysis of large-scale genome-wide data. *PLoS One*. 2014;9:e93766. doi: 10.1371/journal.pone.0093766.
23. UK Biobank Analysis Team. Genotyping and quality control for UK biobank. http://biobank.ctsu.ox.ac.uk/showcase/docs/genotyping_qc.pdf. Accessed July 5, 2016.
24. UK Biobank Analysis Team. Genotype imputation and genetic association studies using uk biobank data. http://biobank.ctsu.ox.ac.uk/crystal/docs/impute_ukb_v1.pdf. Accessed July 5, 2016.

25. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience*. 2015;4:7. doi: 10.1186/s13742-015-0047-8.
26. Lee SH, Wray NR, Goddard ME, Visscher PM. Estimating missing heritability for disease from genome-wide association studies. *Am J Hum Genet*. 2011;88:294–305. doi: 10.1016/j.ajhg.2011.02.002.
27. Yang J, Benyamin B, McEvoy BP, Gordon S, Henders AK, Nyholt DR, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet*. 2010;42:565–569. doi: 10.1038/ng.608.
28. Gusev A, Bhatia G, Zaitlen N, Vilhjalmsson BJ, Diogo D, Stahl EA, et al. Quantifying missing heritability at known GWAS loci. *PLoS Genet*. 2013;9:e1003993. doi: 10.1371/journal.pgen.1003993.
29. Lee SH, Yang J, Chen GB, Ripke S, Stahl EA, Hultman CM, et al. Estimation of SNP heritability from dense genotype data. *Am J Hum Genet*. 2013;93:1151–1155. doi: 10.1016/j.ajhg.2013.10.015.
30. Lubitz SA, Brody JA, Bihlmeyer NA, Roselli C, Weng LC, Christophersen IE, et al; NHLBI GO Exome Sequencing Project. Whole exome sequencing in atrial fibrillation. *PLoS Genet*. 2016;12:e1006284. doi: 10.1371/journal.pgen.1006284.
31. Turner SD. Qqman: an R package for visualizing GWAS results using qq and manhattan plots. *bioRxiv*. 2014:005165.
32. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics*. 2010;26:2336–2337. doi: 10.1093/bioinformatics/btq419.
33. Kannel WB, Wolf PA, Benjamin EJ, Levy D. Prevalence, incidence, prognosis, and predisposing conditions for atrial fibrillation: population-based estimates. *Am J Cardiol*. 1998;82(8A):2N–9N.
34. Lonsdale J, Thomas J, Salvatore M, Phillips R, Lo E, Shad S, et al. The genotype-tissue expression (gtex) project. *Nature genetics*. 2013;45:580–585.
35. Lynch M, Walsh B. *Genetics and Analysis of Quantitative Traits*. Sunderland, Mass: Sinauer; 1998.
36. Hofman N, Tan HL, Alders M, Kolder I, de Haij S, Mannens MM, et al. Yield of molecular and clinical testing for arrhythmia syndromes: report of 15 years' experience. *Circulation*. 2013;128:1513–1521. doi: 10.1161/CIRCULATIONAHA.112.000091.
37. Ge T, Chen CY, Neale BM, Sabuncu MR, Smoller JW. Phenome-wide heritability analysis of the UK Biobank. *PLoS Genet*. 2017;13:e1006711. doi: 10.1371/journal.pgen.1006711.
38. Everett BM, Cook NR, Conen D, Chasman DI, Ridker PM, Albert CM. Novel genetic markers improve measures of atrial fibrillation risk prediction. *Eur Heart J*. 2013;34:2243–2251. doi: 10.1093/eurheartj/eht033.
39. Lubitz SA, Yin X, Lin HJ, Kolek M, Smith JG, Trompet S, et al; AF-Gen Consortium. Genetic risk prediction of atrial fibrillation. *Circulation*. 2017;135:1311–1320. doi: 10.1161/CIRCULATIONAHA.116.024143.
40. Tada H, Shiffman D, Smith JG, Sjögren M, Lubitz SA, Ellinor PT, et al. Twelve-single nucleotide polymorphism genetic risk score identifies individuals at increased risk for future atrial fibrillation and stroke. *Stroke*. 2014;45:2856–2862. doi: 10.1161/STROKEAHA.114.006072.
41. Shoemaker MB, Bollmann A, Lubitz SA, Ueberham L, Saini H, Montgomery J, et al. Common genetic variants and response to atrial fibrillation ablation. *Circ Arrhythm Electrophysiol*. 2015;8:296–302. doi: 10.1161/CIRCEP.114.001909.
42. Mommersteeg MT, Brown NA, Prall OW, de Gier-de Vries C, Harvey RP, Moorman AF, et al. Pitx2c and Nkx2-5 are required for the formation and identity of the pulmonary myocardium. *Circ Res*. 2007;101:902–909. doi: 10.1161/CIRCRESAHA.107.161182.
43. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE, et al. Molecular pathway for the localized formation of the sinoatrial node. *Circ Res*. 2007;100:354–362. doi: 10.1161/01.RES.0000258019.74591.b3.
44. Chinchilla A, Daimi H, Lozano-Velasco E, Dominguez JN, Caballero R, Delpón E, et al. PITX2 insufficiency leads to atrial electrical and structural remodeling linked to arrhythmogenesis. *Circ Cardiovasc Genet*. 2011;4:269–279. doi: 10.1161/CIRCGENETICS.110.958116.
45. Kolfschoten IG, van Leeuwen B, Berns K, Mullenders J, Beijersbergen RL, Bernards R, et al. A genetic screen identifies PITX1 as a suppressor of RAS activity and tumorigenicity. *Cell*. 2005;121:849–858. doi: 10.1016/j.cell.2005.04.017.
46. Kong G, Liu Z, Wu K, Zhang Y, Deng Z, Feng W, et al. Strong expression of paired-like homeodomain transcription factor 1 (PITX1) is associated with a favorable outcome in human osteosarcoma. *Tumour Biol*. 2015;36:7735–7741. doi: 10.1007/s13277-015-3512-1.
47. Volodko N, Gordon M, Salla M, Ghazaleh HA, Baksh S. RASSF tumor suppressor gene family: biological functions and regulation. *FEBS Lett*. 2014;588:2671–2684. doi: 10.1016/j.febslet.2014.02.041.
48. Visscher PM, Hemani G, Vinkhuyzen AA, Chen GB, Lee SH, Wray NR, et al. Statistical power to detect genetic (co)variance of complex traits using SNP data in unrelated samples. *PLoS Genet*. 2014;10:e1004269. doi: 10.1371/journal.pgen.1004269.
49. Hemani G, Yang J. Gcta-greml power calculator. http://cnsgenomics.com/shiny/gctaPower/. Accessed June 12, 2017.

