Supplementary Material

Circulating MicroRNAs as Molecular Biomarkers of Atrial Fibrillation Recurrence After Catheter Ablation: A Systematic Review and Candidate-Centered Evidence Map

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Supplementary Methods

Supplementary Method S1. Search update and source coverage

The original search stream produced 1505 records. After removal of 867 duplicates, 638 records were screened and 601 were excluded. Thirty-seven reports were sought; three were not retrieved, 34 full texts were assessed, and 25 were excluded, yielding nine database-search reports. Targeted updating and citation searching added five reports, while reappraisal retained four additional publications. Searches of PubMed/MEDLINE, Embase, Web of Science, Scopus, and the Cochrane Library were updated through 29 August 2026. A supplementary Wanfang search screened 50 records, excluded 29 at title/abstract, assessed 21 full texts, excluded four, and included 17 reports. The final

review retained 35 publications representing 29 independent studies or programmes. The official PRISMA 2020 flow diagram reports the database and other-method routes separately.

Supplementary Method S2. Eligibility, evidence layers, and cohort linkage

Adult atrial fibrillation ablation cohorts measuring circulating microRNAs before or around ablation were eligible. Recurrence-specific publications entered the prognostic evidence layer; longitudinal publications without a recurrence-specific estimate were retained as context. Eligibility was independent of effect direction, statistical significance, risk of bias, study quality, and quantitative usability. Publications were linked before synthesis by comparing author group and institution, recruitment site and period, sample size and AF phenotype, ablation method, biomarker platform, and follow-up characteristics. Shared or probably shared reports received common study/program and recurrence-cohort identifiers; uncertain linkage remained classified as probable. The 35 publications represented 29 linked programs; 26 recurrence-specific publications represented 25 independent recurrence cohorts, with 3309 participants enrolled and 3205 analyzed. Cohort linkage ensured that each independent cohort could contribute at most one estimate to any reviewer-prompted cross-biomarker sensitivity model.

Supplementary Method S3. Effect selection and coding

Adjusted ORs and HRs and their reported 95% confidence intervals were extracted exactly as published and analyzed separately. No outcome-informed direction folding was performed in the revision. The reviewer-prompted restrictive hierarchy selected (1) an author-designated primary prognostic biomarker or analysis, then (2) the most fully adjusted compatible estimate, and then (3) a pre-ablation measure with a prespecified recurrence outcome when otherwise equivalent. If multiple eligible biomarkers remained without a unique author-designated primary estimate, the cohort was excluded from the cross-biomarker sensitivity model rather than selected by title, abstract order, magnitude, direction, or significance. All reports and biomarkers remained in the qualitative and candidate-specific syntheses.

Supplementary Method S4. Statistical and sensitivity analyses

Log-transformed ORs and HRs were modeled separately with restricted maximum likelihood, modified Hartung–Knapp confidence intervals, and t-based prediction intervals. Heterogeneity was described using Q, I^2, and τ^2. The OR model included nine cohorts (n = 1162); the HR model included three cohorts (n = 364). Leave-one-out analyses were repeated for these restricted sets. Because biomarkers, matrices, compartments, time points, and exposure scales were not commensurate, all pooled results were treated as supplementary sensitivity summaries and not as biological class effects. The adverse-profile fold, substitution analyses based on the former title/abstract-order rule, meta-regression, funnel plot, and Egger test were removed from the revision.

Supplementary Method S5. Candidate-specific evidence mapping and certainty

Candidate prioritization was post hoc and applied uniformly. Direct recurrence evidence in at least two analytically distinct samples was required, together with support in at least one additional clinical, mechanistic, analytical, or external-evidence domain. Selection did not depend on effect size, direction, statistical significance, or pooled-model inclusion. Candidate-specific certainty followed GRADE guidance for prognostic-factor research. Mechanistic plausibility was recorded separately and did not upgrade clinical prognostic certainty. Because each candidate had fewer than 10 independent studies, funnel plots and asymmetry tests were not used for candidate-specific publication-bias assessment.

