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Genetic Susceptibility to Atrial Fibrillation Identified via Deep Learning of 12-lead Electrocardiograms

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Abstract

Background: Artificial intelligence (AI) models applied to 12-lead electrocardiogram (ECG) waveforms can predict atrial fibrillation (AF), a heritable and morbid arrhythmia. However, the factors forming the basis of risk predictions from AI models are usually not well understood. We hypothesized that there might be a genetic basis for ECG-AI-based risk estimates.

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Methods: We applied a validated ECG-AI model for predicting incident AF to ECGs from 39,986 UK Biobank participants without AF. We then performed a genome-wide association study (GWAS) of the predicted AF risk and compared it to an AF GWAS and a GWAS of risk estimates from a clinical variable model.

Results: In the ECG-AI GWAS, we identified three signals ($P < 5 \times 10^{-8}$) at established AF susceptibility loci marked by the sarcomeric gene *TTN* and sodium channel genes *SCN5A* and *SCN10A*. We also identified two novel loci near the genes *VGLL2* and *EXT1*. In contrast, the clinical variable model prediction GWAS indicated a different genetic profile. In genetic correlation analysis, the prediction from the ECG-AI model was estimated to have a higher correlation with AF than that from the clinical variable model.

Conclusions: Predicted AF risk from an ECG-AI model is influenced by genetic variation implicating sarcomeric, ion channel, and body height pathways. ECG-AI models may identify individuals at risk for disease via specific biological pathways.

Introduction

Atrial fibrillation (AF) is a heritable arrhythmia associated with substantial morbidity, including stroke, heart failure, dementia, and mortality.¹⁻³ Identifying individuals at high risk of developing AF may enable early detection via cardiac rhythm monitoring and treatment, or behavioral modification to prevent AF altogether. Artificial intelligence (AI) algorithms applied to 12-lead electrocardiogram (ECG) waveforms can predict AF.⁴⁻⁶ Algorithms that predict AF risk from ECGs have practical appeal given the ubiquity and inexpensive nature of ECGs, and lack of requirement for manual data input for risk estimation. Whether risk estimates derived from AI algorithms reflect specific underlying genetic pathways that increase susceptibility to AF is unclear.

Understanding the biological basis for risk estimates from machine learning models could aid model interpretability, rationalize model outputs, promote clinician confidence, and potentially enable identification of individuals with specific mechanistic pathways that lead to AF. We recently developed and validated an AI algorithm for predicting the 5-year risk of new-onset AF using 12-lead ECGs (“ECG-AI”).⁷ In the present study, we conducted genetic association testing with AF risk estimates generated from the ECG-AI model to assess the genetic underpinnings reflected by the output. As a comparator, we assessed the genetic basis of a widely validated clinical risk factor model for predicting AF, the CHARGE-AF score (Cohorts for Aging and Research in Genomic Epidemiology–AF).⁸

Methods

The study overview is presented in Figure 1. Full methods are available in Supplemental Material. The data and code that support the findings of the present study are available from the corresponding author upon reasonable request. We used data from the UK Biobank^{9,10} for the analysis in this article. All participants provided electronic signed consent at recruitment, and the study protocol was approved by the UK Biobank Research Ethics Committee (reference number 11/NW/0382). Use of data (under UK Biobank application

7089) for the current study was approved by the Mass General Brigham (MGB) Institutional Review Board.

Results

Sample characteristics

A flowchart showing the process of selecting participants in the derivation (39,986) and validation (424,411) analysis is presented in Figure 1. The mean age of the 39,986 participants included in the GWAS was 64.0 \pm 7.7 years at the time of ECG acquisition and 52% were female. The median follow-up time (starting from ECG visit) for this subset was 2.8 years (quartile 1: 1.9, quartile 3: 4.3). 510 individuals developed incident AF within 5 years of follow-up, corresponding to a cumulative incidence of AF of 2.12%. The mean age of the 424,411 participants included in the polygenic risk score application analysis who did not have ECGs was 57.1 \pm 8.1 years at the time of study enrollment and 55% were female. The median follow-up time (starting from study enrollment) for this subset was 11.1 years (quartile 1: 10.4, quartile 3: 11.8). 7,077 individuals developed incident AF within 5 years of follow up, corresponding to a cumulative AF incidence of 1.70%. Participant characteristics are presented in Table 1.

Genome-wide association analyses

The GWAS of ECG-AI predicted AF risk did not demonstrate any inflation ($\lambda_{gc}=1.04$). Four genome-wide significant ($P < 5 \times 10^{-8}$) loci were identified (Figure 2 and Supplemental Table I). Two of the top SNVs were in close proximity to genes previously reported to be associated with AF, including *TTN* and *SCN10A*.^{11,12} The nearest genes at the other two loci were *VGLL2* and *EXT1*. A conditional analysis detected an additional independent association signal at the *SCN5A* locus (Supplemental Table I), which has also been reported to be associated with AF in previous studies.^{11,13} We provide the summary statistics from a prior AF GWAS¹¹ for SNPs in high LD ($r^2 \geq 0.8$) with the top SNPs at the two novel loci implicated in our ECG-AI GWAS (rs9689288 for *VGLL2* and rs35186392 for *EXT1*) in Supplemental Table II. Additionally, we show LocusZoom plots for the two loci comparing between our ECG-AI GWAS and the reference AF GWAS in Supplemental Figure I. Finally, we present the results of an exploratory expression association analysis in which we tested associations between predicted expression of *EXT1* and *VGLL2* with AF, separately (see Supplementary Methods). We observed a nominal association between the expression levels of *EXT1* and AF ($P=0.04$) and a nonsignificant association between *VGLL2* expression and AF ($P=0.17$; Supplemental Table III).