CLINICAL PERSPECTIVE

Atrial fibrillation affects ≈34 million individuals worldwide. Recently, a widespread heritable component underlying atrial fibrillation has been appreciated. Genetic association studies have identified multiple genetic loci related to atrial fibrillation. Yet the aggregate contributions of genetic variation to atrial fibrillation risk remain undefined. We, therefore, estimated atrial fibrillation heritability in the population-based UK Biobank study using genome-wide single-nucleotide polymorphism information in >120 000 individuals, of whom ≈3000 had atrial fibrillation. We observed that ≈22% of the variance in atrial fibrillation risk was accounted for by additive genetic variation. The proportion of variance explained by genetic factors was similar for both men and women. We further observed that nearly one third of the additive genetic variance of atrial fibrillation was explained by genomic variation at known atrial fibrillation loci, putative cardiac arrhythmia, and putative cardiomyopathy gene regions. Our findings underscore the notion that genetic variation contributes substantially to atrial fibrillation risk. Nevertheless, established disease loci for atrial fibrillation, arrhythmias, and cardiomyopathies do not fully account for genetic susceptibility to atrial fibrillation. Future genetic discovery efforts in large data sets and using whole genome sequencing may help elucidate the genetic basis of this complex and morbid condition.

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Heritability of Atrial Fibrillation

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Fields and codes utilized to define atrial fibrillation in the UK Biobank dataset.