Supplementary Table S1. Search strategy

Database	Search string	Notes
PubMed/MEDLINE	("Atrial Fibrillation"[Mesh] OR "atrial fibrillation"[Title/Abstract] OR "atrial flutter"[Title/Abstract]) AND ("Catheter Ablation"[Mesh] OR "catheter ablation"[Title/Abstract] OR "radiofrequency ablation"[Title/Abstract] OR cryoablation[Title/Abstract] OR cryoballoon[Title/Abstract] OR "pulmonary vein isolation"[Title/Abstract]) AND ("MicroRNAs"[Mesh] OR microRNA[Title/Abstract] OR miRNA[Title/Abstract] OR miR[Title/Abstract] OR "micro RNA"[Title/Abstract])	Inception to 29 August 2026; no language restriction.
Embase.com	('atrial fibrillation'/exp OR 'atrial fibrillation':ti,ab,kw OR 'atrial flutter':ti,ab,kw) AND ('catheter ablation'/exp OR 'catheter ablation':ti,ab,kw OR 'radiofrequency ablation':ti,ab,kw OR cryoablation:ti,ab,kw OR cryoballoon:ti,ab,kw OR 'pulmonary vein isolation':ti,ab,kw) AND ('microRNA'/exp OR microRNA:ti,ab,kw OR miRNA:ti,ab,kw OR miR:ti,ab,kw OR 'micro RNA':ti,ab,kw)	Embase.com syntax; inception to 29 August 2026.
Web of Science Core Collection	TS=(("atrial fibrillation" OR "atrial flutter") AND (ablation OR "catheter ablation" OR "radiofrequency ablation" OR cryoablation OR cryoballoon OR "pulmonary vein isolation") AND (microRNA OR miRNA OR miR OR "micro RNA"))	Topic search; inception to 29 August 2026.
Scopus	TITLE-ABS-KEY("atrial fibrillation" OR "atrial flutter" OR "atrial tachyarrhythmia") AND TITLE-ABS-KEY(ablation OR "catheter ablation" OR "radiofrequency ablation" OR cryoablation OR cryoballoon OR "pulmonary vein isolation" OR "pulsed field ablation") AND TITLE-ABS-KEY(microRNA OR miRNA OR mir OR "micro RNA")	Advanced Search. No unsupported wildcard or special-character construction.
Cochrane CENTRAL	("atrial fibrillation" OR "atrial flutter"):ti,ab,kw AND (ablation OR "catheter ablation" OR "radiofrequency ablation" OR cryoablation OR cryoballoon OR "pulmonary vein isolation"):ti,ab,kw AND (microRNA OR miRNA OR miR OR "micro RNA"):ti,ab,kw	CENTRAL/Trials; inception to 29 August 2026.

Database	Search string	Notes
Wanfang	English translation of Chinese-language query: (atrial fibrillation OR atrial flutter) AND (catheter ablation OR radiofrequency ablation OR cryoablation OR cryoballoon OR pulmonary vein isolation) AND (microRNA OR miRNA OR miR)	Chinese-language terms were used in Wanfang; English translation shown for font portability. Supplementary search screened 50 records; 17 publications were eligible.
Citation and source re-evaluation	Backward and forward citation checking of eligible reports and relevant reviews; re-evaluation of the other searched sources.	No further eligible reports beyond the Wanfang update.

Note: Searches used three mandatory concepts—atrial fibrillation, catheter ablation, and microRNA. Recurrence, matrix, OR/HR availability, effect direction, significance, risk of bias, and study quality were assessed after retrieval and were not search exclusions. The final evidence base was frozen on 29 August 2026.

Supplementary Table S2A. PRISMA 2020 checklist (official template)

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	Title
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	Abstract
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	Introduction, final paragraph
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	Introduction, final paragraph
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	Methods 2.2; Supplementary Table S4
Information sources	6	Specify all databases, registers, websites, organizations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	Methods 2.3; Supplementary Table S1

Section and Topic	Item #	Checklist item	Location where item is reported
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	Methods 2.3; Supplementary Table S1
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	Methods 2.4
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	Methods 2.4
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	Methods 2.4
Data items	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	Methods 2.4
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	Methods 2.6

Section and Topic	Item #	Checklist item	Location where item is reported
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	Methods 2.8
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	Methods 2.8–2.9; Supplementary Methods S3–S4
Synthesis methods	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	Methods 2.8–2.9; Supplementary Methods S3–S4
Synthesis methods	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	Methods 2.8–2.9; Supplementary Methods S3–S4
Synthesis methods	13d	Describe any methods used to synthesise results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	Methods 2.8–2.9; Supplementary Methods S3–S4
Synthesis methods	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	No formal investigation performed; rationale in Methods 2.9
Synthesis methods	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesised results.	Methods 2.8–2.9; Supplementary Methods S3–S4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	Methods 2.6; Supplementary Method S5; Supplementary Table S11
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	Methods 2.6; Results 3.6; Supplementary Table S11
RESULTS			

Section and Topic	Item #	Checklist item	Location where item is reported
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	Results 3.1; Figure 1
Study selection	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	Results 3.1; Supplementary Table S4
Study characteristics	17	Cite each included study and present its characteristics.	Results 3.2; Tables 1–2
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	Results 3.3; Supplementary Figure S3
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	Table 2; Figure 2
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	Results 3.3 and 3.5; Table 2; Figure 2
Results of syntheses	20b	Present results of all statistical syntheses conducted. If a meta-analysis was conducted, present, for each, the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	Results 3.5; Supplementary Table S8; Supplementary Figure S1
Results of syntheses	20c	Present results of all investigations of possible causes of heterogeneity among study results.	No formal investigation performed; interpretation in Results 3.5 and Discussion
Results of syntheses	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	Supplementary Table S8; Supplementary Figure S2

Section and Topic	Item #	Checklist item	Location where item is reported
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	Results 3.6; Supplementary Table S11
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	Results 3.6; Supplementary Table S11
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	Discussion
Discussion	23b	Discuss any limitations of the evidence included in the review.	Discussion
Discussion	23c	Discuss any limitations of the review processes used.	Discussion, limitations
Discussion	23d	Discuss implications of the results for practice, policy, and future research.	Discussion; Conclusions
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	Methods 2.1; OSF registration statement
Registration and protocol	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	Methods 2.1
Registration and protocol	24c	Describe and explain any amendments to information provided at registration or in the protocol.	Methods 2.1; dated search amendment
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	Funding
Competing interests	26	Declare any competing interests of review authors.	Competing interests
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	Data availability; Supplementary Material

Note: Official PRISMA 2020 checklist template. Locations refer to the complete updated manuscript and supplementary material.

