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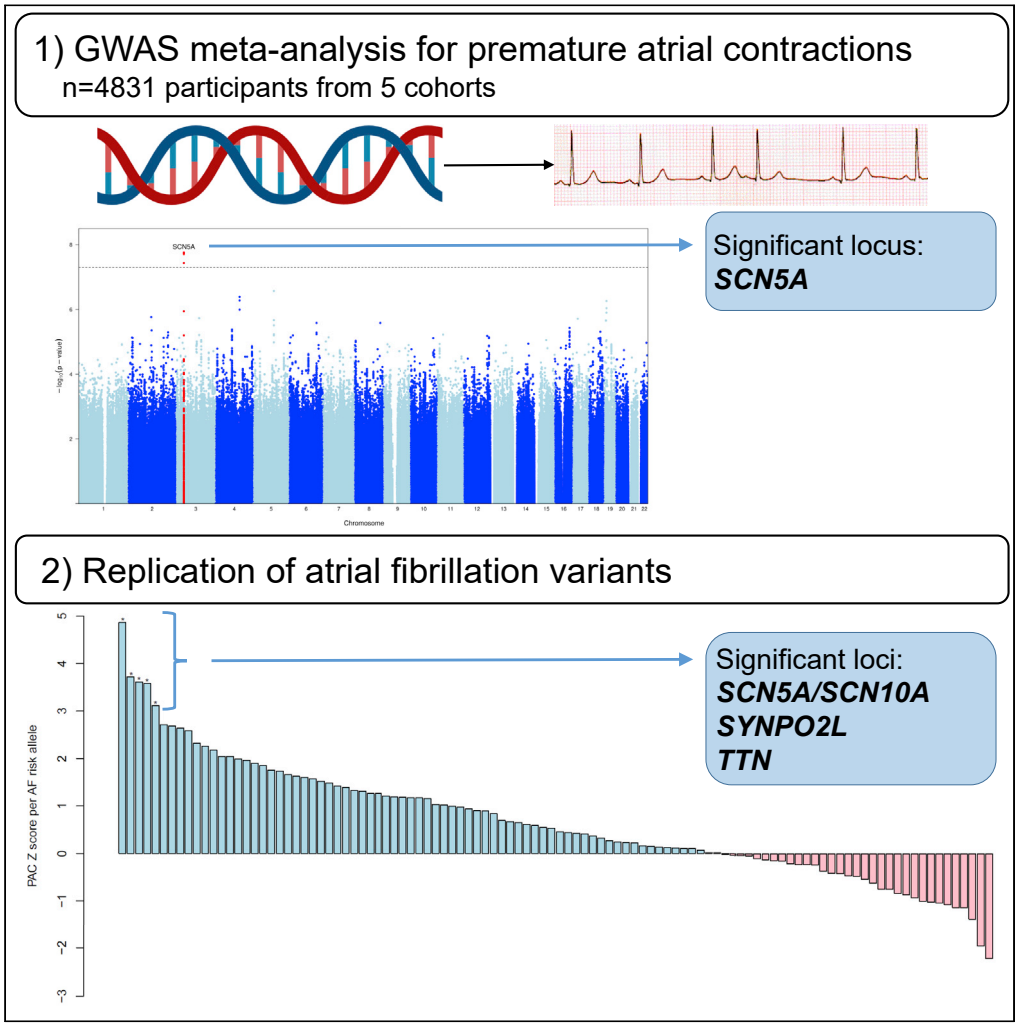

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Highlights

Variants in *SCN5A* are associated with premature atrial contractions (PAC) frequency

Other atrial fibrillation (AF) risk variants are also associated with PAC frequency

Both shared and distinct genetic mechanisms exist for PAC frequency and AF

Thériault et al., iScience *25*, 105210
October 21, 2022
https://doi.org/10.1016/j.isci.2022.105210

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Genome-wide analyses identify *SCN5A* as a susceptibility locus for premature atrial contraction frequency

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SUMMARY

Premature atrial contractions (PACs) are frequently observed on electrocardiograms and are associated with increased risks of atrial fibrillation (AF), stroke, and mortality. In this study, we aimed to identify genetic susceptibility loci for PAC frequency. We performed a genome-wide association study meta-analysis with PAC frequency obtained from ambulatory cardiac monitoring in 4,831 individuals of European ancestry. We identified a genome-wide significant locus at the *SCN5A* gene. The lead variant, rs7373862, located in an intron of *SCN5A*, was associated with an increase of 0.12 [95% CI 0.08–0.16] standard deviations of the normalized PAC frequency per risk allele. Among genetic variants previously associated with AF, there was a significant enrichment in concordance of effect for PAC frequency (n = 73/106, p = 5.1×10^{-5}). However, several AF risk loci, including *PITX2*, were not associated with PAC frequency. These findings suggest the existence of both shared and distinct genetic mechanisms for PAC frequency and AF.