Field	Description	Algorithm elements
20002	Non-cancer illness code, self-reported	atrial fibrillation – 1471, atrial flutter - 1483
20004	Operation code	cardioversion - 1524
41202	Diagnoses - main ICD10	atrial fibrillation or flutter - I48, I48.0, I48.1, I48.2, I48.3, I48.4, I48.9
41204	Diagnoses - secondary ICD10	atrial fibrillation or flutter - I48, I48.0, I48.1, I48.2, I48.3, I48.4, I48.9
41203	Diagnoses - main ICD9	427.3, 427.31, 427.32
41205	Diagnoses - secondary ICD9	427.3, 427.31, 427.32
41200	Operative procedures - main OPCS	AVN ablation- K57.1, atrial fibrillation ablation, flutter ablation, and cardioversion - K62.1, K62.2, K62.3, K62.4
41210	Operative procedures - secondary OPCS	AVN ablation - K57.1, atrial fibrillation ablation, flutter ablation, and cardioversion - K62.1, K62.2, K62.3, K62.4
40002	Contributory (secondary) causes of death: ICD10	atrial fibrillation or flutter - I48, I48.0, I48.1, I48.2, I48.3, I48.4, I48.9
40001	Underlying (primary) cause of death: ICD10	atrial fibrillation or flutter - I48, I48.0, I48.1, I48.2, I48.3, I48.4, I48.9

Supplemental Table 2. Top genetic variants previously associated with atrial fibrillation in an independent analysis used to develop a genetic risk score.

SNP	Risk Allele	Weight
rs72700118	A	0.1324
rs3771537	A	0.085
rs2540949	A	0.0794
rs2288327	G	0.089
rs337711	T	0.0701
rs2967791	T	0.0717
rs4946333	G	0.0744
rs7508	A	0.0879
rs35176054	A	0.1291
rs75190942	A	0.1592
rs11264280	T	0.1154
rs520525	A	0.113
rs11718898	C	0.0733
rs6843082	G	0.3706
rs12664873	T	0.0782
rs1997572	G	0.0973
rs7026071	T	0.0907
rs7915134	C	0.1133
rs11598047	G	0.1618
rs883079	T	0.1084
rs1152591	A	0.0821
rs74022964	T	0.1112
rs2106261	T	0.1845
rs6800541	T	0.0632
rs11047543	G	0.0944

Score				
N of variants	Mean ± SD in AF cases	Mean ± SD in referents	Effect size (Standard Error)	P-value
25	2.38 ± 0.39	2,23 ± 0.36	1.12 (0.05)	1.4×10^{-112}

Supplemental Table 3. Genes previously implicated in atrial fibrillation pathogenesis (n=37).

Gene	Reference	Gene	Reference
ACE	1	*KCNN3*	2
AGT	3, 4	*KCNQ1*	5-7
ANK2	8	*LMNA*	9, 10
C9ORF3	2	*MYOZ1*	2
CAND2	11	*NEURL1*	2
CAV1	2	*NPPA*	12
CAV3	2	*NUP155*	13
GATA4	14-17	*PITX2*	2
GATA5	18, 19	*PRRX1*	2
GATA6	20, 21	*SCN10A*	22
GJA1	11	*SCN1B*	23, 24
GJA5	25, 26	*SCN2B*	24
HCN4	2	*SCN3B*	27
IL6R	28	*SCN5A*	29-35
KCNA5	36	*SYNE2*	2
KCNE1	37-39	*SYNPO2L*	2
KCNE2	40	*TBX5*	11, 41
KCNH2	42, 43	*ZFHX3*	2
KCNJ2	44		

Supplemental Table 4. Genes previously implicated in cardiomyopathies and cardiac arrhythmias (n=53).

Gene	Reference	Gene	Reference
ABCC9	45	*MYBCP3*	46
ACTC1	46	*MYH6*	46
ACTN2	46	*MYH7*	45
CACNA1C	46	*MYL2*	45
CACNB2	46	*MYL3*	45
CASQ2	46	*MYOZ2*	47
CSRP3	46	*PKP2*	45-48
DES	45-48	*PLN*	46
DMD	45, 47	*PRKAG2*	46
DSC2	45-48	*PSEN1*	45
DSG2	45-48	*PSEN2*	45
DSP	45-48	*RYR2*	46
DTNA	45	*SCN1B*	46
EYA4	45	*SCN5A*	46
GLA	45	*SGCD*	45
GPD1L	46	*TAZ*	45
JPH2	45	*TCAP*	45
JUP	45-48	*TGFB3*	45
KCNE1	47	*TMEM43*	46
KCNE2	47	*TPM1*	45
KCNE3	47	*TMPO*	45
KCNH2	46	*TNNC1*	45
KCNJ2	46	*TNNI3*	45
KCNQ1	46	*TNNT2*	45
LAMP2	46	*TTN*	49
LDB3	45	*VCL*	45
LMNA	46		

Supplemental Table 5. Loci uniquely associated with atrial fibrillation in the UK Biobank.