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Supplementary Table S2B. PRISMA 2020 for Abstracts checklist (official template)

Section and Topic	Item #	Checklist item	Reported (Yes/No)
TITLE			
Title	1	Identify the report as a systematic review.	Yes
BACKGROUND			
Objectives	2	Provide an explicit statement of the main objective(s) or question(s) the review addresses.	Yes
METHODS			
Eligibility criteria	3	Specify the inclusion and exclusion criteria for the review.	Yes
Information sources	4	Specify the information sources (e.g. databases, registers) used to identify studies and the date when each was last searched.	Yes
Risk of bias	5	Specify the methods used to assess risk of bias in the included studies.	Yes
Synthesis of results	6	Specify the methods used to present and synthesise results.	Yes
RESULTS			
Included studies	7	Give the total number of included studies and participants and summarise relevant characteristics of studies.	Yes
Synthesis of results	8	Present results for main outcomes, preferably indicating the number of included studies and participants for each. If meta-analysis was done, report the summary estimate and confidence/credible interval. If comparing groups, indicate the direction	Yes

Section and Topic	Item #	Checklist item	Reported (Yes/No)
		of the effect (i.e. which group is favoured).	
DISCUSSION			
Limitations of evidence	9	Provide a brief summary of the limitations of the evidence included in the review (e.g. study risk of bias, inconsistency and imprecision).	Yes
Interpretation	10	Provide a general interpretation of the results and important implications.	Yes
OTHER			
Funding	11	Specify the primary source of funding for the review.	Yes
Registration	12	Provide the register name and registration number.	Yes

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Supplementary Table S3. Translational evidence-mapping framework

Domain	Operational use in the review	Contribution to candidate classification
Direct recurrence evidence	Evidence from an AF-ablation cohort with AF/atrial tachyarrhythmia recurrence.	Mandatory clinical relevance.
Analytically distinct replication	At least two independent cohorts or separately recruited derivation and validation samples.	Mandatory safeguard against isolated findings.
Adjusted prognosis	Candidate retained in a multivariable prognostic model with an interpretable effect estimate.	Additional support domain.
Mechanistic concordance	Experimental support distinguished from computational enrichment and phenotype-level association.	Additional support domain; not proof of clinical prediction.
Analytical feasibility	Matrix, mature-arm identity, processing, normalization, and assay transferability considered separately.	Additional support domain.
External evidence	Support from a non-overlapping AF biomarker or mechanistic evidence body.	Additional support domain.
Prohibited selection criteria	Effect magnitude, direction, statistical significance, pooled-model inclusion, and title/abstract order.	Not used for candidate membership.

Note: The candidate-centered framework was developed post hoc, then applied uniformly to all microRNAs. It organizes evidence for prospective validation and does not establish a clinical ranking.

Supplementary Table S4. Contextual reports and full-text exclusions with prespecified decisions

Study	Year	Main focus	Decision	Reason for exclusion from the main prognostic synthesis
Liu et al. [68]	2012	Plasma miR-150 and AF diagnosis	Contextual only	Evaluated AF versus controls/paroxysmal AF/persistent AF; no post-ablation recurrence outcome.
Liu et al. [23]	2016	miR-409-3p/miR-432 before and after ablation	Contextual only	Pre/post-ablation expression and AF versus controls; not a prognostic recurrence study.
Chen et al. [69]	2021	Circulating miR-21, left atrial fibrosis, persistent AF	Contextual only	Evaluated persistent AF versus sinus rhythm and fibrosis by MRI; no ablation recurrence endpoint.
Xiao et al. [70]	2021	hsa-miR-4443, THBS1, TGF-beta/alpha-SMA/collagen signaling	Contextual only	Mechanistic fibrosis study and AF versus controls; no catheter ablation recurrence outcome.
Geurts et al. [71]	2022	Rotterdam Study: plasma miRNAs and prevalent/incident AF	Contextual only	Population-based AF risk study; not post-ablation recurrence.
Bai et al. [72]	2022	Exosome-derived miR-122-5p and postoperative AF after CABG	Excluded	Postoperative AF after cardiac surgery, not recurrence after AF catheter ablation.
Siwaponanan et al. [73]	2022	Large extracellular vesicle-derived miRNA signature for nonvalvular AF	Contextual only	AF versus non-AF controls; diagnostic/biological biomarker study, not recurrence after ablation.
Yue et al. [24]	2021	Ion-channel-related miRNAs before/after RFA	Contextual only	Pre/post-RFA remodeling study; no extractable prognostic OR/HR for recurrence.
Shen et al. [74]	2018	rs12976445 polymorphism, miR-125a and IL-6R	Mechanistic context only	Genetic/tissue-mechanistic focus; not circulating miRNA recurrence after ablation.
Xie et al. [75]	2023	Serum exosome miRNA-seq distinguishing AF and AF-associated ischemic stroke	Excluded	Stroke differentiation study; not ablation recurrence.
Zhang et al. [76]	2023	miR-145 and intracardiac thrombosis in rheumatic heart disease with AF	Excluded	RHD/AF thrombosis outcome; not post-ablation recurrence.