INTRODUCTION

Premature atrial contractions (PACs) are a form of ectopic atrial activity in which the action potential is generated by the atrial myocardium. They are frequently observed on electrocardiograms (ECG) and detectable in up to 99% of individuals over 50 years of age undergoing 24-h (24h) Holter monitoring, which is considered the gold standard for PAC quantification in clinical practice (Conen et al., 2012). Although generally considered to be benign, PACs are an independent predictor of incident atrial fibrillation (AF) (Dewland et al., 2013) and have been associated with increased risks of stroke and death (Larsen et al., 2015; Lin et al., 2015).

The etiology of PACs remains uncertain, but several risk factors have been identified, including older age, cardiovascular disease, greater height, and the lack of physical activity (Conen et al., 2012). Contrary to AF, for which many susceptibility loci have been identified (Roselli et al., 2018), little is known about the genetic architecture of PAC frequency. The identification of risk loci would improve the understanding of the biological mechanisms leading to PACs, as well as the relationship between PACs and AF. This information could potentially lead to the development of novel preventive therapies.

In this study, we aimed to identify common genetic variants associated with PAC frequency measured by ambulatory ECG monitoring by performing a genome-wide association study (GWAS) meta-analysis. We also compared the genetic architectures of PAC frequency and AF.

RESULTS

GWAS meta-analysis

Five cohorts with a total of 4,831 individuals of European ancestry were included in the analysis (Table 1). Mean age varied from 35.5 to 72.6 years. The proportion of men was between 37% and 54%. The estimated number of PACs per hour before normalization ranged from 0 to 1482, with a median between 0 and 4.1 per

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Continued

Table 1. Clinical characteristics of individuals included from each cohort

	GAPP	SAPALDIA	CHS	MESA	BHS
N	1897	1194	833	502	405
ECG monitoring	Holter	Holter	Holter	Zio	Holter
Duration (h)	24.0 (0.1)	22.3 (2.1)	21.7 (1.0)	305.6 (67.4)	20.0 (2.0)
Age (years)	35.5 (5.2)	59.9 (6.0)	71.3 (4.6)	72.6 (7.9)	47.7 (15.1)
Sex (n male, %)	879 (46.3)	643 (53.9)	342 (41.1)	245 (48.8)	149 (36.8)
Height (m)	1.72 (0.09)	1.68 (0.09)	1.65 (0.09)	1.68 (0.10)	1.63 (0.09)
BMI (kg/m^2)	24.6 (3.8)	26.5 (4.2)	26.6 (4.1)	27.8 (5.2)	26.0 (5.0)
Hypertension (n, %)	254 (13.4)	629 (52.7)	421 (50.5)	278 (55.4)	134 (33.1)
Diabetes (n, %)	0 (0)	67 (5.6)	109 (13.1)	74 (14.7)	24 (5.9)
Myocardial infarction (n, %)	0 (0)	8 (0.7)	0 (0)	7 (1.4)	8 (2.0)
Heart Failure (n, %)	0 (0)	18 (1.5)	0 (0)	1 (0.2)	NA
PAC frequency (n/h) – mean (SD)	0.57 (3.6)	8.6 (40.4)	29.4 (108.4)	31.9 (100.5)	10.9 (63.0)
PAC frequency (n/h) - median (IQR)	0.13 (0.25)	1.2 (2.6)	3.0 (10.0)	4.1 (14.9)	0 (0.86)

Continuous variables are expressed as mean (standard deviation) unless stated otherwise. GAPP: Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors; SAPALDIA: Swiss Cohort Study on Air Pollution and Lung Diseases in Adults, CHS: Cardiovascular Health Study; MESA: Multi-Ethnic Study of Atherosclerosis; BHS: Baependi Heart Study; BMI: body mass index, PAC: premature atrial contraction, SD: standard deviation, IQR: interquartile range. See also Table S1.

cohort. All participant samples were genotyped using a genome-wide array, and data were imputed with a reference panel selected for each cohort (Table S1).

A total of 9,643,192 variants were meta-analyzed, including 6,807,074 variants common to all five cohorts. There was no evidence of inflation in the individual cohorts (lambda between 0.992 and 1.073) or in the meta-analysis (lambda of 1.011) (Figure S1). Five variants at the 3p22.2 locus reached the genome-wide significance threshold (Figures 1 and 2). The lead variant, rs7373862, is located in an intron of the gene for sodium voltage-gated channel alpha subunit 5 (*SCN5A*). The direction of effect was concordant in all five cohorts, and there was no significant heterogeneity (p = 0.27) (Figure 3). A list of variants with $p < 1 \times 10^{-5}$ is available in Table S2. We retrieved a related phenotype in UK Biobank from hospital records, namely the presence of atrial premature depolarization. The risk allele at the lead variant (rs7373862-A) was nominally associated with this phenotype (n = 328 cases and 408,021 controls; OR = 1.22 [95% CI: 1.03, 1.43], p = 0.020).