				Primary analysis			Association in prior AFGen European ancestry analysis[50]		
Locus	**SNP**	**RA / REA**	**Nearest gene**	**RAF (%)**	**Odds ratio (95%CI)**	**P-value**	**RAF (%)**	**Odds ratio (95%CI)**	**P-value**
5q31	rs31209	A/T	*PITX1*	62	1.17 (1.11-1.24)	1.7×10^{-8}	64	1.00 (0.97-1.02)	0.84
12p12	rs117640426	C/T	*RASSF8*	1	1.70 (1.42-2.03)	8.7×10^{-9}	3	0.96 (0.86-1.08)	0.53

*RA = risk allele; NRA = non-risk allele; RAF = risk allele frequency

Supplemental Table 6. Association results at *RASSF8* locus stratified by genotyping arrays

Array	Locus	SNP	RA/NRA	Sample size	Odds ratio (95%CI)	P-value
UK BiLEVE Axiom	12p12	rs117640426	C/T	41007	2.05 (1.56-2.68)	2.21×10^{-07}
UK Biobank Axiom	12p12	rs117640426	C/T	78185	1.49 (1.17-1.89)	0.001288

*RA = risk allele; NRA = non-risk allele; RAF = risk allele frequency

Supplemental Table 7. Number of individuals and variants included in genomic strata in which heritability was calculated.

Genomic stratum	Number of individuals (AF cases)	Number of variants
Overall sample	120,286 (2,987)	811,488
By age of onset		
Early-onset AF (≤65 years old)	90,667 (2,068)	811,488
Older-onset AF (>65 years old)	29,619 (919)	811,488
By Sex		
Men	56,936 (2,064)	811,488
Women	63,350 (923)	811,488
By minor allele frequency		
Low-frequency variants	120,286 (2,987)	383,495
Common variants	120,286 (2,987)	427,993
By established atrial fibrillation loci		
Known loci (n=25)	120,286 (2,987)	6,789
Remaining genome	120,286 (2,987)	804,699
By atrial fibrillation susceptibility gene regions		
Known atrial fibrillation loci (n=25) and putative atrial fibrillation genes (n=37)	120,286 (2,987)	7,508
Other genomic regions	120,286 (2,987)	803,980
By atrial fibrillation, cardiac arrhythmia, and cardiomyopathy susceptibility gene regions		
Known atrial fibrillation loci (n=25), putative atrial fibrillation genes, or cardiac arrhythmia or cardiomyopathy gene regions (n=82)	120,286 (2,987)	9,590
Other genomic regions	120,286 (2,987)	801,898

