

PITX2 rs67249485 and age, not the postoperative inflammatory response, predict new-onset atrial fibrillation after cardiac surgery with cardiopulmonary bypass: a prospective genotyped cohort study (INFLACOR)

Aneta Szofer-Sendrowska

University Clinical Centre in Gdańsk

Martyna Kaja Sendrowska

Amsterdam University Medical Center

Damian Sendrowski

`damian.sendrowski@upsl.edu.pl`

Pomeranian University in Słupsk

Bartosz Słomiński

Medical University of Gdańsk

Krzysztof Lewandowski

Medical University of Gdańsk

Maciej Brzeziński

Medical University of Gdańsk

Romuald Lango

Medical University of Gdańsk

Maciej M. Kowalik

Medical University of Gdańsk

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Abstract

New-onset atrial fibrillation is the most frequent arrhythmia after cardiac surgery and is widely attributed to the inflammatory response to cardiopulmonary bypass, yet whether measured inflammation or constitutional predisposition governs individual risk is unsettled. In the prospective INFLACOR cohort, designed to relate perioperative inflammation to postoperative complications, we contrasted first-day postoperative inflammatory markers with variants at atrial-fibrillation loci. Of 525 adults undergoing first-time cardiac surgery on cardiopulmonary bypass, 364 without pre-existing atrial fibrillation formed the analytical sample. Four variants were genotyped: rs67249485 at the 4q25 locus upstream of *PITX2*, rs2359171 in *ZFHX3*, rs883079 in *TBX5* and rs11773845 in *CAV1*. A treated first episode from the second postoperative day onwards occurred in 115 patients (31.6%). Only rs67249485 was associated with the arrhythmia: risk-allele frequency was 23.5% in patients who fibrillated and 15.7% in those who did not (allelic risk ratio 1.38, 95% confidence interval 1.08 to 1.76; heterozygote risk ratio 1.55). No postoperative inflammatory marker, and none of ten inflammation-related variants genotyped under the original protocol, distinguished the groups. In multivariable analysis only age (odds ratio 1.71 per decade, 1.36 to 2.16) and the heterozygous *PITX2* genotype (odds ratio 1.91, 1.19 to 3.06) remained independent predictors. The arrhythmia was associated with early respiratory failure, acute kidney injury and delirium but not with mortality at 30 days, one year or five years. Susceptibility appears to be set by the patient's constitution rather than by the inflammatory response to the operation.

ClinicalTrials.gov NCT01020409, registered 24 November 2009, retrospectively registered.

Introduction

New-onset atrial fibrillation (NOAF) is the most common complication of cardiac surgery. Depending on the procedure and on the intensity of rhythm monitoring, transient atrial fibrillation is reported in 15% to 60% of patients (Chatterjee et al. 2026; Prasetyadhi et al. 2026). Although frequently self-limiting, it prolongs intensive-care and hospital stay, raises the cost of treatment, and has been linked to stroke, to a range of early complications and to reduced long-term survival (Thorén et al. 2020; Kawczynski et al. 2025; Chatterjee et al. 2026). Predicting which patient will fibrillate therefore remains a practical concern for the surgical team, and the most recent society guidance continues to describe risk stratification as the weakest link in the management of the arrhythmia (Chatterjee et al. 2026).

Two explanations have competed for primacy. The first is inflammatory. Cardiopulmonary bypass (CPB) provokes a systemic inflammatory response, and the resulting cytokine surge, pericardial injury and oxidative stress have been proposed as the proximate trigger of postoperative atrial fibrillation ever since the temporal association between complement activation, C-reactive protein and postoperative arrhythmia was first described (Bruins et al. 1997). Experimental work has since given the hypothesis a plausible molecular substrate in the atrial NLRP3-CaMKII axis (Heijman et al. 2020), and the concept of an inflamed, remodelled atrial cardiomyopathy has become central to contemporary thinking about the arrhythmia (Goette et al. 2024).

The second explanation is constitutional. Genome-wide association studies have repeatedly implicated the chromosome 4q25 region adjacent to *PITX2*, which encodes a paired-like homeodomain transcription factor governing left-atrial identity (Gudbjartsson et al. 2007; Roselli et al. 2018). In the mouse, *Pitx2* prevents atrial arrhythmia by repressing a left-sided pacemaker programme (Wang et al. 2010), and reduced *Pitx2c* expression in the adult left atrium increases the inducibility of atrial fibrillation together with complex changes in gene expression (Kirchhof et al. 2011). Reduced *PITX2* dosage alters microRNA signalling, ion-channel expression, calcium homeostasis and electrical remodelling in the left atrium, and a growing body of experimental and clinical work links individual 4q25 variants to distinct electrophysiological phenotypes and to differing responses to treatment (Jiménez-Sábado and Hove-Madsen 2025). Variants at this locus, first identified in ambulatory populations, were subsequently shown to predict atrial fibrillation after coronary artery bypass grafting (Body et al. 2009), in mixed cardiac-surgery cohorts (Sigurdsson et al. 2015) and in nationwide biobank analyses (Christensen et al. 2023). Related loci, including *ZFHX3* (Benjamin et al. 2009) and innate-immune genes such as *LY96* (Kertai et al. 2015), have also been tied to the arrhythmia, and combined genetic and clinical scores (Kolek et al. 2015) as well as genome-wide polygenic risk scores (Kertai et al. 2021) have been proposed to sharpen prediction. The 4q25 variant examined here, rs67249485, lies in the intergenic region upstream of *PITX2*, emerged from multi-ethnic genome-wide association analysis (Roselli et al. 2018), and has been shown in Mendelian randomisation to drive the genetic association between susceptibility to atrial fibrillation and left-atrial enlargement with reduced left-atrial ejection fraction (van de Vegte et al. 2021).