Credible set and annotation

The 95% credible set included five variants, all in linkage disequilibrium ($R^2 > 0.95$) (Table S3). To verify if these variants are associated with a change in the expression of nearby genes, we queried publicly available expression quantitative trait loci (eQTL) datasets in several tissues, including heart chambers. The lead variant is a weak eQTL for *SCN5A* in thyroid ($p = 5.1 \times 10^{-11}$) and lung ($p = 2.0 \times 10^{-5}$) tissues, according to the Genotype-Tissue Expression (GTEx) project (Table S3). The risk allele rs7373862-A for PAC frequency is associated with higher expression of *SCN5A* in these tissues. As for regulatory elements, two variants, rs3924120 and rs3935184, showed DNase peaks in cardiac tissues. Notably, rs3935184 is located in a DNase peak for the right atrium and is predicted to lie in a gene enhancer region for the right ventricle, according to a model including five chromatin marks (Kundaje et al., 2015) (Table S3).

Replication of variants associated with AF

We verified the effect of previously identified genome-wide significant variants for AF obtained following conditional analysis in a meta-analysis of 537,409 individuals of European ancestry (Roselli et al., 2018). Out of 107 variants, 106 were available in the PAC frequency meta-analysis. One variant (rs187585530) was excluded because the allele frequency was lower than 0.01. Five variants were significantly associated with PAC frequency at a false-discovery rate threshold of 5% (Tables 2 and S4 and Figure S2), including three variants at the *SCN5A/SCN10A* locus (rs7374540, rs7373065, and rs6790396). Variant rs7374540 is in linkage disequilibrium with the PAC frequency lead variant rs7373862 ($R^2 = 0.808$ in European individuals

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https://doi.org/10.1016/j.isci.2022.105210

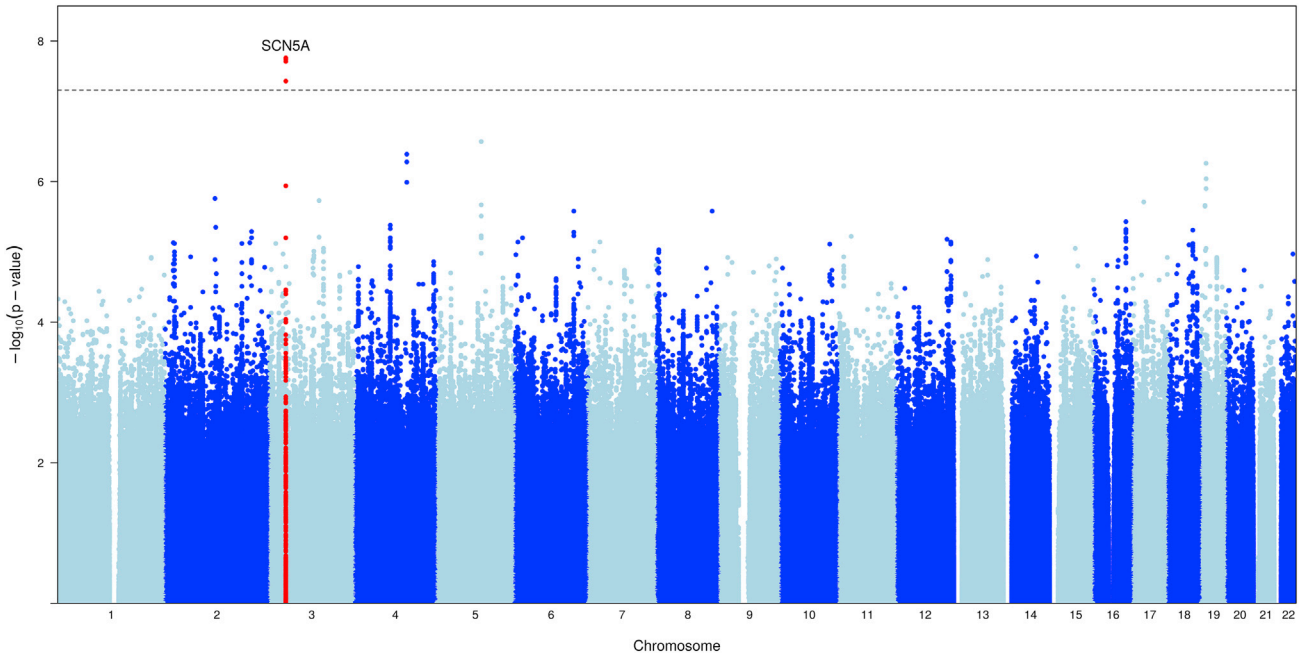

Figure 1. Manhattan plot of the meta-analysis of premature atrial contraction frequency