Study	Year	Main focus	Decision	Reason for exclusion from the main prognostic synthesis
Nopp et al. [77]	2023	miR-411-5p and MACE in AF patients	Excluded	Prognosis of MACE/cardiovascular death in AF, not recurrence after ablation.
Siwaponanan et al. [78]	2023	Small EV levels and miR-126 in AF with diabetes mellitus	Excluded/contextual	Compares AF with versus without diabetes; no ablation recurrence endpoint.
Tsai et al. [79]	2024	Exosomal miRNA changes after restoration to sinus rhythm	Contextual/exploratory	Very small rhythm-control study including RFCA/cardioversion; no OR/HR for post-ablation recurrence.
Xu et al. [80]	2024	miR-499 and IL-1beta in dilated cardiomyopathy with AF	Excluded	DCM with AF; no catheter ablation recurrence outcome.
Sathipati et al. [81]	2024	miRNA signature predicting POAF after CABG	Excluded	Postoperative AF after CABG; preprint; outside post-ablation AF recurrence PICOT.
Li et al. [82]	2024 abstract	Serum miR-122 and LA substrate in AF	Contextual only	Reports substrate association; no robust recurrence-specific prognostic estimate.
Tak et al. [83]	2025	Circulating extracellular RNAs, remodeling and history of AF in ACS survivors	Contextual only	ACS cohort; prevalent AF/remodeling phenotype, not ablation recurrence.
Xue et al. [84]	2025	Exosomal miR-320c and atrial cardiomyopathy/fibrosis	Contextual only	Atrial cardiomyopathy/fibrosis biomarker; no recurrence after catheter ablation.
Lian et al. [85]	2026	lncRNA NEAT1, AMI-AF and miR-624-5p/SAT1	Excluded	lncRNA/AMI-AF mechanistic study; not circulating miRNA recurrence after ablation.
Góra et al. [86]	2024	miRNA expression in persistent versus paroxysmal AF	Excluded	AF phenotype comparison; not recurrence after ablation.
Nakano et al. [87]	2023	Genomic/genetic context of AF	Excluded	Review/genetic context; not original circulating miRNA recurrence study.
Biomedicines review [15]	2025	Narrative review of miRNAs after AF ablation	Excluded from primary evidence	Review article; useful for background only, not primary data.

Study	Year	Main focus	Decision	Reason for exclusion from the main prognostic synthesis
Lage et al. abstract [88]	2022	Preliminary miR-451a abstract	Duplicate	Superseded by the full-text Lage et al. 2023 study.
Sustr et al. abstract [89]	2022	Preliminary miR-206 abstract	Duplicate	Superseded by the full-text Sustr et al. 2024 study.
Cui et al. [51]	2015	Pre/post-ablation circulating miRNA regulation	Contextual—eligible	Longitudinal evidence layer; likely overlapping Beijing n=30 programme.
Zhao et al. [52]	2015	Peripheral-blood miRNA profile before/after ablation	Contextual—eligible	Longitudinal evidence layer; likely overlapping Beijing programme.
Nan and Yang [53]	2015	TBX5/NKX2.5 regulatory miRNAs before/after ablation	Contextual—eligible	Longitudinal evidence layer; likely overlapping Beijing programme.
Xu et al. [54]	2016	Coronary-sinus miRNA expression before/after ablation	Contextual—eligible	Longitudinal evidence layer; likely overlapping Beijing programme.
Wang et al. [55]	2015	Ion-channel-regulating miRNAs before/after ablation	Contextual—eligible	Longitudinal evidence layer; likely overlapping Beijing programme.
Li and Lu [56]	2017	Plasma miR-150 before/after ablation	Contextual—eligible	Independent longitudinal evidence layer; no recurrence association.
Xiao et al. [90]	2020	miRNA-328/223 around surgical ablation	Excluded	Wrong intervention: surgical radiofrequency ablation during valve surgery, not catheter ablation.
Huang et al. [91]	2026	Echocardiography and serum miR-26	Excluded	Non-separable intervention: mixed pharmacologic cardioversion and radiofrequency ablation.
Review article (5c) [92]	2021	Review of microRNAs and atrial fibrillation	Excluded	Wrong report type: no original cohort.
Xu et al. (8c) [93]	2014	Coronary-sinus versus peripheral miRNA expression	Excluded	Wrong evidence layer: no recurrence association and no eligible longitudinal pre/post-ablation analysis.

Note: Eligibility was determined only by prespecified criteria. Contextual reports are included in publications in the longitudinal evidence layer. Full-text exclusions receive one objective primary reason; effect direction, significance, risk of bias, study quality, and quantitative usability were not exclusion criteria.

Supplementary Table S5. Criteria used for candidate microRNA prioritization and translational evidence mapping

Criterion	Operational definition	Role in candidate prioritization
Direct recurrence evidence	Evidence in an AF-ablation cohort with post-ablation AF/atrial tachyarrhythmia recurrence.	Mandatory.
Analytically distinct replication	Evidence in at least two independent cohorts or separately recruited derivation and validation samples.	Mandatory; avoids isolated signals.
Adjusted prognosis	Candidate retained in a multivariable prognostic model with an interpretable effect estimate.	One possible additional support domain.
Mechanistic concordance	Experimental target/pathway support or clearly labeled computational/phenotype-level evidence relevant to atrial remodeling.	One possible additional support domain; not proof of prediction.
Analytical feasibility	Measurable circulating signal with sufficiently described matrix, mature-arm identity, processing, and normalization for prospective assay development.	One possible additional support domain.
External evidence	Support from a separate AF biomarker or mechanistic evidence body without reusing an overlapping recurrence cohort.	One possible additional support domain.
Exclusions from prioritization logic	Effect magnitude, direction, statistical significance, pooled-model membership, and title/abstract order.	Never used to select candidates.