AF: atrial fibrillation

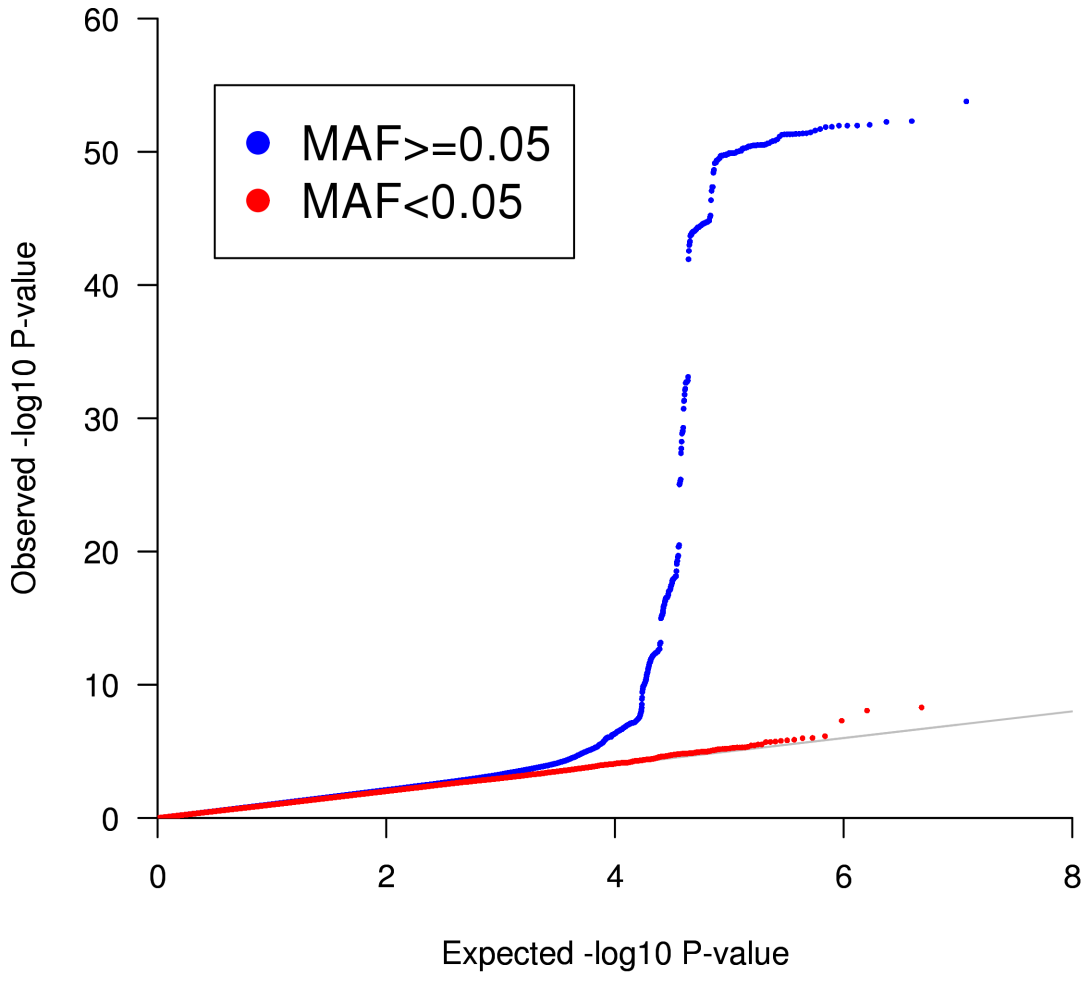
Supplemental Table 8. Power calculation for heritability estimates stratified by age subgroup.

Group	Cases (n)	Controls (n)	AF Prevalence	h^2_g	Power
Overall sample	2,987	117,299	2.5%	0.20	1.00
				0.15	1.00
				0.10	0.99
				0.05	0.75
Age-stratified					
Early-onset AF (≤ 65 years)	2,068	88,599	2.3%	0.20	1.00
				0.15	0.99
				0.10	0.96
				0.05	0.47
Older-onset AF (> 65 years)	919	28,700	3.1%	0.20	0.86
				0.15	0.63
				0.10	0.33
				0.05	0.12

Power calculations based on the method designed by Visscher et al.[51] and available online (http://cnsgenomics.com/shiny/gctaPower/). Power estimates (probability of estimating a SNP-heritability that is greater than zero) to detect various h^2_g are listed above. Our overall and early onset AF groups have 80% power to detect h^2_g = 0.10 and lower, whereas in the older-onset AF group power is limited to higher h^2_g estimates (~ 0.20).

Supplemental Figure 1. QQ plot summarizing genome-wide variation associated with atrial fibrillation.

Variants are stratified by minor allele frequencies (MAF). Low frequency variants (MAF 0.01-0.05) are indicated in red, and common variants (MAF ≥ 0.05) are indicated in blue. Genomic inflation factors (λ) are 1.01 and 1.03 for low frequency and common variation, respectively). N= 8,325,606 variants overall (2,405,829 low frequency, and 5,919,777 common).



