MicroRNA as Biomarkers for Platelet Function and Maturity in Patients with Cardiovascular Disease

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Abstract

Patients with cardiovascular disease (CVD) are at increased risk of suffering myocardial infarction. Platelets are key players in thrombus formation and, therefore, antiplatelet therapy is crucial in the treatment and prevention of CVD. MicroRNAs (miRs) may hold the potential as biomarkers for platelet function and maturity. This systematic review was conducted using the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). To identify studies investigating the association between miRs and platelet function and maturity in patients with CVD, PubMed and Embase were searched on October 13 and December 13, 2020 without time boundaries. Risk of bias was evaluated using a standardized quality assessment tool. Of the 16 included studies, 6 studies were rated "good" and 10 studies were rated "fair." In total, 45 miRs correlated significantly with platelet function or maturity (rho ranging from −0.68 to 0.38, all $p < 0.05$) or differed significantly between patients with high platelet reactivity and patients with low platelet reactivity (*p*-values ranging from 0.0001 to 0.05). Only four miRs were investigated in more than two studies, namely miR-223, miR-126, miR-21 and miR-150. Only one study reported on the association between miRs and platelet maturity. In conclusion, a total of 45 miRs were associated with platelet function or maturity in patients with CVD, with miR-223 and miR-126 being the most frequently investigated. However, the majority of the miRs were only investigated in one study. More data are needed on the potential use of miRs as biomarkers for platelet function and maturity in CVD patients.

Keywords
- ► MicroRNAs
- ► biomarker
- ► platelet function test
- ► prognosis
- ► cardiovascular disease

Introduction

Cardiovascular diseases (CVDs) account for a considerable percentage of morbidity and mortality.[1,2] Platelets are essential for repairing endothelial damage and maintaining primary haemostasis.[3] However, due to plaque rupture and platelet-mediated thrombosis, patients with CVD may experience myocardial infarction.[4–6] Accordingly, platelet inhibitors are considered to be the cornerstone in prevention and treatment of CVD caused by atherosclerosis.[7–9] However, despite prophylactic antiplatelet therapy, patients remain at

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high risk of recurrent cardiovascular events.[10–13] The insufficient effect of platelet inhibitors is probably caused by multiple mechanisms such as genetic variability, drug interactions, and increased platelet turnover.[14,15] Hence, there is a great need for biomarkers to identify these high-risk patients. Altered platelet function and the amount of newly formed platelets, termed immature platelets, are of great importance for the risk of recurrent cardiovascular events.[15,16] Immature platelets, contrary to mature platelets, contain ribonucleic acid (RNA) providing the ability to produce proteins important for platelet function.[17–19]

MicroRNAs (miRs) are small noncoding RNA[20] involved in gene regulation[21,22] and present in many cells including platelets.[23] Owing to their stability in the circulation, miRs may hold a potential as biomarkers.[21] Previous studies indicate that miRs contribute to the regulation of both platelet formation[24,25] and platelet function.[26,27]

The objective of this systematic review was to summarize and discuss the current literature on miRs potentially associated with platelet function and maturity in patients with CVD.

Methods

Study Design and Search Strategy

The systematic review was conducted using a predefined protocol following the guidelines of Preferred Reporting items for Systematic Reviews and Meta-analyses (PRISMA),[28] and was registered in PROSPERO International Prospective Register of Systematic Reviews (https://www.crd.york.ac.-ul/prospero/;Record-ID: 220095). A systematic search of all original research without time boundaries was performed in the databases PubMed/Medline and Embase/Ovid on October 13, 2020 and updated on December 13, 2020. Additional articles were found in reference lists of identified studies and by using the "shared references" search in the Web of Science database. Authors of identified appropriate studies were contacted to obtain additional data not reported.

The PubMed search string was as follows: ((((micrornas) AND ((((((((((blood platelets) OR ("platelet function"[All Fields])) OR ("platelet function tests"[MeSH Terms])) OR ("platelet activation"[MeSH Terms])) OR (platelet aggregation)) OR (platelet adhesiveness)) OR (mean platelet volume)) OR ("immature platelets"[All Fields])) OR ("immature platelet fraction"[All Fields])) OR ("immature platelet count"[All Fields]))) AND (((cardiovascular disease) OR (arteriosclerosis)) OR ("coronary artery disease"[MeSH Terms]))) NOT (("Animals"[Mesh]) NOT ("Humans"[Mesh]))). The Embase search string was as follows: 'microrna'/exp AND ('thrombocyte'/exp OR 'thrombocyte function'/exp OR 'thrombocyte adhesion'/exp OR 'thrombocyte aggregation'/exp OR 'thrombocyte activation'/exp OR 'mean platelet volume'/exp OR 'immature platelet count'/exp OR 'immature platelet count' OR 'immature platelet fraction'/exp OR 'immature platelet fraction') AND ('cardiovascular disease'/exp OR 'cardiovascular disease' OR 'coronary artery disease'/exp OR 'coronary artery disease') NOT (('animal'/exp OR 'animal' OR 'nonhuman'/exp OR 'nonhuman') NOT (('animal'/exp OR 'animal' OR 'nonhuman'/exp OR 'nonhuman') AND ('human'/exp OR 'human'))) AND [english]/lim AND ('article'/it OR 'article in press'/it OR 'review'/it).