The INFLACOR (INFLammation After Cardiac OpeRation) study was designed to test the inflammatory hypothesis directly, by relating clinical variables, circulating cytokines and inflammation-related gene variants to eight postoperative complications, atrial fibrillation among them (Kowalik et al. 2018; Kowalik et al. 2020; Kowalik et al. 2026). The present analysis uses that cohort to ask a focused question: in patients without prior atrial fibrillation, is NOAF after on-pump surgery predicted by the measured postoperative inflammatory response, by variants at loci associated with atrial fibrillation in the general population, or by neither? The aim was to identify pre-, intra- and postoperative risk factors for NOAF, with particular attention to postoperative inflammatory markers and to the selected susceptibility variants, and to examine the interdependence of the factors identified. With respect to the four loci, the analysis is a replication study: it tests, in the setting of cardiac surgery, associations established in ambulatory populations, using for the 4q25 locus a marker (rs67249485) different from the classic markers rs2200733 and rs10033464 used in the earlier candidate-variant study of postoperative atrial fibrillation (Body et al. 2009).

Material and methods

Study design, ethics and population

INFLACOR was a prospective observational cohort study conducted at a single tertiary academic centre between 22 October 2009 and 16 March 2011, enrolling 525 adult patients scheduled for first-time open-

chest cardiac surgery with CPB (Kowalik et al. 2018; Kowalik et al. 2020; Kowalik et al. 2026). Patients undergoing off-pump procedures, reoperations or emergency operations, and those younger than 18 years or unwilling to consent, were excluded. All participants were adult Polish patients of European ancestry (Kowalik et al. 2018). All procedures involving human participants were performed in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments. The protocol was approved by the Independent Bioethics Committee for Scientific Research of the Medical University of Gdańsk (NKEBN/358/2007) and registered at ClinicalTrials.gov on 24 November 2009 (NCT01020409), after enrolment had begun on 22 October 2009; the registration was therefore retrospective. Written informed consent was obtained from every participant. The size of the original cohort was calculated with the Kelsey formula to give 80% power, at a two-sided α of 0.05, to detect a genotype effect with an odds ratio of at least 1.65 ($n = 492$, rounded up to 525 to allow for losses) (Kowalik et al. 2020). The present analysis is reported in accordance with the STROBE statement for observational studies (von Elm et al. 2007); the completed checklist is provided as Online Resource 1. The additional items recommended for genetic association studies by the STREGA extension (Little et al. 2009), namely genotyping methods and quality control, Hardy-Weinberg equilibrium, the genetic model examined and the characteristics of the source population, are addressed in the text and mapped, item by item, in the same checklist.

For this analysis, three patients were excluded for insufficient data, and patients with pre-existing permanent ($n = 111$; 21.2%) or paroxysmal ($n = 47$; 9.0%) atrial fibrillation were removed so that a genuine new-onset phenotype could be studied. The final analytical sample comprised 364 patients (69.3% of the cohort), in whom the group developing a first postoperative atrial-fibrillation episode (NOAF) was compared with the group that remained in sinus rhythm. Sex was recorded as the biological sex documented in the hospital record and was entered as a candidate variable; no analysis was stratified by sex, because sex was not associated with the endpoint on univariate testing.

Endpoints and candidate variables

The primary endpoint was the occurrence of NOAF during the postoperative course. NOAF was defined as a first episode of atrial fibrillation arising after operation in a patient with no history of the arrhythmia, and of sufficient clinical significance to prompt treatment, whether with an antiarrhythmic drug, with rate control or by cardioversion. Cardiac rhythm was monitored from the operation until hospital discharge, by continuous telemetry in the cardiac surgical intensive care unit and by electrocardiography after transfer to the ward. The observation window for the arrhythmia began on the second postoperative day and ended at discharge; episodes confined to the day of operation or to the first postoperative day were not counted. Patients not meeting the criterion for NOAF formed the comparison group. Secondary endpoints were the association of NOAF with early postoperative morbidity, namely stroke, perioperative myocardial infarction, acute circulatory failure, acute kidney injury, acute respiratory failure, systemic inflammatory response and delirium, and with 30-day, one-year and five-year mortality. Acute respiratory

failure was defined as a $\text{PaO}_2/\text{FiO}_2$ ratio below 300 mmHg, acute kidney injury as an AKIN stage above 1, and delirium as an acute disturbance of consciousness requiring pharmacological treatment.

Preoperative candidate variables comprised demographic data (age, sex, body mass index [BMI]); comorbidities (diabetes, chronic kidney disease, chronic obstructive pulmonary disease, coronary artery disease, prior myocardial infarction, peripheral atherosclerosis, hypertension, prior stroke); medications (acetylsalicylic acid, statins, β -blockers, angiotensin-converting-enzyme inhibitors or angiotensin-receptor blockers); laboratory values (creatinine, estimated glomerular filtration rate [eGFR], C-reactive protein, white-blood-cell count); and echocardiographic left-atrial diameter and left-ventricular ejection fraction. Intraoperative variables were operation, CPB and aortic cross-clamp times, procedure type and the number of transfused blood-product units. Postoperative first-day clinical and laboratory variables included interleukin-6, soluble intercellular adhesion molecule-1, soluble E-selectin, C-reactive protein, creatine kinase-MB, white-blood-cell count and the genotyped polymorphisms. Interleukin-6, soluble intercellular adhesion molecule-1 and soluble E-selectin were measured in serum obtained three hours after admission to intensive care, stored at -80°C and assayed by bead-based flow cytometry (Cytometric Bead Array Flex Sets on a FACSCanto II cytometer, BD Biosciences, San Diego, CA, USA) (Kowalik et al. 2018).

Genotyping

Beyond the ten inflammation-related single-nucleotide polymorphisms (SNPs) genotyped under the original INFLACOR protocol (Kowalik et al. 2018), four further variants previously associated with atrial fibrillation in the general population were genotyped for this analysis. These were selected for the highest reported effect sizes or epigenetic relevance, and for a minor-allele frequency of at least 20%: *PITX2* rs67249485 (4q25, intergenic, upstream of *PITX2*), *ZFHX3* rs2359171, *TBX5* rs883079 and *CAV1* rs11773845. Three of these genes (*PITX2*, *ZFHX3*, *TBX5*) encode transcription factors, while *CAV1* encodes the structural myocardial protein caveolin-1.