Supplemental References

1. Bedi M, McNamara D, London B, Schwartzman D. Genetic susceptibility to atrial fibrillation in patients with congestive heart failure. *Heart Rhythm*. 2006;3:808-812.
2. Ellinor PT, Lunetta KL, Albert CM, Glazer NL, Ritchie MD, Smith AV, et al. Meta-analysis identifies six new susceptibility loci for atrial fibrillation. *Nature genetics*. 2012;44:670-675.
3. Tsai CT, Hwang JJ, Chiang FT, Wang YC, Tseng CD, Tseng YZ, et al. Renin-angiotensin system gene polymorphisms and atrial fibrillation: A regression approach for the detection of gene-gene interactions in a large hospitalized population. *Cardiology*. 2008;111:1-7.
4. Wang Q-S, Li Y-G, Chen X-D, Yu J-F, Wang J, Sun J, et al. Angiotensinogen polymorphisms and acquired atrial fibrillation in chinese. *Journal of electrocardiology*. 2010;43:373-377.
5. Chen Y-H, Xu S-J, Bendahhou Sd, Wang X-L, Wang Y, Xu W-Y, et al. Kcnq1 gain-of-function mutation in familial atrial fibrillation. *Science*. 2003;299:251-254.
6. Das S, Makino S, Melman YF, Shea MA, Goyal SB, Rosenzweig A, et al. Mutation in the s3 segment of kcnq1 results in familial lone atrial fibrillation. *Heart rhythm*. 2009;6:1146-1153.
7. Otway R, Vandenberg JI, Guo G, Varghese A, Castro ML, Liu J, et al. Stretch-sensitive kcnq1 mutation: A link between genetic and environmental factors in the pathogenesis of atrial fibrillation? *Journal of the American College of Cardiology*. 2007;49:578-586.

8. Cunha SR, Hund TJ, Hashemi S, Voigt N, Li N, Wright P, et al. Defects in ankyrin-based membrane protein targeting pathways underlie atrial fibrillation. *Circulation*. 2011;124:1212-1222.

9. Fatkin D, MacRae C, Sasaki T, Wolff MR, Porcu M, Frenneaux M, et al. Missense mutations in the rod domain of the lamin a/c gene as causes of dilated cardiomyopathy and conduction-system disease. *New England Journal of Medicine*. 1999;341:1715-1724.

10. Sebillon P, Bouchier C, Bidot LD, Bonne G, Ahamed K, Charron P, et al. Expanding the phenotype of lmna mutations in dilated cardiomyopathy and functional consequences of these mutations. *Journal of Medical Genetics*. 2003;40:560-567.

11. Sinner MF, Tucker NR, Lunetta KL, Ozaki K, Smith JG, Trompet S, et al. Integrating genetic, transcriptional, and functional analyses to identify five novel genes for atrial fibrillation. *Circulation*. 2014;130:1225-1235.

12. Hodgson-Zingman DM, Karst ML, Zingman LV, Heublein DM, Darbar D, Herron KJ, et al. Atrial natriuretic peptide frameshift mutation in familial atrial fibrillation. *New England Journal of Medicine*. 2008;359:158-165.

13. Zhang X, Chen S, Yoo S, Chakrabarti S, Zhang T, Ke T, et al. Mutation in nuclear pore component nup155 leads to atrial fibrillation and early sudden cardiac death. *Cell*. 2008;135:1017-1027.

14. Jiang J-Q, Shen F-F, Fang W-Y, Liu X, Yang Y-Q. Novel gata4 mutations in lone atrial fibrillation. *International journal of molecular medicine*. 2011;28:1025-1032.

15. Posch MG, Boldt L-H, Polotzki M, Richter S, Rolf S, Perrot A, et al. Mutations in the cardiac transcription factor gata4 in patients with lone atrial fibrillation. *European journal of medical genetics*. 2010;53:201-203.
16. Wang J, Sun Y-M, Yang Y-Q. Mutation spectrum of the gata4 gene in patients with idiopathic atrial fibrillation. *Molecular biology reports*. 2012;39:8127-8135.
17. Yang Y-Q, Wang M-Y, Zhang X-L, Tan H-W, Shi H-F, Jiang W-F, et al. Gata4 loss-of-function mutations in familial atrial fibrillation. *Clinica chimica acta*. 2011;412:1825-1830.
18. Wang X-H, Huang C-X, Wang Q, Li R-G, Xu Y-J, Liu X, et al. A novel gata5 loss-of-function mutation underlies lone atrial fibrillation. *International journal of molecular medicine*. 2013;31:43-50.
19. Yang Y-Q, Wang J, Wang X-H, Wang Q, Tan H-W, Zhang M, et al. Mutational spectrum of the gata5 gene associated with familial atrial fibrillation. *International journal of cardiology*. 2012;157:305-307.
20. Li J, Liu W-D, Yang Z-L, Yang Y-Q. Novel gata6 loss-of-function mutation responsible for familial atrial fibrillation. *International journal of molecular medicine*. 2012;30:783-790.
21. Yang Y-Q, Li L, Wang J, Zhang X-L, Li R-G, Xu Y-J, et al. Gata6 loss-of-function mutation in atrial fibrillation. *European journal of medical genetics*. 2012;55:520-526.
22. Jabbari J, Olesen MS, Yuan L, Nielsen JB, Liang B, Macri V, et al. Common and rare variants in scn10a modulate the risk of atrial fibrillation. *Circulation: Cardiovascular Genetics*. 2014;8:64-73.