In the GWAS of CHARGE-AF predicted risk, minimal genomic inflation was observed ($\lambda_{gc}=1.10$) which was likely due to polygenicity rather than population stratification, as implicated by the LD score regression intercept (1.0107). Nineteen loci were identified in the GWAS of CHARGE-AF predicted risk (Figure 2 and Supplemental Table IV), none of which have previously been reported in association with AF. Traits associated with lead SNVs at these loci mainly consist of body size measurements and phenotypes related to the clinical factors included in the CHARGE-AF score calculation (Supplemental Table IV

and Supplemental Table V). No secondary association signals were detected in a conditional analysis.

The LocusZoom plots for risk loci identified in the two GWAS are presented in Supplemental Figure II and Supplemental Figure III, respectively. We also compared the summary statistics of the independent significant lead SNVs in the ECG-AI risk GWAS to that in the CHARGE-AF risk GWAS, and vice versa (Supplemental Table VI). No variants exceed the genome-wide significance threshold in the GWAS of 5-year incident AF with the same covariates (Figure 2).

Heritabilities and genetic correlations

Using individual level genomic and phenotypic data, the estimated heritability (h^2) was 13.0% (s.e. 1.4%) for ECG-AI risk and 36.5% (s.e. 1.4%) for CHARGE-AF risk. Genetic correlations with AF were estimated to be 35.3% (s.e. 13.7%) for ECG-AI risk and 18.9% (s.e. 8.6%) for CHARGE-AF risk. We further estimated the genetic correlation between the predicted AF risks from ECG-AI and CHARGE-AF, and found a significant correlation of 39.3% (s.e. 4.5%). As a comparator, we also calculated the heritabilities and genetic correlations using GWAS summary statistics with LD score regression,¹⁴ and the estimates were similar in magnitude to those calculated from individual-level data. Detailed results are provided in Figure 3 and Supplemental Table VII.

Polygenic risk scores and incident AF

We calculated two polygenic risk scores (PRS) for the 424,411 eligible participants using predicted AF risk GWAS results. Each one standard deviation (SD) increase in the PRS of rank-based inverse normal transformed (R-INT) ECG-AI risk ($PRS_{\text{ECG-AI}}$) and the PRS of R-INT CHARGE-AF risk ($PRS_{\text{CHARGE-AF}}$) were significantly associated with 5-year incident AF ($PRS_{\text{ECG-AI}}$ hazard ratio [HR] 1.07, 95% CI 1.04 - 1.09, $P = 3.0 \times 10^{-8}$; and $PRS_{\text{CHARGE-AF}}$ HR 1.12, 95% CI 1.09 - 1.14, $P = 3.4 \times 10^{-19}$). When included in the same model, both remained significantly associated with 5-year incident AF ($PRS_{\text{ECG-AI}}$ HR 1.06, 95% CI 1.04 - 1.09, $P = 1.1 \times 10^{-6}$; and $PRS_{\text{CHARGE-AF}}$ HR 1.11, 95% CI 1.08 - 1.14, $P = 1.1 \times 10^{-17}$). The C-index of models testing the performance of $PRS_{\text{ECG-AI}}$, $PRS_{\text{CHARGE-AF}}$, and the two scores together are presented in Supplemental Table VIII. We observed that the C-index was comparable using $PRS_{\text{ECG-AI}}$ and $PRS_{\text{CHARGE-AF}}$ and was highest when including the two scores; the pattern of discrimination is similar to that reported in the original report of the derivation of the ECG-AI score.⁷

We did not observe a significant interaction between the two PRSs ($P = 0.97$). We also plotted the cumulative risk of AF stratified by high (10%), middle (80%), and low (10%) groupings of the PRS distributions and observed separation between groups (Supplemental Figure IV). Due to a difference in the ancestral composition of the GWAS discovery set (White British: 96.6%) and PRS testing set (White British: 87.2%), we repeated the above analysis in the subset of the PRS testing set comprising White British participants only. We observed similar results (Supplemental Table IX and Supplemental Figure V).