Blood for genotyping and cytokine assays was drawn three hours after admission to the postoperative intensive care unit; cells were separated by cooled centrifugation and genomic DNA was extracted from leukocytes with a Genomic Midi AX kit (A&A Biotechnology, Poland) (Kowalik et al. 2018). The ten inflammation-related variants were amplified in two multiplex polymerase chain reactions and genotyped by single-base primer extension (SNaPshot Multiplex Kit, Life Technologies, Foster City, CA, USA) with capillary electrophoresis on an Applied Biosystems 3130 Genetic Analyzer and GeneMapper 4.0 analysis; genotypes of selected samples were confirmed by independent amplification and bidirectional Sanger sequencing (Kowalik et al. 2018). The four atrial-fibrillation-associated variants were genotyped from the same DNA by the same single-base extension protocol in the Department of Biology and Medical Genetics, Medical University of Gdańsk; genotypes were obtained for 358 of the 364 patients in the analytical sample (call rate 98.4%). Investigators performing genetic, cytokine and laboratory testing were unaware of clinical data and outcome measures (Kowalik et al. 2026). Genotype distributions were

tested for departure from Hardy-Weinberg equilibrium in the whole genotyped sample by the χ^2 goodness-of-fit test.

Statistical analysis

Continuous variables were compared with the two-sample t test or one-way analysis of variance when normally distributed (Bartlett's test) and with the Kruskal-Wallis test otherwise; categorical variables were compared with the χ^2 test. Variables associated with NOAF at $p < 0.05$ were examined for mutual dependence by cross-tabulation. Genetic associations were assessed by comparing risk-allele and genotype frequencies (Mantel-Haenszel test; χ^2 test for trend). Because no homozygote for the *PITX2* risk allele was observed, the genotypic comparison for that variant reduced to a dominant model, heterozygous carriers against non-carriers. Independent variables were entered into backward stepwise multivariable logistic regression to derive a discriminative model; a Bonferroni correction was applied where genotype variants were included. Discrimination was summarised by the area under the receiver-operating-characteristic curve (ROC-AUC), and the age threshold maximising the Youden index was determined.

For the association of NOAF with early complications, odds ratios and their 95% confidence intervals (CI) were calculated from the observed frequencies by the Woolf method, and group frequencies were compared with the χ^2 test; the Fisher exact test was used where any expected cell count was below five. Mortality was analysed in the 363 patients for whom vital status was available at all three time points. Analyses were performed with Epi Info 7 (Centers for Disease Control and Prevention, Atlanta, GA, USA) and Statistica 12 (StatSoft, Tulsa, OK, USA).

Use of a language model

A large language model (Claude, Anthropic) was used to assist with language editing and with formatting of the manuscript to the journal's requirements. The authors reviewed and edited all output and take full responsibility for the content.

Results

Incidence and univariate risk factors

The parent cohort has been characterised in detail elsewhere (Kowalik et al. 2018; Kowalik et al. 2026): among the 484 patients of the most recent report the mean age was 65 ± 11 years, 45% were women, mean body mass index was 27.5 ± 5.0 kg/m², and single-valve surgery with or without additional procedures predominated, because elective isolated coronary artery bypass grafting is performed off-pump at the centre and was therefore not represented. The analytical sample of the present study differs in composition from the parent cohort, since patients with pre-existing atrial fibrillation were excluded; its clinical characteristics by outcome are given in Online Resource 2. NOAF developed in 115 of 364

patients (31.6%). On univariate testing (Online Resource 2), NOAF was associated with age, BMI, eGFR, coronary artery disease, procedure type together with its correlates (operation, bypass and aortic cross-clamp times), the number of transfused red-cell and fresh-frozen-plasma units, treatment with acetylsalicylic acid, statins and β -blockers, left-atrial diameter in women, and *PITX2* rs67249485. Sex, hypertension, diabetes, chronic kidney disease, chronic obstructive pulmonary disease, angiotensin-pathway inhibitors, preoperative creatinine and white-cell count, and left-ventricular ejection fraction were not associated with the outcome. Twelve variables were carried forward to cross-tabulation: age, BMI, eGFR, coronary artery disease, procedure type, red-cell and plasma transfusion, the three medications, left-atrial diameter and *PITX2* rs67249485.

Genetic risk factors

DNA suitable for genotyping the four atrial-fibrillation-associated variants was available in 358 patients (98.4%; NOAF + $n = 113$, NOAF – $n = 245$). Genotype distributions of *ZFHX3* rs2359171, *TBX5* rs883079 and *CAV1* rs11773845 conformed to Hardy-Weinberg expectation in the whole genotyped sample ($p = 0.17$, 0.17 and 0.93 , respectively). The distribution of *PITX2* rs67249485 did not ($p < 0.001$): no TT homozygote was observed, whereas about 12 would have been expected from a T-allele frequency of 18.2%, a discrepancy considered further under Limitations.

Of the four variants, only *PITX2* rs67249485 was associated with NOAF, in both the allelic and the genotypic test (Table 1, Fig. 1). The risk allele (T) was carried more often by patients who developed NOAF than by those who did not (23.5% versus 15.7%; risk ratio 1.38, 95% CI 1.08–1.76; $p = 0.013$). Because the homozygous risk genotype did not occur, risk was confined to heterozygous carriers (53 of 113 NOAF+ versus 77 of 245 NOAF– patients), in whom the genotypic risk ratio was 1.55 (95% CI 1.1–2.1; Mantel-Haenszel $p = 0.005$). Expressed as an incidence, NOAF occurred in 40.8% of heterozygous carriers (53 of 130) and in 26.3% of non-carriers (60 of 228) (Fig. 2). The remaining three variants showed no association: *ZFHX3* rs2359171 ($p = 0.284$), *TBX5* rs883079 ($p = 0.482$) and *CAV1* rs11773845 ($p = 0.669$).