Eligibility Criteria and Study Selection

Inclusion and exclusion criteria were pre-specified before the literature search. Inclusion criteria were the following: (1) studies examining patients with CVD; (2) studies examining the expression of miRs; and (3) studies presenting original data evaluating platelet function (determined by adhesion, activation or aggregation), maturity (determined by immature platelet count, immature platelet fraction or mean platelet volume [MPV]) or both. Exclusion criteria were the following: (1) letters, conference abstracts, editorials and letters without original data, guidelines, meta-analysis or comments without original data; (2) case reports with less than five patients; (3) animal and in vitro studies; and (4) non-English publications.

After removing duplicates, 20 random abstracts were distributed between two authors, OBP and AMH, and screened for relevance to evaluate inclusion criteria. The remaining abstracts were screened by OBP. Studies considered relevant or unclear were assessed in full-text review. By choosing a random sample of 10 of the full text, the study selection was tested independently by OBP and AMH. All disagreements were discussed by all authors to reach consensus. OBP performed the rest of the study selection.

Data Extraction

Data extraction was performed by OBP and verified by AMH.

Measurements of platelet function were reported as platelet activation or platelet aggregation. Platelet activation analyses included measurements of soluble markers of platelet activation (expression of activation-dependent platelet markers) and platelet aggregation as functional analyses. Platelet maturity was reported as MPV. As immature platelets are larger than mature platelets, increasing MPV values correspond to an increased number of immature platelets.[16,17,29]

To evaluate the potential risk of bias, OBP and AMH independently evaluated each study using the standardized quality assessment tool for observational cohort and cross-sectional studies from the National Heart, Lung, and Blood Institute of the National Institutes of Health rating the quality of studies as good, fair or poor.[30] In accordance with most of the included studies, the ranking was performed with platelet function or maturity as exposure and the miR levels as outcome. Disagreements were discussed to reach consensus.

Results

►Fig. 1 shows the inclusion and exclusion flow diagram. After removal of duplicates, 335 records were screened by title and abstract. During this screening process, 297 records were excluded and the remaining 38 records were assessed for eligibility in full-text papers. Among these, 22 records were excluded in accordance with the eligibility criteria, leaving 16 studies for inclusion in the systematic review.