The genome-wide significant locus is shown in red. The dashed line represents the genome-wide significance threshold ($p < 5 \times 10^{-8}$). See also Figure S1 and Table S2.

from 1000 Genomes), and the risk allele for AF (rs7374540-A) correlates with the allele associated with a higher PAC frequency (rs7373862-A). Variant rs6480708, located in an intron of *SYNPO2L-AS1*, near *SYNPO2L*, and rs2288327, located in an intron of *TTN*, were also associated with PAC frequency (p respectively 0.00020 and 0.0019). On the other hand, the lead AF variant at the *PITX2* locus, rs6847935, was not associated with PAC frequency (p = 0.94). Out of the 106 variants, 73 (68.9%) had a concordant direction of effect between AF and PAC frequency, which is significantly higher than expected by chance ($p = 5.1 \times 10^{-5}$) (Figure S3).

DISCUSSION

We performed the first genetic association study for PAC frequency measured from ambulatory monitoring and identified a robust and reproducible association with common variants located in an intron of *SCN5A*. Among genetic variants previously associated with AF, there was a significant enrichment in concordance of effect for PAC frequency.

SCN5A encodes the alpha subunit of the main cardiac sodium channel Nav1.5, a large transmembrane protein with four homologous domains. It maintains the normal inward sodium current in the heart and has an important role in the fast depolarization phase that enables initiation of the excitation–contraction coupling cascade, for proper conduction of the electrical impulses in the heart (Li et al., 2018). Genetic variants in *SCN5A* have been associated with several arrhythmias, including AF, Brugada syndrome, long QT syndrome, sick sinus syndrome, ventricular fibrillation, and atrioventricular block, along with contractile dysfunction and dilated cardiomyopathy (Akai et al., 2000; Benito et al., 2008; Kapplinger et al., 2010; Wang et al., 2002; Wilde and Amin, 2018). Both gain- and loss-of-function variants located in different coding regions of the protein have been linked to atrial arrhythmias (Li et al., 2018; Wilde and Amin, 2018). *SCN5A* is therefore an obvious candidate causal gene for PAC frequency, although the specific causal variant and mechanism cannot be established with certainty based on our data. Some of the identified SNPs could have a regulatory function, as suggested by modest effects on gene expression in non-cardiac tissues and the presence of regulatory elements in cardiac tissues (GTEx Consortium, 2020; Dong and Boyle, 2019). The SNPs could also tag a rare mutation impacting the protein, although this interpretation is less likely given the consistency of the association among several European ancestry populations from Europe, North America, and South America.