Table 1

Association of the four atrial-fibrillation-associated variants with new-onset atrial fibrillation (NOAF)

Gene and variant (risk/other allele)	Genotypes, NOAF+ (n)	Genotypes, NOAF- (n)	Risk-allele frequency, NOAF+ versus NOAF- (%)	Allelic risk ratio (95% CI)	p^a	Heterozygous genotype, NOAF+ versus NOAF- (%)	p^b
<i>PITX2</i> rs67249485 (T/A)	AA 60 / AT 53 / TT 0	AA 168 / AT 77 / TT 0	23.5 versus 15.7	1.38 (1.08–1.76)	0.013	46.9 versus 31.4	0.005
<i>ZFHX3</i> rs2359171 (A/T)	TT 81 / AT 32 / AA 0	TT 166 / AT 73 / AA 6	14.2 versus 17.4	0.84 (0.61–1.16)	0.284	28.3 versus 29.8	0.266
<i>TBX5</i> rs883079 (T/C)	CC 13 / CT 51 / TT 49	CC 30 / CT 94 / TT 121	65.9 versus 68.6	0.92 (0.74–1.16)	0.482	45.1 versus 38.4	0.498
<i>CAV1</i> rs11773845 (A/C)	CC 22 / AC 53 / AA 38	CC 40 / AC 122 / AA 83	57.1 versus 58.8	0.95 (0.77–1.19)	0.669	46.9 versus 49.8	0.669
Genotyping was available in 358 of 364 patients (98.4%): NOAF + n = 113, NOAF - n = 245. Genotypes are listed as other-allele homozygote / heterozygote / risk-allele homozygote. Hardy-Weinberg equilibrium in the whole genotyped sample (χ^2 goodness of fit): <i>PITX2</i> $p < 0.0001$; <i>ZFHX3</i> $p = 0.17$; <i>TBX5</i> $p = 0.17$; <i>CAV1</i> $p = 0.93$							
^a Allelic Mantel-Haenszel test. ^b Genotypic χ^2 test for trend							
CI confidence interval, NOAF new-onset atrial fibrillation							

Postoperative inflammatory markers

None of the first-day postoperative inflammatory variables was associated with NOAF, and none of the ten inflammation-related polymorphisms of the original protocol was associated with the arrhythmia. The systemic inflammatory response, present in a comparable proportion of both groups (33.0% versus 34.9%), did not distinguish patients who developed NOAF.

Interdependence of risk factors and the multivariable model

Cross-tabulation showed that coronary artery disease, itself a significant risk factor, was in turn associated with statin, acetylsalicylic-acid and β -blocker use, and with age, BMI, eGFR, procedure type and transfusion, so that these variables were mutually entangled. The single exception was *PITX2* rs67249485, which showed no cross-association with any other risk factor and thus behaved as a genuinely independent variable.

On the basis of these dependencies, collinear terms (medications, eGFR, procedure type, transfusion) were not entered into the regression. Age, BMI, coronary artery disease, left-atrial diameter and *PITX2* were offered to backward stepwise logistic regression; the final model retained only two independent predictors (Table 2): age (per 10-year increment, odds ratio 1.71, 95% CI 1.36–2.16) and the heterozygous *PITX2* genotype (odds ratio 1.91, 95% CI 1.19–3.06). The effect of the *PITX2* variant was broadly uniform across age strata (weighted risk ratio 1.44, 95% CI 1.08–1.92; $p = 0.018$), whereas the age effect became most pronounced beyond 80 years (χ^2 for trend $p = 0.0001$); the Youden index identified an at-risk age of 64 years or older. A three-level composite of these two discriminators yielded a ROC-AUC of 0.644.

Table 2
Independent predictors of new-onset atrial fibrillation in the multivariable logistic-regression model

Variable	Odds ratio (95% CI)	β (SE)	p^a
Age (per 10 years)	1.71 (1.36–2.16)	0.54 (0.12)	0.000004
<i>PITX2</i> rs67249485 (heterozygous)	1.91 (1.19–3.06)	0.65 (0.24)	0.015

Candidate variables offered to the model were age, body mass index, coronary artery disease, left-atrial diameter and *PITX2* rs67249485. The area under the receiver-operating-characteristic curve of the three-level composite model was 0.644

^a p value after Bonferroni correction

CI confidence interval, SE standard error

New-onset atrial fibrillation and postoperative outcomes

NOAF was associated with several early complications (Table 3, Fig. 3). Acute respiratory failure was more than twice as frequent in patients with NOAF (15.7% versus 6.8%; odds ratio 2.53, 95% CI 1.25–5.12; $p = 0.008$), as was acute kidney injury (28.7% versus 13.7%; odds ratio 2.54, 95% CI 1.48–4.38; $p = 0.0006$). The strongest association was with postoperative delirium (22.6% versus 5.6%; odds ratio 4.90, 95% CI 2.45–9.82; $p < 0.001$). Perioperative myocardial infarction showed a non-significant trend (11.3% versus 6.0%; $p = 0.079$), while stroke, acute circulatory failure and the systemic inflammatory response did not differ. Mortality did not differ between groups at any time point: 2.7% versus 3.6% at 30 days, 7.1% versus 7.2% at one year, and 24.1% versus 23.5% at five years.