23. Hayashi K, Konno T, Tada H, Tani S, Liu L, Fujino N, et al. Functional characterization of rare variants implicated in susceptibility to lone atrial fibrillation. *Circulation: Arrhythmia and Electrophysiology*. 2015:CIRCEP-114.

24. Watanabe H, Darbar D, Kaiser DW, Jiramongkolchai K, Chopra S, Donahue BS, et al. Mutations in sodium channel β1-and β2-subunits associated with atrial fibrillation. *Circulation: Arrhythmia and Electrophysiology*. 2009;2:268-275.

25. Firouzi M, Ramanna H, Kok B, Jongsma HJ, Koeleman BPC, Doevendans PA, et al. Association of human connexin40 gene polymorphisms with atrial vulnerability as a risk factor for idiopathic atrial fibrillation. *Circulation research*. 2004;95:e29-e33.

26. Gollob MH, Jones DL, Krahn AD, Danis L, Gong X-Q, Shao Q, et al. Somatic mutations in the connexin 40 gene (gja5) in atrial fibrillation. *New England Journal of Medicine*. 2006;354:2677-2688.

27. Olesen MS, Jespersen T, Nielsen JB, Liang B, Møller DV, Hedley P, et al. Mutations in sodium channel β-subunit scn3b are associated with early-onset lone atrial fibrillation. *Cardiovascular research*. 2011;89:786-793.

28. Schnabel RB, Kerr KF, Lubitz SA, Alkylbekova EL, Marcus GM, Sinner MF, et al. Large-scale candidate gene analysis in whites and african americans identifies il6r polymorphism in relation to atrial fibrillation the national heart, lung, and blood institute's candidate gene association resource (care) project. *Circulation: Cardiovascular Genetics*. 2011;4:557-564.

29. Benito B, Brugada R, Perich RM, Lizotte E, Cinca J, Mont L, et al. A mutation in the sodium channel is responsible for the association of long qt syndrome and familial atrial fibrillation. *Heart Rhythm*. 2008;5:1434-1440.

30. Darbar D, Kannankeril PJ, Donahue BS, Kucera G, Stubblefield T, Haines JL, et al. Cardiac sodium channel (scn5a) variants associated with atrial fibrillation. *Circulation*. 2008;117:1927-1935.

31. Ellinor PT, Nam EG, Shea MA, Milan DJ, Ruskin JN, MacRae CA. Cardiac sodium channel mutation in atrial fibrillation. *Heart Rhythm*. 2008;5:99-105.

32. Li Q, Huang H, Liu G, Lam K, Rutberg J, Green MS, et al. Gain-of-function mutation of na v 1.5 in atrial fibrillation enhances cellular excitability and lowers the threshold for action potential firing. *Biochemical and biophysical research communications*. 2009;380:132-137.

33. Makiyama T, Akao M, Shizuta S, Doi T, Nishiyama K, Oka Y, et al. A novel scn5a gain-of-function mutation m1875t associated with familial atrial fibrillation. *Journal of the American College of Cardiology*. 2008;52:1326-1334.

34. McNair WP, Ku L, Taylor MRG, Fain PR, Dao D, Wolfel E, et al. Scn5a mutation associated with dilated cardiomyopathy, conduction disorder, and arrhythmia. *Circulation*. 2004;110:2163-2167.

35. Olson TM, Michels VV, Ballew JD, Reyna SP, Karst ML, Herron KJ, et al. Sodium channel mutations and susceptibility to heart failure and atrial fibrillation. *Jama*. 2005;293:447-454.