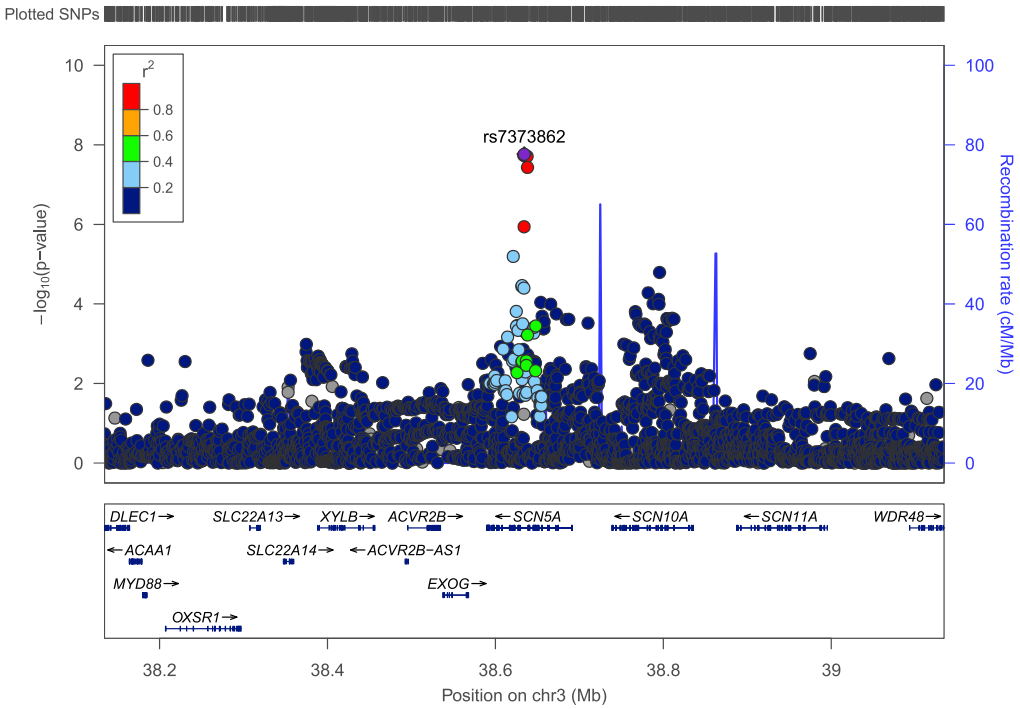

Figure 2. Regional plot at the genome-wide significant locus associated with premature atrial contraction frequency

The lead variant rs7373862 is depicted by a purple diamond. Linkage disequilibrium in relation to the lead SNP is shown using the color scale. See also Table S3.

To our knowledge, a single GWAS was previously published for a closely related phenotype. Napier et al. performed a multi-ancestry GWAS meta-analysis in 42,976 individuals for supraventricular ectopy, defined as one or more supraventricular ectopic beats (a synonym of PACs) on a standard 10-s, twelve-lead electrocardiogram (Napier et al., 2018). Although they did not identify any genome-wide significant association, the lead variant, rs3922844 ($p = 1.5 \times 10^{-7}$), was located in the *SCN5A* gene, about 10 kilobases from our lead SNP. The identification of a genome-wide significant signal at this locus in our study with a smaller sample size underscores the importance of optimizing phenotype measurement to maximize power, in this case by using continuous PAC frequency from ambulatory monitoring.

We also observed associations between PAC frequency and previously reported AF risk variants. In addition to *SCN5A*, variants in two other loci, near *SYNPO2L* and *TTN*, showed a significant association with PAC frequency after correction for multiple testing. Interestingly, the proteins coded by these genes are

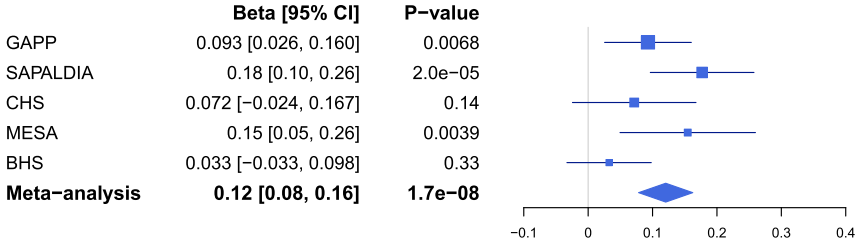

Figure 3. Forest plot representing the effect of the lead variant rs7373862 on premature atrial contraction frequency in each cohort

Beta represents the effect of each risk allele (rs7373862-A) on the normalized number of premature atrial contractions per hour, one unit corresponding to one standard deviation. GAPP: Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors; SAPALDIA: Swiss Cohort Study on Air Pollution and Lung Diseases in Adults, CHS: Cardiovascular Health Study; MESA: Multi-Ethnic Study of Atherosclerosis; BHS: Baependi Heart Study.

Table 2. Replication of selected variants previously associated with atrial fibrillation

Variant	Chr	Pos	RA	NRA	RR AF	*P* AF	Z PAC	*P* PAC	Candidate gene(s)
rs7374540	3	38634142	A	C	1.06	1.7×10^{-12}	4.866	1.1×10^{-6}	*SCN5A*
rs6480708	10	75420114	C	A	1.12	5.8×10^{-27}	3.720	0.00020	*SYNPO2L*
rs7373065	3	38710315	T	C	1.26	3.9×10^{-15}	3.614	0.00030	*SCN5A, SCN10A*
rs6790396	3	38771925	G	C	1.06	5.7×10^{-15}	3.581	0.00034	*SCN10A*
rs2288327	2	179411665	G	A	1.11	3.5×10^{-23}	3.110	0.0019	*TTN*
rs6847935	4	111696651	T	A	1.48	1.9×10^{-427}	0.070	0.94	*PITX2*

Chr: chromosome; Pos: position (GRCh 37); RA: risk allele; NRA: non risk allele; RR AF: relative risk for atrial fibrillation from Roselli et al. (2018); *P* AF: *P* for atrial fibrillation from Roselli et al. (2018); Z PAC: Z score for premature atrial contraction frequency from the current meta-analysis; *P* PAC: *P* for premature atrial contraction frequency from the current meta-analysis. See also Figures S2 and S3 and Table S4.

expressed in the heart and are involved in sarcomere organization (Clausen et al., 2021). Loss-of-function variants in both genes have been associated with AF in humans (Ahlberg et al., 2018; Clausen et al., 2021), and transgenic animal models exhibit cardiac fibrosis and electrical abnormalities (Ahlberg et al., 2018; van Eldik et al., 2017). These mechanisms could be common to PAC frequency and AF. Also, variants near *TTN* have recently been associated with left atrial passive emptying fraction, further supporting the role of this gene in atrial function (Ahlberg et al., 2021).