Note: Candidate prioritization was post hoc. The same rule was applied to all microRNAs after the framework was formalized; no effect-size, direction, significance, or pooled-model threshold was used.

Supplementary Table S6. Phenotype-oriented interpretation of candidate circulating microRNAs

Clinical or biological phenotype	Most relevant microRNA candidates	Rationale	Evidence strength
Atrial fibrosis / low-voltage substrate	miR-21-5p, miR-29b-3p, miR-10b-5p, miR-320a	Fibrosis, collagen deposition, extracellular matrix remodeling, and atrial substrate severity are central mechanisms of recurrence.	miR-21-5p is the strongest; others are biologically plausible but less replicated.
Enlarged left atrium / structural remodeling	miR-21-5p, miR-150-5p, miR-29b-3p	Left atrial enlargement reflects structural remodeling and may interact with fibrosis and inflammation-related microRNA patterns.	Moderate biological support; limited subgroup-specific evidence.

Clinical or biological phenotype	Most relevant microRNA candidates	Rationale	Evidence strength
Persistent AF / substrate-driven recurrence	miR-21-5p, miR-29b-3p, miR-206, miR-320a	Persistent AF is typically more substrate-dependent than paroxysmal AF; fibrosis and myocardial remodeling signals may be more relevant.	Biologically plausible; formal subgroup meta-analysis not possible.
Inflammatory or immune-remodeling phenotype	miR-150-5p, miR-155	These candidates are linked to inflammatory/immune remodeling pathways and may complement fibrosis-oriented signals.	Exploratory to moderate support.
Early recurrence / acute remodeling phenotype	miR-206, miR-451a, miR-483-5p, miR-320a	Early recurrence may reflect active remodeling, procedural substrate, or unstable atrial biology.	Study-specific signals; requires prospective replication.
Extracellular vesicle or intercellular communication phenotype	miR-20b-5p, miR-330-3p, miR-320c	Extracellular vesicle-related signals may capture cell-to-cell communication and atrial remodeling biology.	Mostly contextual or qualitative evidence.

Note: This phenotype-oriented mapping is descriptive and hypothesis-generating. Published studies did not provide consistent patient-level or subgroup-specific effect estimates by left atrial size, hypertension, AF phenotype, or fibrosis burden; therefore, formal subgroup meta-analysis was not performed.

Supplementary Table S7. Corrected candidate-specific molecular and translational evidence map

Candidate	Direct recurrence evidence	Molecular context	Source/carrier status	Principal limitation	Interpretive status
miR-21-5p (legacy miR-21)	Multiple recurrence reports across peripheral and intracardiac compartments; direction varied by compartment, assay, and coding [26,29,30,36,44,48].	Cardiomyocyte–fibroblast cross-talk and experimental atrial profibrotic signaling involving miR-21 and Spry1 [30,57].	Measured in serum or plasma, including arterial, venous, peripheral, and left-atrial blood; total circulating abundance does not establish atrial or extracellular-vesicle origin.	Mature-arm identity, matrix, compartment, platelet/hemolysis effects, and direction coding.	Priority for prospective validation; certainty very low.
miR-150-5p (legacy miR-150)	Recurrence-specific and longitudinal evidence, including model-based estimates and post-ablation kinetics [22,38,42,50,56].	Experimental evidence links miR-150 to inhibition of cardiac-fibroblast activation through c-Myb [58].	Circulating source and carrier remain unresolved; arm-unspecified legacy labels are not mature-arm confirmation.	Legacy labels, concentration scaling, normalization, and possible blood-cell/platelet contribution.	Priority for prospective validation; certainty very low.

Candidate	Direct recurrence evidence	Molecular context	Source/carrier status	Principal limitation	Interpretive status
miR-29b-3p (legacy miR-29b)	Recurrence-specific evidence in several reports; one estimate had a non-computable confidence bound [35,41,50].	Antifibrotic extracellular-matrix regulation; experimental evidence includes collagen-related programs and PDGF-B signaling with effects on fibrosis and Cx43 in an atrial model [59,60].	Serum/plasma measurements may reflect systemic matrix turnover; concordance with atrial tissue or fibroblast release has not been demonstrated in recurrence cohorts.	Mature-arm attribution, assay scale, attrition, and one zero confidence bound.	Priority for prospective validation; certainty very low.
miR-451a	Lower left-atrial abundance was associated with recurrence, whereas greater scar burden was positively associated with recurrence; a second cohort reported an adjusted inverse association [31,40].	The clinical signal is phenotype-level and aligns with atrial scar/fibrosis; no atrial target pathway has been experimentally confirmed in the recurrence setting.	Strongly erythrocyte-enriched; measured abundance is vulnerable to occult hemolysis and may not represent myocardial release [61,62].	Hemolysis, intracardiac versus peripheral sampling, and small event counts.	Priority for prospective validation; certainty very low.
miR-10b-5p	Two-phase discovery and separately recruited internal validation within one program; lower expression was associated with recurrence [39].	In silico target enrichment implicated FOXO, p53, cellular senescence, and circadian pathways; these are hypotheses rather than demonstrated causal mechanisms [39].	Plasma biomarker with no validated atrial cellular source, carrier fraction, or tissue-expression concordance.	Single-center validation, discovery multiplicity, mixed ablation technologies, and no experimental target validation.	Priority for prospective validation; certainty very low.
miR-320a (reported also as miR-320a-3p)	Adjusted recurrence estimates in two cohorts with different matrices, labels, sampling details, and model consistency [40,47].	Extracellular miR-320a-3p is elevated in paroxysmal AF, supporting a remodeling-associated signal; atrial target-level mechanisms remain unvalidated [63].	Reported in serum/plasma and as extracellular circulating RNA; extracellular-vesicle enrichment was not established in the recurrence cohorts.	Matrix and mature-arm differences, unclear sampling time, internal model inconsistency, and limited mechanistic specificity.	Priority for prospective validation; certainty very low.