36. Olson TM, Alekseev AE, Liu XK, Park S, Zingman LV, Bienengraeber M, et al. Kv1. 5 channelopathy due to kcna5 loss-of-function mutation causes human atrial fibrillation. *Human molecular genetics*. 2006;15:2185-2191.

37. Fatini C, Sticchi E, Genuardi M, Sofi F, Gensini F, Gori AM, et al. Analysis of mink and enos genes as candidate loci for predisposition to non-valvular atrial fibrillation. *European heart journal*. 2006;27:1712-1718.

38. Lai L-P, Su M-J, Yeh H-M, Lin J-L, Chiang F-T, Hwang J-J, et al. Association of the human mink gene 38g allele with atrial fibrillation: Evidence of possible genetic control on the pathogenesis of atrial fibrillation. *American Heart Journal*. 2002;144:485-490.

39. Prystupa A, Dzida G, Myśliński W, Małaj G, Lorenc T. Mink gene polymorphism in the pathogenesis of lone atrial fibrillation. *Kardiologia polska*. 2006;64:1205-1211.

40. Yang Y, Xia M, Jin Q, Bendahhou S, Shi J, Chen Y, et al. Identification of a kcne2 gain-of-function mutation in patients with familial atrial fibrillation. *The American Journal of Human Genetics*. 2004;75:899-905.

41. Raychaudhuri S, Plenge RM, Rossin EJ, Ng ACY, Purcell SM, Sklar P, et al. Identifying relationships among genomic disease regions: Predicting genes at pathogenic snp associations and rare deletions. *PLoS genetics*. 2009;5:e1000534.

42. Hong KUI, Bjerregaard P, Gussak I, Brugada R. Short qt syndrome and atrial fibrillation caused by mutation in kcnh2. *Journal of cardiovascular electrophysiology*. 2005;16:394-396.

43. Sinner MF, Pfeufer A, Akyol M, Beckmann B-M, Hinterseer M, Wacker A, et al. The non-synonymous coding ikr-channel variant kcnh2-k897t is associated with atrial fibrillation: Results from a systematic candidate gene-based analysis of kcnh2 (herg). *European heart journal*. 2008;29:907-914.

44. Xia M, Jin Q, Bendahhou S, He Y, Larroque M-M, Chen Y, et al. A kir2. 1 gain-of-function mutation underlies familial atrial fibrillation. *Biochemical and biophysical research communications*. 2005;332:1012-1019.

45. Hershberger RE, Lindenfeld J, Mestroni L, Seidman CE, Taylor MRG, Towbin JA, et al. Genetic evaluation of cardiomyopathy--a heart failure society of america practice guideline. *Journal of cardiac failure*. 2009;15:83-97.

46. Ackerman MJ, Priori SG, Willems S, Berul C, Brugada R, Calkins H, et al. Hrs/ehra expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies this document was developed as a partnership between the heart rhythm society (hrs) and the european heart rhythm association (ehra). *Heart rhythm : the official journal of the Heart Rhythm Society*. 2011;8:1308-1339.

47. Gollob MH, Blier L, Brugada R, Champagne J, Chauhan V, Connors S, et al. Recommendations for the use of genetic testing in the clinical evaluation of inherited cardiac arrhythmias associated with sudden cardiac death: Canadian cardiovascular society/canadian heart rhythm society joint position paper. *The Canadian journal of cardiology*. 2011;27:232-245.

48. Marcus FI, McKenna WJ, Sherrill D, Basso C, Bauce B, Bluemke DA, et al. Diagnosis of arrhythmogenic right ventricular cardiomyopathy/dysplasia: Proposed modification of the task force criteria. *Circulation*. 2010;121:1533-1541.

49. Herman DS, Lam L, Taylor MR, Wang L, Teekakirikul P, Christodoulou D, et al. Truncations of titin causing dilated cardiomyopathy. *N Engl J Med*. 2012;366:619-628.

50. Christophersen IE, Rienstra M, Roselli C, Yin X, Geelhoed B, Barnard J, et al. Large-scale analyses of common and rare variants identify 12 new loci associated with atrial fibrillation. *Nature genetics*. 2017;49:946-952.

51. Visscher PM, Hemani G, Vinkhuyzen AA, Chen GB, Lee SH, Wray NR, et al. Statistical power to detect genetic (co)variance of complex traits using snp data in unrelated samples. *PLoS genetics*. 2014;10:e1004269.