Table 3

Association of new-onset atrial fibrillation (NOAF) with early postoperative complications and mortality

Outcome	NOAF+ (%)	NOAF- (%)	Odds ratio (95% CI)	p
Postoperative delirium	22.6	5.6	4.90 (2.45–9.82)	< 0.001
Acute circulatory failure	2.6	0.8	3.31 (0.55–20.07)	0.183 ^a
Acute kidney injury	28.7	13.7	2.54 (1.48–4.38)	0.0006
Acute respiratory failure	15.7	6.8	2.53 (1.25–5.12)	0.008
Perioperative myocardial infarction	11.3	6.0	1.99 (0.91–4.33)	0.079
Stroke	2.6	2.4	1.08 (0.27–4.42)	1.000 ^a
Systemic inflammatory response	33.0	34.9	0.92 (0.58–1.47)	0.723
30-day mortality	2.7	3.6	0.74 (0.20–2.79)	0.762 ^a
One-year mortality	7.1	7.2	1.00 (0.42–2.36)	0.992
Five-year mortality	24.1	23.5	1.03 (0.61–1.74)	0.901
Morbidity comparisons: NOAF + n = 115, NOAF – n = 249. Mortality comparisons: NOAF + n = 112, NOAF – n = 251				
^a Fisher exact test; all other comparisons by χ^2 test				
CI confidence interval				

Discussion

The central result of this analysis is that, in a cohort assembled expressly to test the inflammatory hypothesis, NOAF after on-pump cardiac surgery was not predicted by the measured postoperative inflammatory response but by two constitutional characteristics of the patient: advancing age and heterozygosity for a single variant at the 4q25/*PITX2* locus. Neither the first-day inflammatory markers nor the ten inflammation-related polymorphisms of the original protocol distinguished the patients who fibrillated, whereas *PITX2* rs67249485 was the only variable that survived cross-tabulation as a genuinely independent factor and, together with age, the only one retained in the multivariable model.

The 4q25/*PITX2* locus in postoperative atrial fibrillation

This finding sits within a now-substantial literature implicating the 4q25/*PITX2* locus in postoperative atrial fibrillation. Variants in this region, discovered in ambulatory cohorts (Gudbjartsson et al. 2007; Roselli et al. 2018), were shown by Body et al. (2009) to predict atrial fibrillation after coronary artery bypass grafting, an observation extended to broader cardiac-surgery populations (Sigurdsson et al. 2015) and to nationwide biobank data (Christensen et al. 2023). Our result reproduces that association

with a further 4q25 variant, rs67249485, and is consistent with the interpretation that reduced *PITX2* dosage predisposes the left atrium to arrhythmia irrespective of the acute surgical milieu (Jiménez-Sábado and Hove-Madsen 2025). The classic markers rs2200733 and rs10033464 were not genotyped, so the relation of rs67249485 to them within this cohort cannot be stated; in populations of European ancestry the 4q25 signals segregate as more than one independent haplotype and are associated with different atrial phenotypes and treatment responses (Jiménez-Sábado and Hove-Madsen 2025), which argues against treating the locus as a single unit. The absence of any association for *ZFHX3*, *TBX5* and *CAV1*, despite their established links to atrial fibrillation in large populations (Benjamin et al. 2009; Roselli et al. 2018), most likely reflects the modest size of a surgical cohort relative to the effect sizes of common variants rather than a true null.

The variant that emerged here deserves comment in molecular terms. rs67249485 lies in the intergenic region upstream of *PITX2* that harbours the strongest common-variant signals for atrial fibrillation (Gudbjartsson et al. 2007; Roselli et al. 2018), a gene desert in which risk variants are thought to act through regulatory elements controlling gene expression in atrial cardiomyocytes (van Ouwertkerk et al. 2019). In Mendelian randomisation with 84 atrial-fibrillation variants as instruments, rs67249485 alone drove the causal estimate linking genetic susceptibility to atrial fibrillation with larger left-atrial volumes and lower left-atrial ejection fraction (van de Vegte et al. 2021), which places this particular variant on the structural as much as the electrical side of *PITX2* biology. Left-atrial diameter was associated with NOAF on univariate testing in our cohort but was incompletely recorded and did not enter the final model, so a mediating role of atrial size in the genotype effect could not be examined. Experimental work adds a further layer that bears directly on a cohort exposed to CPB. *Pitx2* restrains a left-sided pacemaker programme (Wang et al. 2010), its reduced expression in the adult atrium promotes inducible atrial fibrillation (Kirchhof et al. 2011), it activates the antioxidant response after cardiac injury (Tao et al. 2016), and oxidative stress is sufficient to produce mitochondrial and electrophysiological dysfunction and atrial fibrillation in *Pitx2* haploinsufficient mice (Subati et al. 2025). Together, these observations suggest a reading of our data that reconciles the two competing hypotheses rather than choosing between them. The inflammatory and oxidative stimulus of CPB is delivered to nearly every patient, and the systemic inflammatory response was indeed present in one third of both groups. What separated the patients who fibrillated from those who did not was not the intensity of that stimulus, as measured on the first postoperative day, but the constitution of the atrium receiving it, indexed here by age and by *PITX2* genotype. On this reading the postoperative markers describe a trigger that is close to universal, whereas genotype and age describe a substrate that is not.

Genotype and clinical prediction

The value of adding genotype to clinical prediction is supported by combined genetic and clinical models (Kolek et al. 2015) and, more directly, by polygenic risk scores. In 1047 patients of European ancestry a score built from 2746 atrial-fibrillation-associated variants was independently associated with postoperative atrial fibrillation, although it added little to clinical discrimination (Kertai et al. 2021). Our data echo both halves of that observation: the genetic term contributed independently of every clinical

covariate, yet the two-factor model reached a ROC-AUC of only 0.644. That figure is a realistic reflection of what constitutional factors alone can achieve, and it is close to the discrimination of established clinical instruments, whether validated prognostic models developed in national cohorts (Chung et al. 2023) or the POAF score, whose C-statistic of 0.60 improved only to 0.63 when thirteen pathophysiological biomarkers were added (Noordzij et al. 2025). Genotype and biomarkers appear to be operating at a similar, and similarly modest, level of incremental value.