Contributing components of ECG-AI predicted AF risk

Informed by the genetic signals from our common variant analysis, we tested the causal effects of P wave duration and body height on ECG-AI predicted risk using a two sample Mendelian Randomization (MR) approach. We extracted GWAS summary statistics from studies that did not include UK Biobank samples for P wave duration and body height (see Supplemental Methods). The P wave was selected because (1) it had the greatest impact on ECG-AI predicted risk indicated by saliency maps and a median waveform analysis in our previous study,⁷ (2) previous literature suggests an association between ECG P-wave duration and AF risk,¹⁵ and (3) it has been linked to *SCN5A/SCN10A* in previous genetic studies¹⁶ and an overlap between genetic variants associated with the P-wave and AF has been reported.¹⁷ Body height was selected because (1) it has been linked to *VGLL2* and *EXT1* loci,^{18,19} (2) is an established risk factor for AF,^{20,21} and (3) there is published evidence showing that neural networks applied to 12-lead ECGs can predict body size measurements.²²

A significant and plausible causal effect of P wave duration on ECG-AI risk was supported by four out of the five methods we used, with MR effect sizes ranging from 0.017 to 0.023. The causal effect of height on ECG-AI risk was supported by all five methods, with MR effect sizes ranging from 0.085 to 0.123. Results are presented in Figure 4.

Additionally, to discern what ECG features were responsible for associations with observed genes, in exploratory analyses we plotted the median ECG waveforms for individuals in the highest and lowest 1% of the regional PRS for top loci (Supplemental Figure VI). We note that differences are observable but are subtle between risk groups. Given that ECGs were only available for a subset of the UK Biobank participants (N=39,986 in the current study), we anticipate this approach will be more informative as the ECG sample size increases.

Discussion

To facilitate the interpretability of a validated deep-learning model that predicts AF risk from 12-lead ECGs, we assessed the genetic basis of risk predictions generated from the ECG-AI model. Despite the fact that individuals did not have AF at the time of ECG acquisition, we identified variants at three established AF susceptibility loci – *TTN*, *SCN5A*, and *SCN10A* – and at two novel loci that implicate body size measurements – *VGLL2* and *EXT1*. In contrast, our GWAS of CHARGE-AF derived AF risk did not identify any signals previously reported in a GWAS for AF, but identified loci linked to component clinical risk factors for AF that are included in the risk model. Our MR analyses provide supporting evidence that P wave duration and body height are predictive factors for AF that were captured by the ECG-AI model. Broadly, our findings imply that estimates of disease risk from deep learning models that use raw physiologic data, and risk scores more generally, are influenced by genetic susceptibility. In turn, such deep learning models may have the potential to identify individuals at risk for disease via specific genetic pathways.

Deep learning models of 12-lead ECGs for the identification of individuals with a high likelihood of AF have been reported⁵⁻⁷ but the interpretability and representations that underlie the risk estimates generated by the models have not been explained. We previously

observed that ECG-AI estimates for AF risk are largely influenced by the P wave, a period corresponding to atrial depolarization and repolarization.⁷ Moreover, we have previously reported that both ECG-AI and clinical risk for AF are complementary.⁷ Here, we extend these observations by identifying genetic signals that have been associated with P wave duration,¹⁷ and documenting the distinct genetic profiles underlying risk estimates generated by ECG-AI and a clinical risk factor model. Our findings are consistent with the previous observation that ECG-AI and CHARGE-AF models are complementary in terms of predictive utility,⁷ which may be attributed to the different biological pathways captured by different risk prediction models.

Our findings have two major implications. First, risk estimates from the ECG-AI model are influenced by genetic mechanisms that are more specific to AF than are those from a clinical risk factor model. Specifically, loss-of-function variants in *TTN* have been associated with a substantially increased risk for AF^{23,24} and common variants at this locus have been associated with AF.¹¹ *TTN* encodes titin, an integral protein involved in sarcomere development, structural integrity, and contractility.²⁵ The ECG-AI GWAS also identified variation at the *SCN5A* and *SCN10A* loci. Both *SCN5A* and *SCN10A* encode the alpha subunits of voltage gated sodium channels and *SCN5A* is essential for myocyte depolarization. Genetic variants at these loci have been described in association with AF and ECG traits in prior GWAS^{11,16,26,27} and in rare familial forms of AF.^{13,28-30} *VGLL2* encodes for the vestigial like family member 2a protein, is critical for skeletal muscle development and contains an interacting domain for TEAD1, a member of the Hippo signaling pathway.³¹ *EXT1* encodes a protein involved in the production of heparan sulfate³², and has been implicated in the development of the outflow tract.³³ We note that the *EXT1* and *VGLL2* loci are relative gene-dense loci, and we have focused here on the nearest genes as a means of prioritization.

In contrast, the genetic signals for CHARGE-AF risk were not specific to AF but reflected the diverse genetic mechanisms underlying the component risk factors in the model, including body height, body weight, blood pressure, and smoking status. Notably, the ECG-AI model loci linked to body size – *VGLL2* and *EXT1* – differ from the CHARGE-AF model loci linked to body size, implying that the manifestation of body size on the ECG may reflect different dimensions of body size from those measured conventionally using height and weight. The relative specificity of the ECG-AI model for genetic mechanisms underlying AF is further supported by our observation that the genetic correlation with AF from a prior GWAS was greater with ECG-AI predicted risk than with CHARGE-AF predicted risk.