On the other hand, other AF susceptibility loci showed no significant association with PAC frequency. Moreover, >30% of the AF-associated variants had a discordant direction of effect. Notably, the lead SNP at the strongest AF risk locus, *PITX2*, showed no association with PAC frequency. These findings suggest that the genetic mechanisms involved in the two phenotypes are not identical.

In conclusion, we identified a novel association at the *SCN5A* locus with PAC frequency and the existence of genetic determinants shared between AF and PAC frequency. More studies are needed to further define the biological mechanisms by which cardiac sodium channels and sarcomere organization influence ectopic atrial activity and to elucidate the complete genetic architecture of PAC frequency.

Limitations of the study

First, the distribution of PAC frequency was highly skewed and varied considerably among the cohorts. The use of ambulatory monitoring data and normalization within each cohort produced a robust phenotype adapted to each population. Second, only individuals of European ancestry were included in the analyses due to a low sample size for other ancestries; further studies are needed with other ancestries. The power to detect variants with a small effect was limited by the modest sample size for a genome-wide association study, due to the limited availability of ambulatory electrocardiographic monitoring in large population-based cohort studies. This is nevertheless the first study evaluating genetic determinants of PAC frequency as a continuous variable obtained from ambulatory monitoring.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2022.105210.

ACKNOWLEDGMENTS

We thank the GAPP staff and all GAPP study participants for their important contributions. The GAPP study was supported by the Liechtenstein Government, the Swiss Heart Foundation, the Swiss Society of Hypertension, the University of Basel, the University Hospital Basel, the Hanela Foundation, the Mach-Gaensslen Foundation, Schiller AG, and Novartis.

The study could not have been done without the help of the SAPALDIA study participants, technical and administrative support, and the medical teams and field workers at the local study sites: Study directorate: NM Probst-Hensch (PI; e/g); T Rochat (p), C Schindler (s), N Künzli (e/exp), JM Gaspoz (c) Scientific team: JC Barthélémy (c), W Berger (g), R Bettschart (p), A Bircher (a), C Brombach (n), PO Bridevaux (p), L Burdet (p), Felber Dietrich D (e), M Frey (p), U Frey (pd), MW Gerbase (p), D Gold (e), E de Groot (c), W Karrer (p), F Kronenberg (g), B Martin (pa), A Mehta (e), D Miedinger (o), M Pons (p), F Roche (c), T Rothe (p), P Schmid-Grendelmeyer (a), D Stolz (p), A Schmidt-Trucksäss (pa), J Schwartz (e), A Turk (p), A von Eckardstein (cc), E Zemp Stutz (e). Scientific team at coordinating centers: M Adam (e), I Aguilera (exp), S Brunner (s), D Carballo (c), S Caviezel (pa), I Curjuric (e), A Di Pascale (s), J Dratva (e), R Ducret (s), E Dupuis Lozeron (s), M Eeftens (exp), I Eze (e), E Fischer (g), M Foraster (e), M Germond (s), L Grize (s), S Hansen (e), A Hensel (s), M Imboden (g), A Ineichen (exp), A Jeong (g), D Keidel (s), A Kumar (g), N Maire (s), A Mehta (e), R Meier (exp), E Schaffner (s), T Schikowski (e), M Tsai (exp). SAPALDIA was funded by the Swiss National Science Foundation (grants no 33CS30-177506/1, 33CS30-148470/1&2, 33CSCO-134276/1, 33CSCO-108796, 324730_135673, 3247BO-104283, 3247BO-104288, 3247BO-104284, 3247-065896, 3100-059302, 3200-052720, 3200-042532, 4026-028099, PMPDP3_129021/1, PMPDP3_141671/1), the Federal Office for the Environment, the Federal Office of Public Health, the Federal Office of Roads and Transport, the canton's government of Aargau, Basel-Stadt, Basel-Land, Geneva, Luzern, Ticino, Valais, and Zürich, the Swiss Lung League, the canton's Lung League of Basel Stadt/Basel Landschaft, Geneva, Ticino, Valais, Graubünden and Zurich, Stiftung ehemals Bündner Heilstätten, SUVA, Freiwillige Akademische Gesellschaft, UBS Wealth Foundation, Talecris Biotherapeutics GmbH, Abbott Diagnostics, Klinik Barmelweid, Hirslanden Klinik Aarau, European Commission 018996 (GABRIEL), Wellcome Trust WT 084703MA, Exposomics EC FP7 grant(Grant agreement No: 308610).