The negative inflammatory result

The negative inflammatory result deserves emphasis rather than apology. INFLACOR was instrumented to detect an inflammatory contribution, yet none was found for NOAF. This does not exclude a mechanistic role for inflammation in the initiation of individual episodes; the atrial NLRP3-CaMKII substrate described in surgical patients is a compelling account of how an inflamed atrium becomes arrhythmogenic (Heijman et al. 2020). Our finding argues something narrower but clinically consequential: at the level of routinely measurable perioperative markers, inflammation is not what separates the patients who will fibrillate from those who will not. That conclusion is not isolated. Studies reporting positive associations have generally examined preoperative rather than postoperative inflammatory status, whether a composite inflammatory burden index measured before coronary surgery (Osmanaj et al. 2026) or preoperative cytokine profiles (Staicu et al. 2025), which is consistent with inflammation marking a pre-existing atrial substrate rather than being generated by the operation. A recent systematic review of 52 studies likewise concluded that inflammatory and oxidative-stress signals remain promising but unvalidated, while advanced age and left-atrial enlargement are the predictors that recur across cohorts (Prasetyadhi et al. 2026). A comparable picture emerges outside cardiac surgery, where perioperative inflammation is regarded as a plausible but still unproven determinant of the arrhythmia (Richter et al. 2026). The clinically useful corollary of our data is that the strongest determinants of NOAF risk are fixed before the patient reaches the operating theatre.

New-onset atrial fibrillation and early outcome

NOAF was, however, far from an isolated electrical event. It travelled with acute respiratory failure, acute kidney injury and, most strikingly, delirium, the last carrying a nearly fivefold association. Whether NOAF causes or merely marks this adverse course cannot be settled by these data, but its value as an early signal of a complicated recovery is clear. This morbidity did not translate into a mortality difference at 30 days, one year or five years. That null result contrasts with a reconstructed individual-patient-data meta-analysis in which early postoperative atrial fibrillation was significantly associated with late mortality, though not with late stroke, after adjustment for age, sex, comorbidity and type of surgery (Kawczynski et al. 2025), and with long-term registry data linking the arrhythmia to stroke, heart failure and death (Thorén et al. 2020). Two explanations seem plausible. The deliberate exclusion of patients with paroxysmal atrial fibrillation removed precisely the group in whom a postoperative episode is most likely to represent unmasked chronic disease, and the resulting sample is underpowered for mortality: with 12 deaths at 30 days, only a very large effect could have been detected. The recent VISION Cardiac Surgery experience, in which the long-term significance of postoperative atrial fibrillation depended on its pattern

and duration and on the underlying atrial substrate rather than on its mere occurrence, points in the same direction (McIntyre et al. 2026). Our data are therefore best read as showing that in a contemporary surgical programme the prognostic weight of an isolated first episode lies in early morbidity and resource use.

Limitations

Several limitations qualify these conclusions. By design, patients with paroxysmal atrial fibrillation were excluded to isolate a new-onset phenotype, which reduced the sample available for genetic association; for a genetic study the cohort is at the lower bound of adequacy, though it was sufficient to identify two independent predictors, one of them genetic. Rhythm was followed by continuous telemetry during intensive care and by electrocardiography thereafter, so detection was unlikely to be the limiting factor. Two features of the endpoint definition nonetheless narrow the phenotype. The observation window opened on the second postoperative day, so an episode confined to the day of operation or to the first postoperative day did not count; and the arrhythmia was identified by the need for treatment rather than by a prespecified electrocardiographic duration threshold, so episodes brief enough to resolve untreated did not count either. What was studied is therefore clinically manifest atrial fibrillation from the second postoperative day onwards, and the incidence of 31.6% is a lower bound relative to cohorts applying the 30-second criterion of current guidance (Van Gelder et al. 2024) to continuous recordings from the moment of operation. Both restrictions act in the same direction and in a manner unrelated to genotype. A patient whose only episode fell outside the window or below the treatment threshold joins the comparison group, which biases the observed associations towards the null rather than away from it; and genotype was unknown to the treating clinicians, so neither the timing of the window nor the threshold for treatment can have varied with *PITX2* status.

A further limitation concerns the genotype distribution of rs67249485. No TT homozygote was found among 358 genotyped patients, whereas roughly a dozen would be expected from the observed allele frequency, and the distribution consequently departed from Hardy-Weinberg equilibrium while the other three variants conformed, as did all ten variants of the original protocol (Kowalik et al. 2018). Chance is an unlikely explanation for a deficit of this size. Selected samples were verified by bidirectional sequencing as part of the genotyping protocol, but that verification was not designed to detect a systematic failure to call one homozygous class, and allele-specific dropout of the TT genotype in the extension assay cannot be excluded. The direction of any resulting bias depends on how a true TT homozygote would have been called: if as AA, the association would be biased towards the null; if as AT, the heterozygote estimate would absorb any homozygote effect, and the reported genotypic risk ratio should then be read as that of risk-allele carriage rather than of heterozygosity in the strict sense. Neither possibility creates an association where none exists, but replication with a validated assay is required before the variant is used for risk stratification.

Left-atrial diameter was incompletely recorded and, most probably for that reason, did not enter the final model despite a univariate association. Resource constraints limited genotyping to four selected variants; a denser panel, haplotype analysis or a polygenic score would almost certainly refine prediction

(Kertai et al. 2021). The cohort was recruited between 2009 and 2011, and perioperative practice has changed since, although the constitutional predictors identified here are by their nature insensitive to such change. Finally, the study was conducted at a single centre in a Polish population of European ancestry, and the findings require replication before they are generalised. Replication of established atrial-fibrillation loci across populations is known to be variable, and genome-wide data from Polish individuals have only begun to appear (Wasiak et al. 2026).

Conclusion

In adults undergoing first-time cardiac surgery on cardiopulmonary bypass, the risk of new-onset atrial fibrillation was determined chiefly by patient age and by heterozygosity for the 4q25 variant rs67249485 upstream of *PITX2*. No evidence was found that the arrhythmia is driven by the postoperative inflammatory parameters measured in this study, which is consistent with a model in which the inflammatory stimulus of bypass is near-universal and the constitutional susceptibility of the atrium is what discriminates. New-onset atrial fibrillation was nonetheless associated with early postoperative acute respiratory failure, acute kidney injury and delirium, marking it as a useful early indicator of a complicated recovery.