Note: Experimental mechanisms, computational pathway enrichment, phenotype-level associations, and source/carrier inference are deliberately separated. Legacy arm-unspecified labels are not treated as mature-arm confirmation.

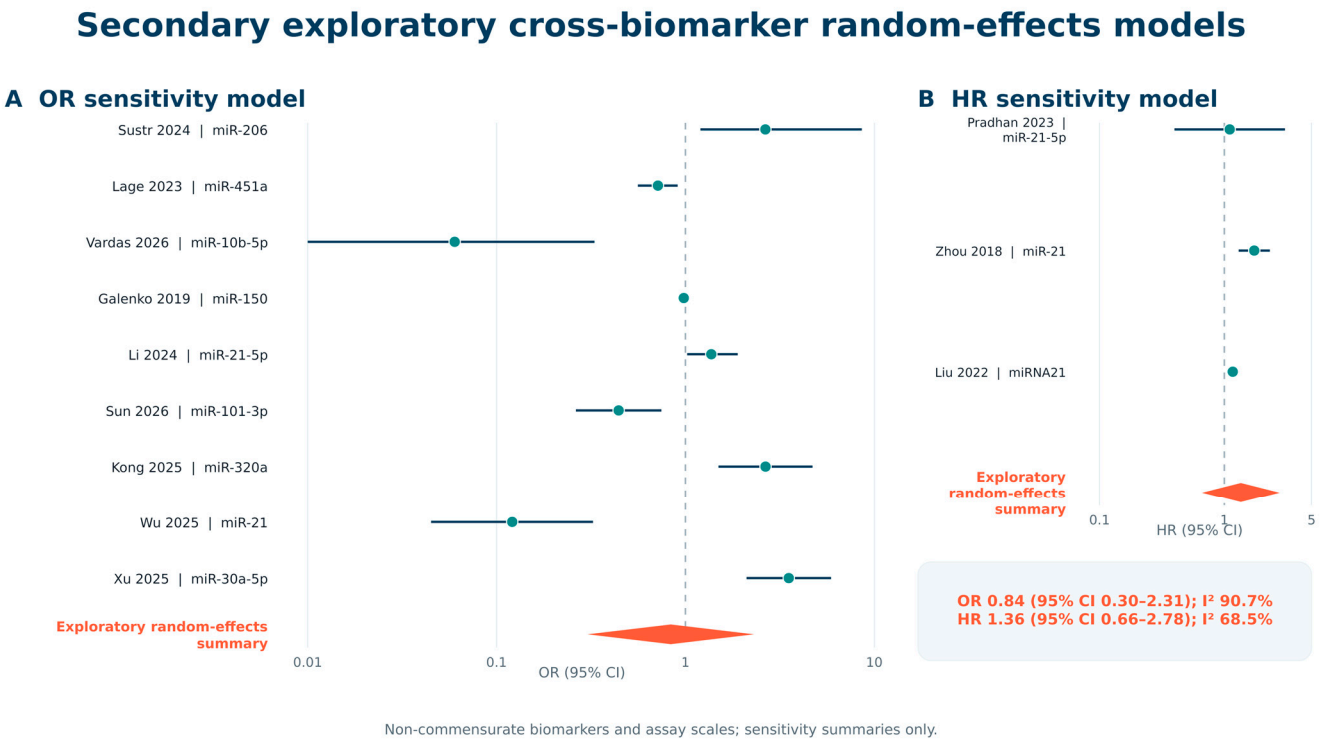
Supplementary Table S8. Reviewer-prompted restrictive OR and HR sensitivity models

Exploratory model	k	n	Summary	95% CI	I^2	τ^2	95% prediction interval	Interpretation
Restrictive OR sensitivity	9	1162	0.84	0.30–2.31	90.7%	1.409	0.04–16.69	Non-commensurate cross-biomarker sensitivity summary; no class-effect interpretation.
Reported HR sensitivity	3	364	1.36	0.66–2.78	68.5%	0.050	0.04–46.36	Sparse, heterogeneous time-to-event sensitivity summary; no class-effect interpretation.