The authors thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts 75N92020D00001, HHSN268201500003I, N01-HC-95159, 75N92020D00005, N01-HC-95160, 75N92020D00002, N01-HC-95161, 75N92020D00003, N01-HC-95162, 75N92020D00006, N01-HC-95163, 75N92020D00004, N01-HC-95164, 75N92020D00007, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-000040, UL1-TR-001079, UL1-TR-001420, UL1-TR-001881, and DK063491 and by grant R01 HL127659. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0.

This CHS research was supported by NHLBI contracts HHSN268201200036C, HHSN268200800007C, HHSN268201800001C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, 75N92021D00006; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, U01HL130114, and HL130114, with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR001881,

and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. N.S. is supported by NHLBI R01HL141989 and by the Laughlin Family.

We thank all participants of the Baependi Heart Study and the local staff for their dedication to this project. This work is funded by NIH/NHLBI (R01HL141881).

AUTHOR CONTRIBUTIONS

S.A., K.G., N.M.P.H., M.I., E.S., E.Z.S., W.S.P., S.R.H. contributed to data collection. S.T., N.M.P.H., M.I., E.S., M.L.B., J.A.B., T.R.A., T.M.B., H.J.L., A.C.P. contributed to data analysis. D.C., L.R., N.M.P.H., N.S., S.R.H., A.C.P., J.E.K. supervised the analysis. S.T. drafted the manuscript. All authors revised the manuscript prior to submission.

DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: June 6, 2022
Revised: August 29, 2022
Accepted: September 22, 2022
Published: October 21, 2022

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Summary statistics of the GWAS meta-analysis for PAC frequency	This paper	https://www.ebi.ac.uk/gwas/: accession number GCST90134637
Software and algorithms		
METAL	Willer et al. (2010)	https://genome.sph.umich.edu/wiki/METAL
SAIGE	Zhou et al. (2018)	https://github.com/weizhouUMICH/SAIGE
CAVIAR	Hormozdiari et al. (2014)	https://github.com/fhormoz/caviar
HaploReg	Ward and Kellis (2012)	https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php
RegulomeDB	Dong and Boyle (2019)	https://regulomedb.org/regulome-search/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Sébastien Thériault (sebastien.theriault@criucpq.ulaval.ca).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Summary statistics of the meta-analysis have been deposited on GWAS Catalog and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.
- This paper does not report original code. All codes used in this study followed the manuals of the software listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODELS AND SUBJECT DETAILS

A total of five population-based cohorts were included in the study: Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (GAPP), Swiss Cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA), Cardiovascular Health Study (CHS), Multi-Ethnic Study of Atherosclerosis (MESA), and Baependi Heart Study (BHS). Only individuals of European ancestry were included due to a low sample size for other ancestries. Individuals with a history of AF, pacemaker implantation or those using antiarrhythmic drugs other than beta-blockers and calcium channel blockers were excluded. Institutional review board approval and informed consent for all participating individuals were obtained at the study level. A summary of the clinical characteristics of the individuals included from each cohort is available in Table 1.

Cohort description

GAPP

Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (GAPP) is a population-based prospective cohort study involving a representative sample of healthy adults aged 25–41 years residing in the Principality of Liechtenstein (Conen et al., 2013). Exclusion criteria were the presence of cardiovascular disease, diabetes, obstructive sleep apnoea and a body mass index >35 kg/m^2.

SAPALDIA

Swiss Cohort Study on Air Pollution And Lung And Heart Disease In Adults (SAPALDIA) is a population-based multi-center study in eight geographic areas representing the range of environmental,

meteorological and socio-demographic conditions in Switzerland (Ackermann-Liebrich et al., 1997; Martin et al., 1997). It was initiated in 1991 (SAPALDIA 1) with a follow-up assessment in 2002 (SAPALDIA 2) and 2010 (SAPALDIA3). This study has specifically been designed to investigate longitudinally lung function, respiratory and cardiovascular health; to study and identify the associations of these health indicators with individual long term exposure to air pollution, other toxic inhalants, lifestyle and molecular factors.

CHS

Cardiovascular Health Study (CHS) is a population-based cohort study of risk factors for coronary heart disease and stroke in adults ≥65 years conducted across four field centers (Fried et al., 1991). The original predominantly European ancestry cohort of 5,201 persons was recruited in 1989–1990 from random samples of the Medicare eligibility lists; subsequently, an additional predominantly African American cohort of 687 persons was enrolled for a total sample of 5,888.