Overall, our finding that predicted risk of AF from an ECG-based deep learning model is influenced by inherited susceptibility raises the possibility that an individual's genetic predisposition to AF is inferable using their raw ECG data alone, even prior to disease onset. Indeed, the GWAS of incident AF did not identify any significant genetic susceptibility loci, underscoring the power of performing a GWAS of models trained to predict risk of a disease, rather than of the disease itself. Future analysis is warranted to assess whether ECG-AI, and other risk models more broadly, can be used to specifically predict which individuals are predisposed to diseases via particular biological mechanisms. Such insights

could theoretically have important implications for the personalization of prevention and therapeutics, identification of individuals with genetic disorders, and the use of deep learning or other risk models as digital biomarkers in addition to general risk prediction models.

Second, the genetic analysis of risk estimates generated by AI models may facilitate the interpretation of output, and underlying representations, of the models which may otherwise be difficult to interpret. Our GWAS, and MR analyses, highlight the fact that our ECG-AI model is influenced by heritable factors related to the P-wave and body size. The fact that ECG-AI can infer anthropometric traits²² suggests that deep learning model estimates from raw physiologic data can be influenced by numerous variables which manifest on the data modality under study. Height has been causally linked to AF risk previously.^{20,21} Future work is warranted to understand which specific ECG features reflect risk factors for AF and how artificial intelligence models learn and predict risk factors for AF. We propose the transfer of clinically-derived models to datasets with genomic information and subsequent genetic association testing, as a means to explore the factors which influence risk estimates from deep learning models. We submit that this approach could serve as a tool for improving model interpretability when the prediction models are otherwise difficult to understand.

We further note that whereas our approach focused on a single model task – the predicted risk of AF – examining the genetic architecture of the model latent space itself may reveal the genetic basis for the ECG representations learned by the model. As the number of samples with both ECG and genomic data increase, we anticipate greater statistical power to identify genetic signals underlying predicted disease risk, including rare large-effect variants. Given the fact that the ECG-AI GWAS implicated known AF risk loci in individuals without AF, the approach we employed may have the potential to identify novel disease-related pathways as sample sizes grow. Moreover, we submit that the increasing availability of large-scale biobank data with raw data acquisition amenable to deep learning will enable examination of the genetic basis of other disease predictions. We anticipate that improved understanding of the relations between biological pathways and deep learning models may facilitate model application in clinical practice by enhancing clinician confidence in model outputs, and facilitating the inference of biological pathways that lead to disease risk in specific individuals.

Our study should be interpreted in the context of the design. First, our phenotyping algorithm used hospitalization and death records to ascertain disease status, which may lead to disease misclassification. However, before applying the ECG-AI model in UKBB, we omitted individuals that were not identified as prevalent AF by the phenotyping algorithm but were indicated as AF by their diagnostic statement accompanying ECG data, to reduce the impact of misclassification. Second, the MGH dataset we used to train the ECG-AI model and the UKBB dataset we used to perform genetic analyses consist predominantly of White British participants, which may limit the generalizability of our findings to populations of other ancestries. It is unclear if the results of the GWAS of ECG-AI reflect the underlying composition of the sample in which it was trained – future analysis of other ECG-AI models for AF prediction are warranted. Third, as additional and larger biorepository datasets emerge, replication of the ECG-AI GWAS will be necessary to

support the utility of our approach for understanding disease prediction models. Functional validation will be required to support the mechanisms by which genetic loci predispose to disease risk. Fourth, risk discrimination of the ECG-AI model in UKBB is moderate. A model with greater discrimination in the discovery sample may increase yield of genetic associations. Fifth, given roughly three years of follow-up after ECG in the UK Biobank, the power of our incident AF analysis may be limited. Sixth, we note that neither the GWAS of the ECG-AI model nor the CHARGE-AF model identified some established AF susceptibility loci, including that upstream of *PITX2*, the predominant AF common variant susceptibility locus. We note that *PITX2* is not identified in any ECG trait GWAS, which implies that the mechanism underlying association with AF for this risk locus does not prominently involve factors that impact the ECG. Our findings highlight the fact that specific genetic susceptibility loci identified when performing association testing using a particular modality will reflect the risk pathways which manifest on that modality. Seventh, when interpreting the genome-wide associations with disease risk estimates generated from prediction models, the signals may reflect confounders rather than causal risk factors for the disease. However, instead of discovering novel causal genes for AF, the current study aims to understand the factors forming the basis of predictions generated from the ECG-AI model. Our analysis identified factors that manifest on the ECG and are interpreted by the neural network to contribute to disease risk. Lastly, 2,000 individuals among the 39,986 participants in the discovery set were related (3rd degree or closer), which may impact the GWAS results generated by the REGENIE software. While REGENIE includes a genetic relatedness matrix in its analysis, which may address this issue to some extent, it is important to note that other factors, such as the degree of relatedness, the size of the dataset, and the underlying genetic architecture of the trait of interest, may still affect the results.