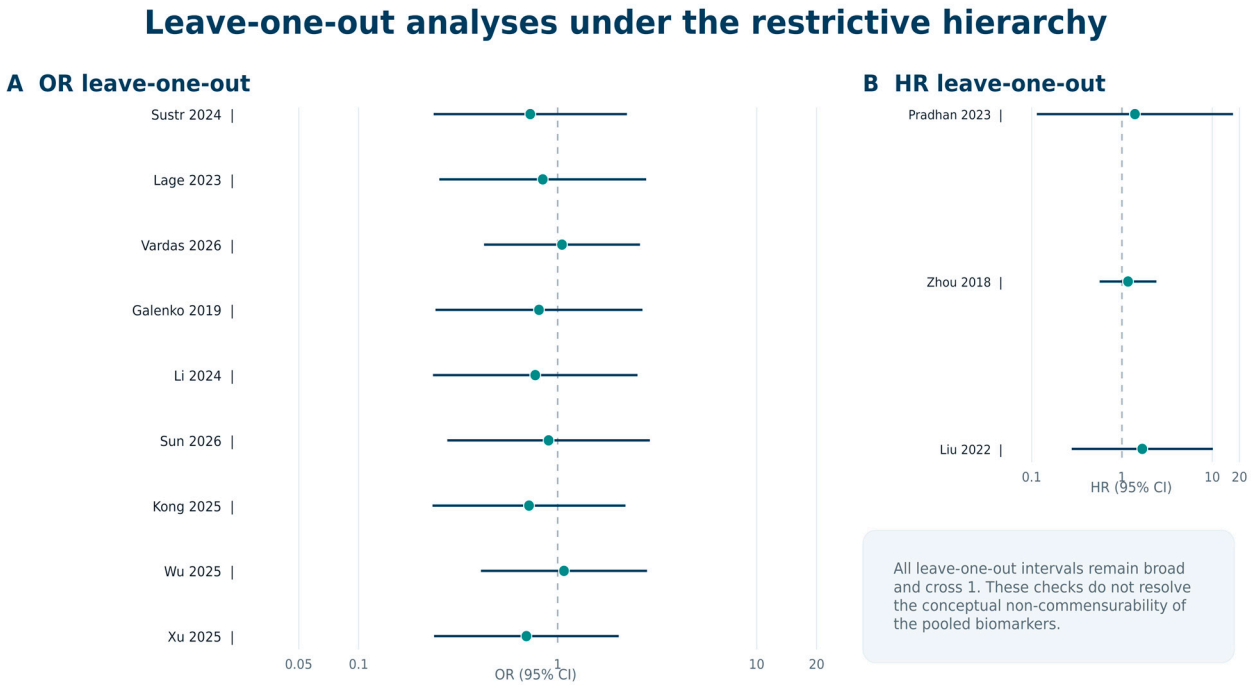
Note: These models are supplementary sensitivity analyses only. Different microRNAs and exposure scales are not commensurate, and the summaries do not estimate a biological class effect.

Supplementary figure legends

Supplementary Figure S1. Secondary exploratory cross-biomarker sensitivity models after application of the reviewer-prompted restrictive hierarchy. The OR model contains nine cohorts (n = 1162) and the HR model contains three cohorts (n = 364). The pooled diamonds are sensitivity summaries only and do not represent a uniform microRNA class effect.



Supplementary Figure S2. Leave-one-out analyses for the restrictive OR and HR sensitivity sets. All intervals remained broad and crossed 1; the analyses do not resolve the conceptual non-commensurability of the biomarkers.



Supplementary Figure S3. QUIPS risk-of-bias traffic-light plot for 26 recurrence-specific reports. Judgments were made after eligibility and were not used to include or exclude reports.

QUIPS risk-of-bias assessment of recurrence-specific reports

	Participation	Attrition	Prognostic factor	Outcome	Confounding	Analysis/ reporting
Lai 2026	High	High	Moderate	Moderate	High	High
Šustr 2024	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Lage 2023	Low	Low	Moderate	Moderate	Moderate	Moderate
Zhan 2024	Moderate	High	Moderate	Moderate	Moderate	High
He 2023	High	High	High	High	High	High
Vardas 2026	Low	Low	Moderate	Low	Moderate	Moderate
Pradhan 2023	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Galenko 2019	Moderate	Moderate	Low	Moderate	Moderate	Moderate
Zhou 2018	Moderate	Moderate	Moderate	Moderate	High	High
Liu 2022	Moderate	Low	Moderate	Moderate	Moderate	Moderate
Ruan 2025	Moderate	Low	High	Moderate	High	High
Vaze 2017	Low	Moderate	Moderate	Moderate	Moderate	Moderate
Harada 2023	Moderate	Low	Moderate	Moderate	High	Moderate
Ren 2025	Moderate	Low	Moderate	Moderate	Moderate	High
Kiliszek 2020	Low	Low	Low	Low	Moderate	Low
Liu 2025 thesis	Low	Moderate	Moderate	Low	Moderate	High
Li 2023	Low	Low	Moderate	Moderate	Moderate	Moderate
Zhou 2024 (CN)	Low	Low	Moderate	Moderate	High	High
Wang 2024	Low	Low	Moderate	Moderate	Moderate	Moderate
Li 2024	Low	Low	Moderate	Moderate	Moderate	Moderate
Wang 2021	Low	Low	Moderate	Moderate	High	High
Sun 2026	Low	Low	Moderate	Moderate	Moderate	Moderate
Kong 2025	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate
Wu 2025	Low	Low	Moderate	High	Moderate	High
Xu 2025	Low	Low	Moderate	Moderate	Moderate	Moderate
Lai 2023 (CN)	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate

Risk-of-bias judgments were made after eligibility and were not used to include or exclude reports.