Declarations

Competing interests.

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval.

All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments. The study was approved by the Independent Bioethics Committee for Scientific Research of the Medical University of Gdańsk (NKEBN/358/2007) and registered at ClinicalTrials.gov on 24 November 2009 (NCT01020409), after enrolment had begun.

Consent to participate.

Written informed consent was obtained from all individual participants included in the study.

Consent to publish.

The manuscript contains no data in a form that permits identification of individual participants.

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Author Contribution

A.S.-S. and M.M.K. conceived the study. A.S.-S., B.S., K.L., R.L. and M.M.K. designed the methodology. A.S.-S., B.S., K.L., M.B. and M.M.K. conducted the investigation, including patient enrolment, clinical data collection, genotyping and laboratory assays. A.S.-S., M.M.K. and M.K.S. curated the data. A.S.-S., B.S. and K.L. performed the statistical analysis. A.S.-S. wrote the main manuscript text. A.S.-S., D.S. and M.K.S. prepared Figures 1-3 and the tables. D.S. administered the submission and corresponds with the journal. R.L. and M.M.K. supervised the work, and M.M.K. acquired the funding. A.S.-S., M.K.S., D.S., B.S., K.L. and M.M.K. revised the manuscript critically for important intellectual content. All authors reviewed and approved the final manuscript.

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Data Availability

Aggregate data (genotype counts and Hardy-Weinberg tests by outcome group, clinical characteristics, postoperative outcomes and the multivariable model) are openly available in the RepOD repository under a CC BY 4.0 licence at <https://doi.org/10.18150/0QSZMB>. Individual-level clinical and genotype data are available from the corresponding author on reasonable request, subject to the privacy and ethical restrictions under which they were collected; the consent obtained from participants does not extend to open deposition of individual genotype data.

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Figures

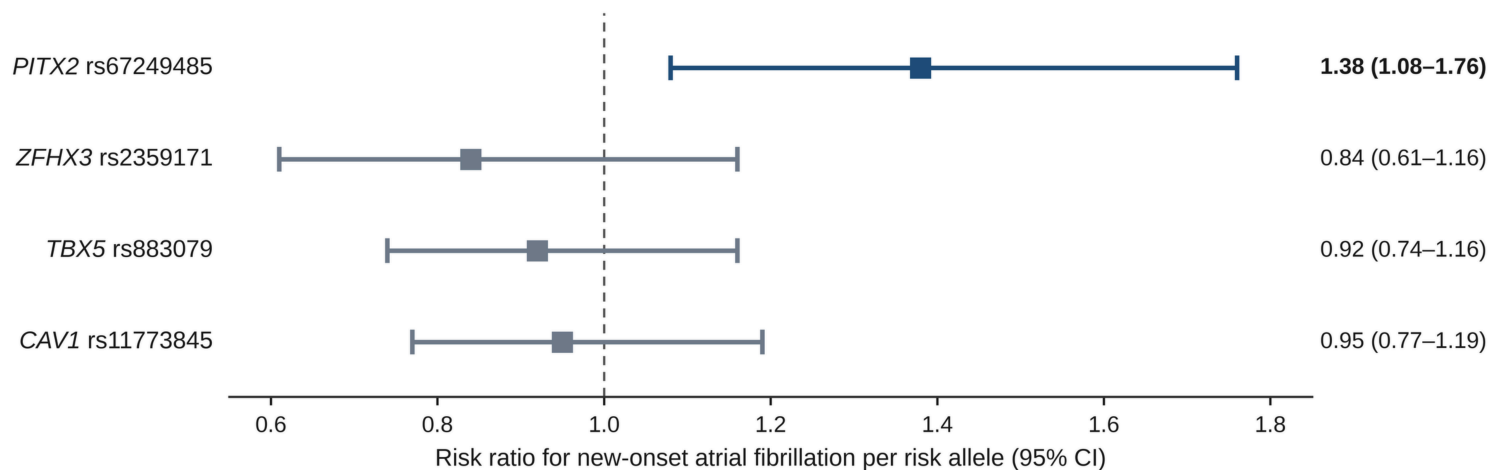


Figure 1

Association of four atrial-fibrillation-associated variants with new-onset atrial fibrillation. Allelic risk ratios with 95% confidence intervals (CI) are shown for *PITX2* rs67249485, *ZFHX3* rs2359171, *TBX5* rs883079 and *CAV1* rs11773845. Squares mark the point estimate and horizontal bars the confidence interval; the dashed line marks a risk ratio of 1. Only *PITX2* rs67249485, shown in dark blue with the estimate in bold, reached statistical significance