In conclusion, we have shown that ECG-AI predicted AF risk reflects inherited predisposition to AF with a genetic background that is more specific for AF risk loci when compared to that for a clinical model. A polygenic risk score constructed using common variants associated with ECG-AI risk was significantly associated with incident AF in a prospective cohort. The interpretability of the ECG-AI model was improved by genetic analyses indicating that P wave duration and body height are likely to be two contributing factors forming the basis of AF risk predictions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

CHARGE-AF:	Cohorts for Aging and Research in Genomic Epidemiology–Atrial Fibrillation.
CNN:	convolutional neural network.
ECG-AI:	an artificial intelligence algorithm for predicting the 5-year risk of new-onset atrial fibrillation using 12-lead ECGs.
LD:	linkage disequilibrium
R-INT:	Rank-based inverse normal transformation.
SNPs:	single nucleotide polymorphisms
SNVs:	single nucleotide variants

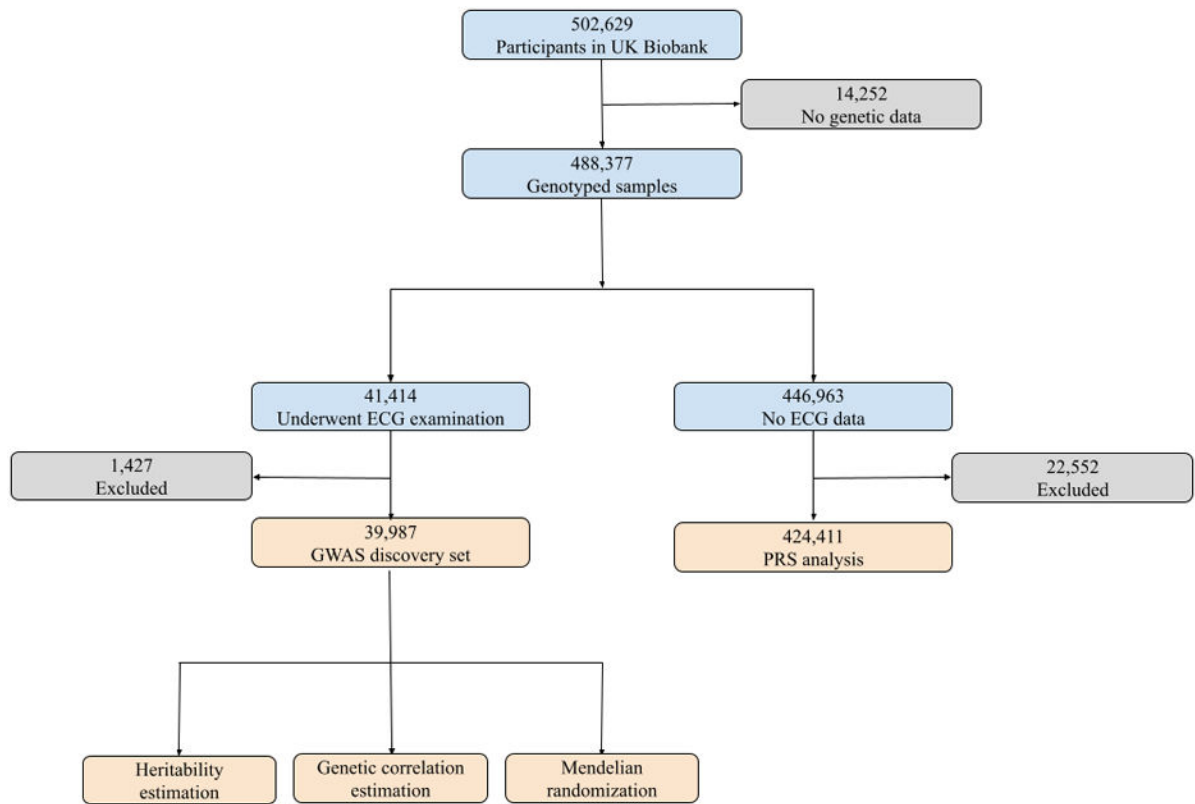
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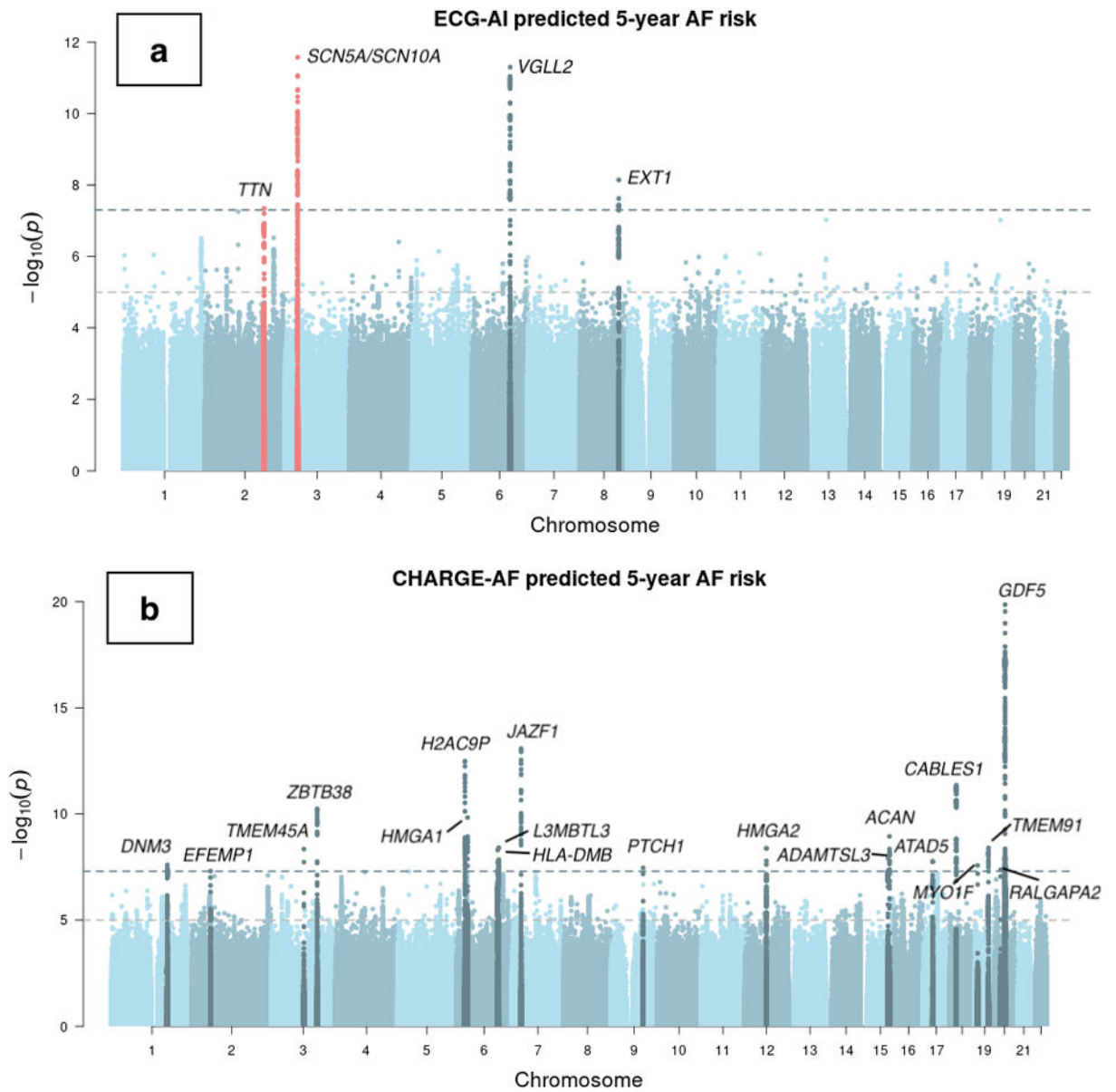
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**Figure 1.**