Supplementary Table S9. Wanfang screening update and full-text decisions

Stage/report	Number	Decision	Prespecified reason
Records screened	50	Screened	All records assessed against the same eligibility criteria
Excluded at title/abstract	29	Excluded	Clearly failed one or more eligibility criteria
Full texts assessed	21	Assessed	No exclusion based on results or quality
Eligible reports	17	Included	11 recurrence-specific; 6 longitudinal/contextual
Xiao 2020	1	Excluded	Surgical rather than catheter ablation
Huang 2026	1	Excluded	Mixed intervention without separable catheter-ablation results
Review (5c)	1	Excluded	No original cohort
Xu 2014 (8c)	1	Excluded	Outside both eligible evidence layers

Other re-evaluated databases yielded no additional eligible reports. The final review retained 35 publications.

Supplementary Table S10. Audit of the reviewer-prompted restrictive effect-selection hierarchy

Study	Candidate used previously	n	Restrictive sensitivity decision	Reason
Šustr 2024	miR-206	48	Included	Author-designated primary prognostic biomarker.
Lage 2023	miR-451a	42	Included	Author-designated primary prognostic biomarker.
Vardas 2026	miR-10b-5p	126	Included	Author-designated primary estimate in the validation phase.
Galenko 2019	miR-150	141	Included	Unique compatible model-based estimate.
Li 2024	miR-21-5p	120	Included	Author-designated primary prognostic biomarker.
Sun 2026	miR-101-3p	205	Included	Author-designated primary prognostic biomarker.
Kong 2025	miR-320a	200	Included	Author-designated primary prognostic biomarker.
Wu 2025	miR-21	125	Included	Unique compatible model-based estimate; published coding retained.
Xu 2025	miR-30a-5p	155	Included	Author-designated primary prognostic biomarker.

Study	Candidate used previously	n	Restrictive sensitivity decision	Reason
Lai 2026	miR-483-5p	122	Excluded from cross-biomarker sensitivity model	Multiple eligible biomarkers without a unique author-designated single-biomarker primary estimate.
Vaze 2017	miR-125a-5p	83	Excluded from cross-biomarker sensitivity model	Multiple eligible biomarkers; the earlier title/abstract-order rule was not retained.
Ren 2025	miRNA-21	126	Excluded from cross-biomarker sensitivity model	Multiple eligible biomarkers without a unique author-designated primary estimate.
Wang 2024	miR-133a-3p	180	Excluded from cross-biomarker sensitivity model	Multiple eligible biomarkers without a unique author-designated primary estimate.
Liu Meichen 2025	miR-320a-3p	150	Excluded from cross-biomarker sensitivity model	Multiple eligible biomarkers without a unique author-designated primary estimate.
Lai 2023	miR-192-5p	254	Excluded from cross-biomarker sensitivity model	Multi-biomarker model without a unique eligible single-biomarker primary estimate.

Supplementary Table S11. Candidate-specific GRADE certainty profiles

GRADE note: Observational prognostic evidence began at high certainty under GRADE guidance for prognostic-factor research. ‘Not serious’ does not establish consistency when only two studies are available, and ‘not assessable’ indicates that a single program cannot support an inconsistency judgment. Mechanistic plausibility did not upgrade clinical prognostic certainty. Publication bias was strongly suspected because candidate-specific bodies were small and selected signals predominated; formal asymmetry tests were not performed because every candidate had fewer than 10 independent studies.

Candidate	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias / upgrading	Final certainty and principal rationale
miR-21-5p / miR-21	Serious	Very serious	Serious	Serious	Strongly suspected; no upgrade	Very low: discordant directions and compartments, heterogeneous assays, and imprecise study-level evidence.
miR-150-5p / miR-150	Serious	Serious	Serious	Serious	Strongly suspected; no upgrade	Very low: legacy labels and non-commensurate concentration/normalization scales limit replication.

Candidate	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias / upgrading	Final certainty and principal rationale
miR-29b-3p / miR-29b	Serious	Not serious*	Serious	Serious	Strongly suspected; no upgrade	Very low: few cohorts, arm/assay differences, attrition, and a non-computable confidence bound.
miR-451a	Serious	Not serious*	Serious	Serious	Strongly suspected; no upgrade	Very low: only two small cohorts, different compartments, and marked susceptibility to hemolysis.
miR-10b-5p	Serious	Not assessable*	Not serious	Serious	Strongly suspected; no upgrade despite a large estimate	Very low: a single-center program with internally separated phases and no external validation.
miR-320a / miR-320a-3p	Serious	Serious	Serious	Serious	Strongly suspected; no upgrade	Very low: matrix, label, sampling, and model-reporting differences across two cohorts.

Supplementary abbreviations

AF, atrial fibrillation; AMI, acute myocardial infarction; CABG, coronary artery bypass grafting; CI, confidence interval; DCM, dilated cardiomyopathy; EV, extracellular vesicle; GRADE, Grading of Recommendations, Assessment, Development and Evaluation; HR, hazard ratio; LVA, low-voltage area; MACE, major adverse cardiovascular events; NGS, next-generation sequencing; OR, odds ratio; POAF, postoperative atrial fibrillation; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses; QUIPS, Quality in Prognosis Studies; RF, radiofrequency; RFCA, radiofrequency catheter ablation; RHD, rheumatic heart disease; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

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