MESA

Multi-Ethnic Study of Atherosclerosis (MESA) is a community-based study of subclinical cardiovascular disease in a sample of 6,814 men and women without cardiovascular disease, aged 45–84 years at baseline (2000–2002) (Bild et al., 2002). Participants were enrolled at six US field centers, and self-identified as Black, Chinese American, Hispanic, or White. At Exam 6 (2016–2018), a subset of participants wore one or two 14-day Zio Patch cardiac monitors (Heckbert et al., 2020). The White participants with Zio Patch data were included in this analysis.

BHS

The Baependi Heart Study was set up in 2005 to develop a longitudinal family-based cohort study that reflects on some of the genetic and lifestyle-related peculiarities of the Brazilian populations, in order to evaluate genetic and environmental influences on CVD risk factor traits (Egan et al., 2016).

METHOD DETAILS

Quantification of PACs

PAC frequency was obtained from 24h monitoring using Holter monitors or 2-week monitoring using Zio patches (one study). Participants with a duration of recording of less than 18 h were excluded. The number of PACs per hour was normalized using rank-based inverse normal transformation with the Rankit method, i.e., the inverse cumulative normal function of (r - 1/2)/n, where r is the rank and n the number of observations.

Genotyping and imputation

Genotyping was performed individually by each cohort using a genome-wide array (Table S1). Individuals with a poor genotype call rate, unusually high heterozygosity or sex mismatch and ancestry outliers (verified using principal component analysis) were excluded. Variants with a poor call rate, marked deviations from Hardy Weinberg equilibrium or a low minor allele frequency were excluded. Imputation was performed using reference panels from 1000 Genomes, Haplotype Reference Consortium (HRC), Trans-Omics for Precision Medicine (TOPMed) or the Genetic Investigation of ANthropometric Traits (GIANT) consortium (Table S1).

Study-level association analyses

Association between each variant and normalized PAC frequency was evaluated using linear regression. An additive model was used with the genotype probability (expected allele dosage) for imputed data. Covariables included age, sex and the first ten genetic principal components, along with study-specific covariates (e.g., center, genotyping platform) (Table S1).

GWAS meta-analysis

The summary statistics from each cohort were examined for signs of inflation and discrepancy in allele frequencies. Variants with an imputation quality score <0.3 or minor allele frequency <0.01 were excluded. A meta-analysis was performed using METAL (Willer et al., 2010) with sample size weighting. The genome-wide significance level of $p < 5.0 \times 10^{-8}$ was used. Heterogeneity was evaluated using Cochran's Q-test.

Replication in UK Biobank

We verified the association of the lead SNP at the genome-wide associated locus with a diagnosis of atrial premature depolarisation in UK Biobank (International Classification of Diseases version-10 code number I49.1). Samples were genotyped with the Affymetrix UK BiLEVE Axiom array or the Affymetrix UK Biobank Axiom Array. Phasing and imputation were performed centrally using a reference panel combining the Haplotype Reference Consortium, UK10k and 1000 Genomes Phase 3 samples (Bycroft et al., 2018). Samples with call rate <95%, outlier heterozygosity rate, gender mismatch, non-white British ancestry, samples with excess third-degree relatives (>10), or not used for relatedness calculation were excluded. The association between the genetic variant and atrial premature depolarisation (as a binary phenotype) was evaluated by mixed modeling using SAIGE version 0.45 (Zhou et al., 2018). A selection of independent, high quality genotyped variants (n = 93,511) was used to derive the genetic relationship matrix in step 1. The models were adjusted for age, sex, and the first ten ancestry-based principal components. The analyses were conducted under UK Biobank data application number 25205.

Fine-mapping

A 95% credible set of variants was established at the genome-wide associated locus with a model assuming a single causal variant in CAVIAR (Hormozdiari et al., 2014). Expression quantitative trait loci (eQTL) were retrieved from The Genotype-Tissue Expression (GTEx) project v8 (GTEx Consortium, 2020) and from the Hsu et al. study (Hsu et al., 2018) for the left atrial appendage. In order to prioritize variants with a potential functional impact, HaploReg v.4.1 (Ward and Kellis, 2012) and RegulomeDB (Boyle et al., 2012; Dong and Boyle, 2019) were used to look at regulatory elements, including DNA accessibility, chromatin marks and states.

Replication of atrial fibrillation variants

We selected independent variants previously reported to be associated with atrial fibrillation in a large meta-analysis (Roselli et al., 2018). A list of 107 genome-wide significant variants obtained following conditional analysis in individuals of European ancestry was used to perform replication in our PAC frequency meta-analysis. A threshold of 5% false-discovery rate was used for statistical significance. The proportion of variants with a concordant direction of effect was evaluated using one-sided Pearson's chi-square test.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses were performed with R version 3.6.0 unless otherwise specified. Two-sided p below 0.05 were considered significant unless otherwise specified.