Study overview. We applied our validated ECG-AI model to samples with ECG data in the UK Biobank. After excluding participants who withdrew consent, had missing CHARGE-AF components, were diagnosed with atrial fibrillation (AF) before ECG examination, did not have follow-up information or failed sample QC procedures, 39,987 remained in the discovery set, in which we performed GWAS and post-GWAS analyses. Among the 446,963 remaining genotyping samples, 424,411 did not withdraw consent, were not < 3rd-degree relatives with individuals in the GWAS set, were not diagnosed with AF before enrollment, had follow-up information, and did not fail sample QC procedures. We calculated polygenic risk scores (PRS) using the GWAS results and associated the PRS with incident AF in this subset.



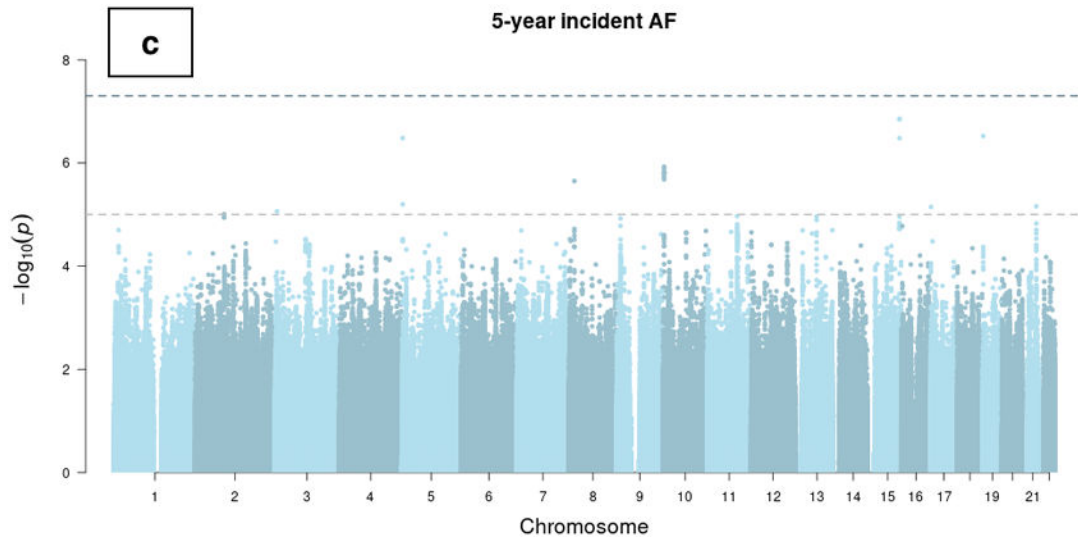
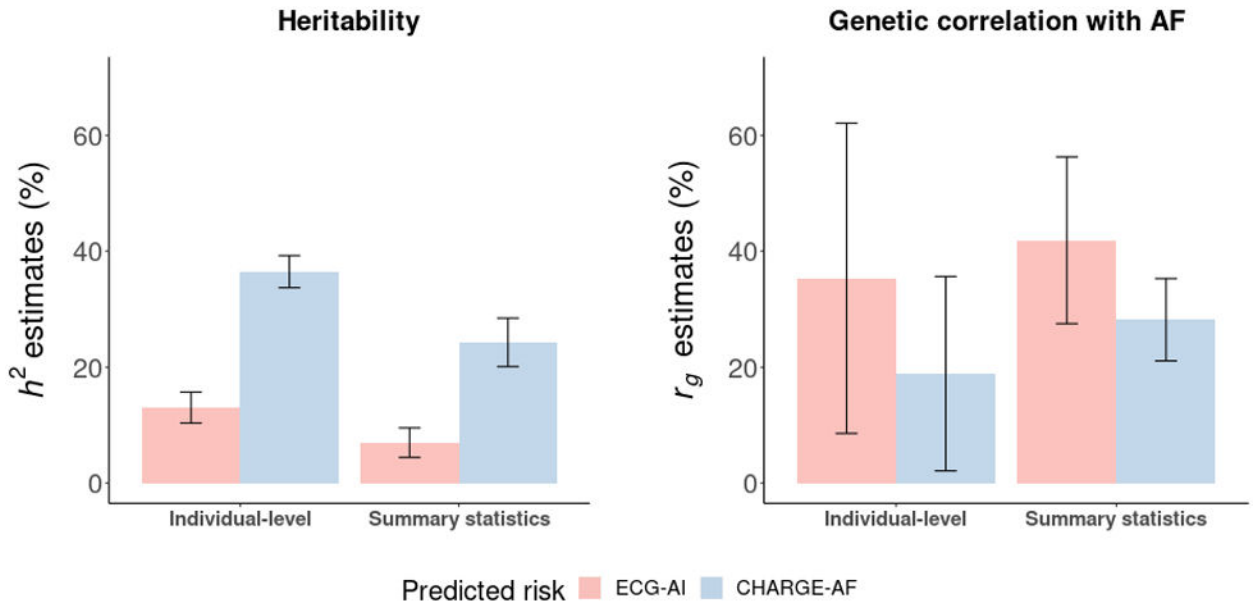


Figure 2.

Manhattan plots of genome-wide association studies of ECG-AI and CHARGE-AF predicted risk of AF, and observed 5-year incident AF in the UK Biobank. Chromosomal variant positions are plotted on the x-axis. The $-\log_{10}(P)$ values are plotted on the y-axis. The genome-wide significance threshold (5×10^{-8}) is indicated by the horizontal dotted line. Variants are colored red near loci that have been reported in a prior atrial fibrillation (AF) GWAS,²¹ and are colored dark blue near loci that have not been reported previously in association with AF. Panels display associations with (a) ECG-AI predicted 5-year risk of AF, (b) CHARGE-AF predicted 5-year risk of AF, and (c) observed incident AF at 5-years in the UK Biobank. 5-year AF risk estimates were rank-based inverse normal transformed prior to analysis (see text).

**Figure 3.**

Heritability and genetic correlation estimates for model predicted 5-year atrial fibrillation (AF) risk. Heritability (h^2) derived from the ECG-AI and CHARGE-AF GWAS are displayed on the left panel. Genetic correlation (r_g) comparing both the ECG-AI and CHARGE-AF GWAS with a prior independent large-scale GWAS of AF,²¹ is displayed on the right panel. ‘Individual-level’ refers to estimates generated from individual-level genetic and phenotypic data. ‘Summary statistics’ refers to estimates generated from GWAS results. Summary statistics for ECG-AI risk and CHARGE-AF risk were extracted from the GWAS in the present study.