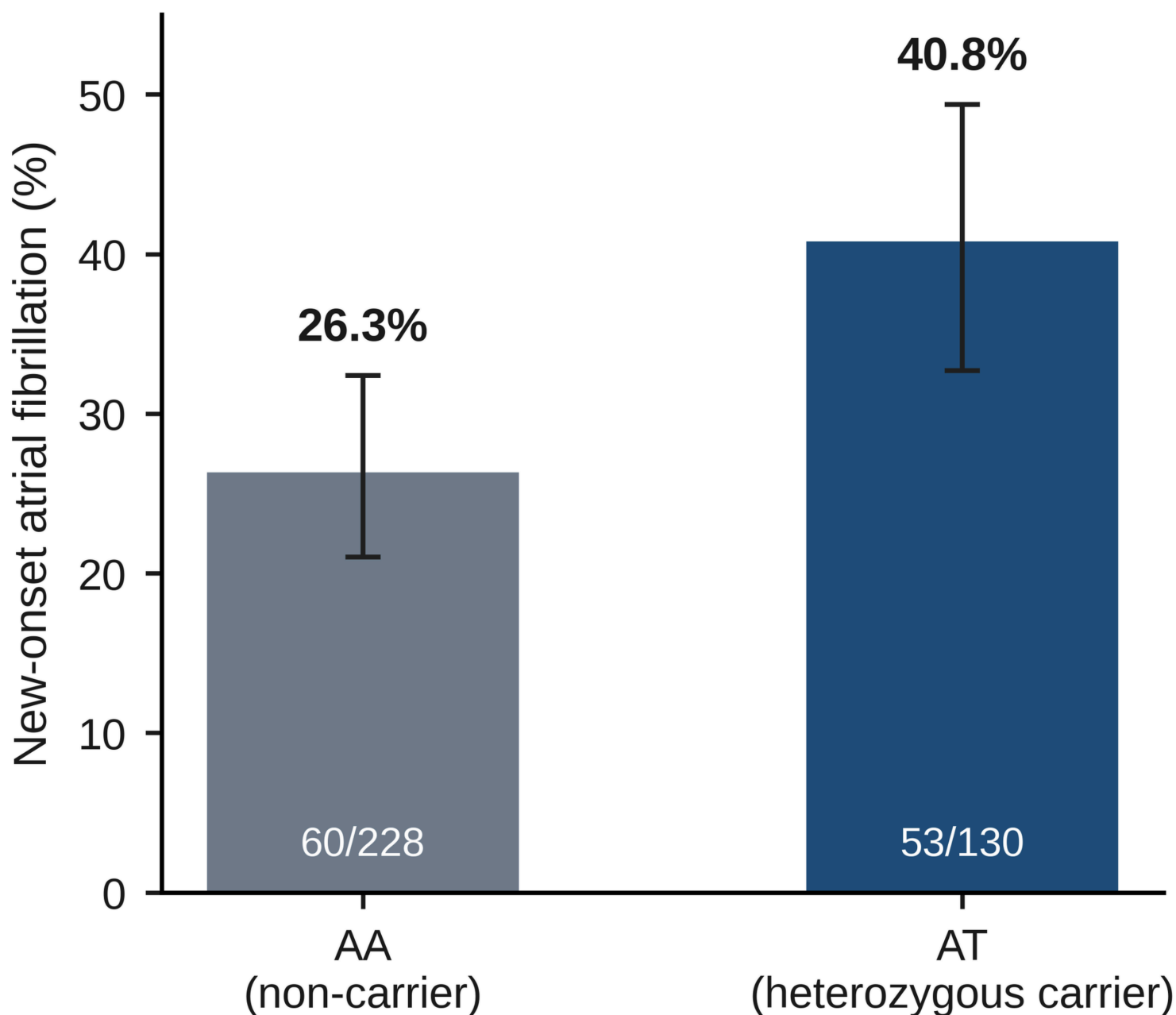


Figure 2

Incidence of new-onset atrial fibrillation according to *PITX2* rs67249485 genotype. Bars show the observed incidence with 95% confidence intervals calculated by the Wilson method; the numerator and denominator of each proportion are given within the bar. Heterozygous carriers of the risk allele (AT) had a higher incidence than non-carriers (AA). The homozygous risk genotype (TT) did not occur in this population

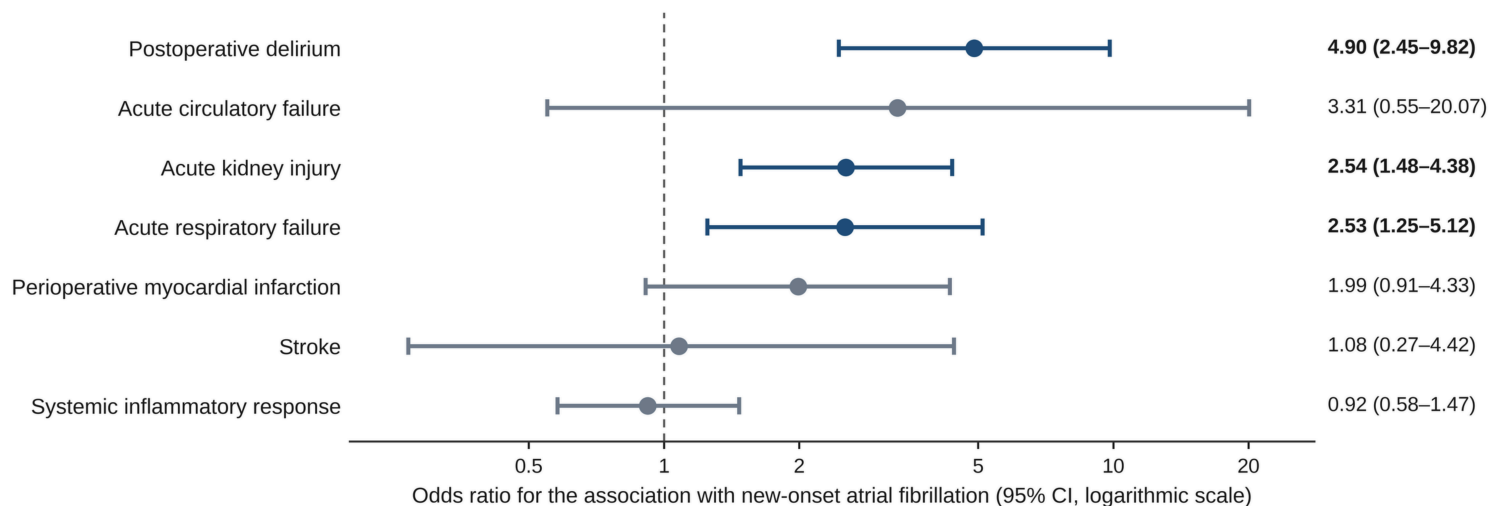


Figure 3

Association of new-onset atrial fibrillation with early postoperative complications. Odds ratios with 95% confidence intervals (CI) are plotted on a logarithmic scale. Circles mark the point estimate and horizontal bars the confidence interval; the dashed line marks an odds ratio of 1. Associations whose confidence interval excludes 1 are shown in dark blue with the estimate in bold

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