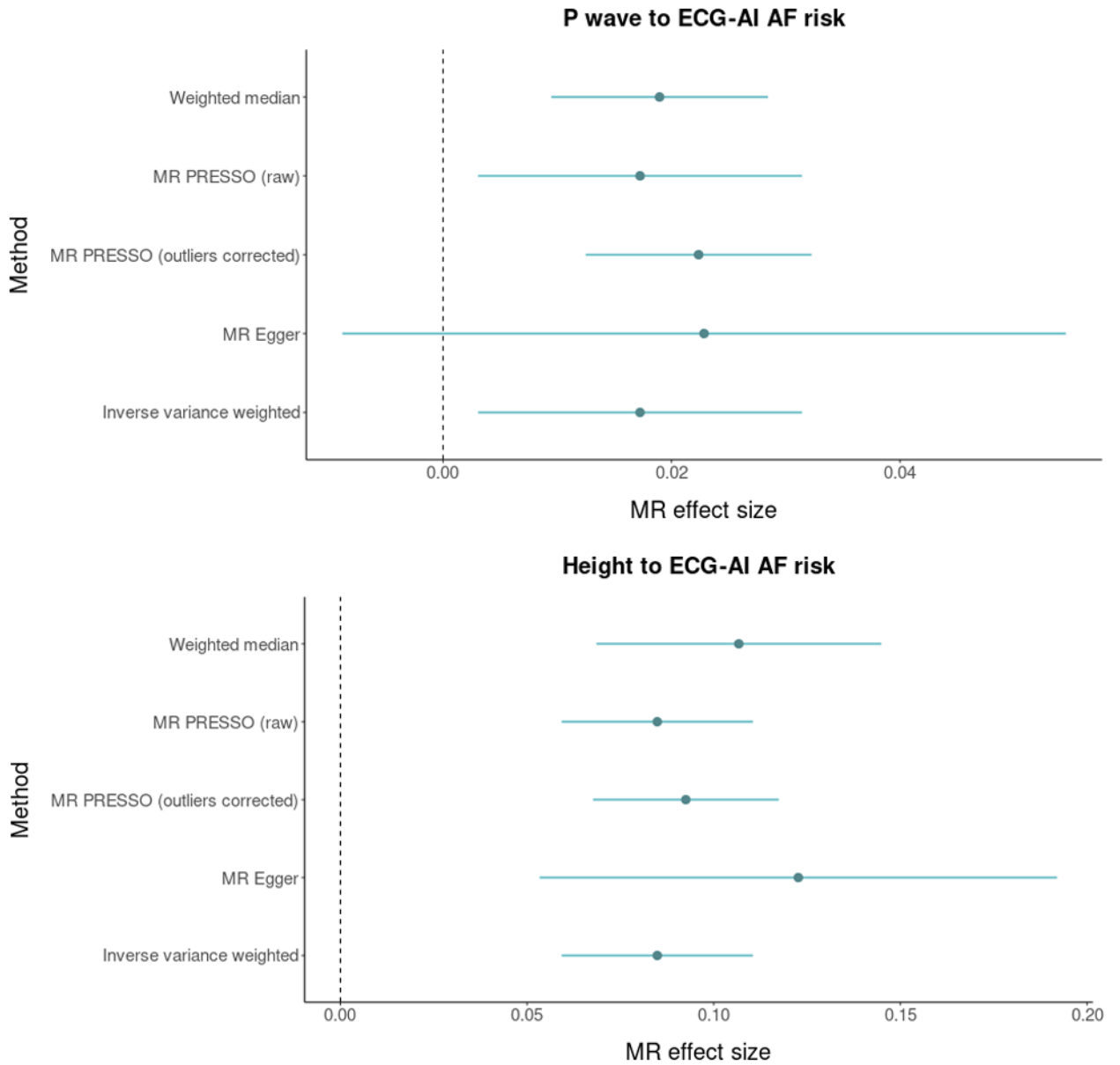


Figure 4. Mendelian Randomization analysis between P wave duration, body height, and atrial fibrillation (AF). The figure displays Mendelian randomization results assessing ECG P wave duration (top panel) and body height (bottom panel) for relations with genetically predicted ECG-AI risk. Mendelian randomization effect sizes are graphed on the x-axis. Dots represent point estimates and bars represent 95% confidence intervals. Dashed gray lines represent zero effect sizes. The five Mendelian randomization methods used in this analysis are shown in the y-axis.

Table 1.

Characteristics of the UK Biobank cohort participants.

Variable	GWAS discovery set (N=39,986)	PRS testing set (N=424,411)
Enrollment age	55.6 (7.6)	57.1 (8.1)
ECG age	64.0 (7.7)	Not applicable
Female	20,809 (52.0%)	232,654 (54.8%)
Race (White British)	38,617 (96.6%)	370,121 (87.2%)
Height (cm)	169.1 (9.2)	168.3 (9.3)
Weight (kg)	76.0 (15.2)	78.0 (16.0)
Systolic blood pressure	138.3 (18.6)	138.1 (18.7)
Diastolic blood pressure	79.1 (10.1)	82.4 (10.2)
Smoking (current)	1,446 (3.6%)	46,433 (11.0%)
Antihypertensive medication use (Yes)	4,275 (10.7%)	88,887 (18.6%)
Diabetes (Yes)	1,546 (3.9%)	11,089 (2.6%)
Heart failure (Yes)	186 (0.5%)	1,701 (0.4%)
Myocardial infarction (Yes)	916 (2.3%)	9,657 (2.3%)

Clinical variables were ascertained at baselines. For the genome-wide association studies (GWAS) discovery set, baseline refers to the time of electrocardiogram (ECG) visit. For the polygenic risk scores (PRS) testing set, baseline refers to the time of study enrollment. Descriptive statistics of enrollment age, female, race, and history of diabetes, heart failure and myocardial infarction for the PRS testing set were calculated using the complete sample (N=424,411). Height, weight, systolic blood pressure and diastolic blood pressure were summarized in a subset with the data available (N=423,044). The sample size for participants who self-reported their smoking status and medication use were 423,919 and 477,644, respectively.