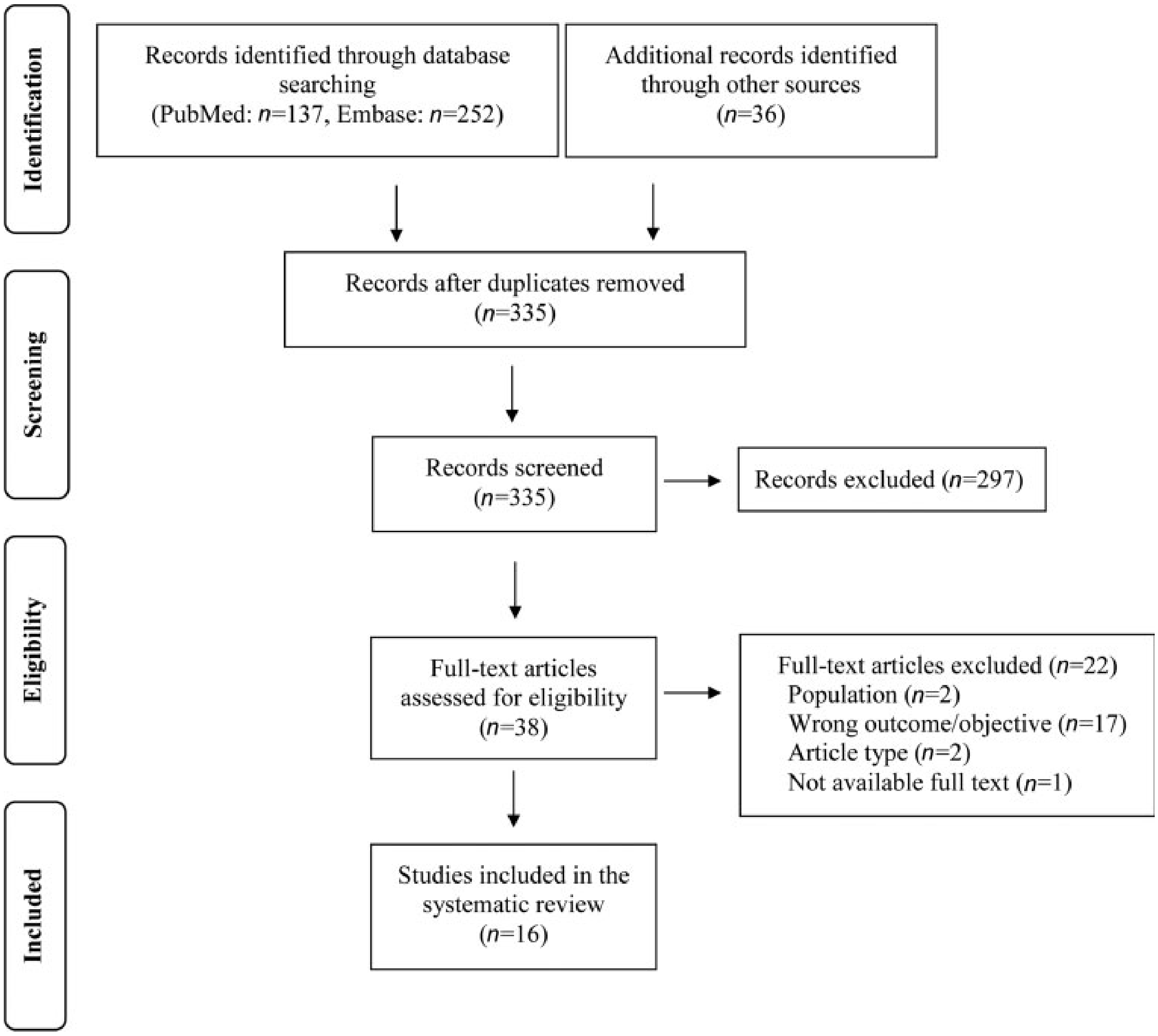

Fig. 1 Flow diagram of the systematic literature search.

miR Analyses

►**Table 1** summarizes characteristics of the miR analyses in the included 16 studies. One study used blood cells,[31] 1 study used leukocyte suspension[32] and 14 studies used plasma samples.[33–46] Among the studies using plasma samples, seven studies used platelet-rich plasma (PRP),[34,36,37,39,42,43,46] whereas two studies used platelet-poor plasma (PPP).[33,35]

To compensate for potential analytical variations,[47] all studies used normalization strategies of the measured miRs. Four studies used high-throughput sequencing to investigate a large number of miRs followed by reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) of a selected number of miRs.[33,39,44,46] The remaining 12 studies used only RT-qPCR to investigate pre-selected miRs.[31,32,34–38,40–43,45]

Subgroups, Laboratory Analyses, and Treatment

In 12 of 16 included studies, the patient cohort was divided into subgroups.[32–37,39,42–46] The methods and cut-offs employed to define subgroups varied between studies. Therefore, we decided to define the patient cohorts as having either high platelet reactivity (HPR) or low platelet reactivity (LPR) corresponding to low and high antiplatelet response.

To categorize patients, 9 of 16 included studies used platelet aggregation measurements (multiple electrode aggregation [MEA], light transmission aggregation [LTA] or optical aggregation),[32,35,37–40,43,44,46] 2 studies used platelet reactivity index (PRI) by vasodilator-stimulated phosphoprotein (VASP) measurements,[36,45] 4 studies used a combination of these[33,34,41,42] and 1 study used MPV.[31]

In five of the included studies, blood samples were obtained at different time points.[33,34,37,40,43] The results were only presented if the studies also investigated the corresponding association between miRNA and platelet function. We denominated the first blood sample as *baseline* and the following blood samples were named after the number of days they were taken after the baseline sample.

All included studies, except one,[31] provided information on antiplatelet therapy. In studies on patients with acute coronary syndrome (ACS), all patients were treated according to guidelines with dual antiplatelet inhibitors consisting

Table 1 MicroRNA analyses in the 16 included studies

Study	Anticoagulant	Sample	Further preparation	RNA extraction	Sequencing	RT-qPCR	Normalization
Becker et al[33]	NA	PPP	NA	Perfect pure + miRNeasy	Yes	Yes	Endogenous miRs
Binderup et al[35]	EDTA	PPP	1 cycle of centrifugation	Nucleospin	No	Yes	miR-39
Chen et al[36]	Citrate	PRP	1 cycle centrifugation + anti-CD45 beads	NA	No	Yes	U6
Chen et al[37]	NA	PRP	Centrifugation[a] NA cycles	High pure	No	Yes	U44 + U48
Chyrchel et al[38]	EDTA	Plasma	NA	mirVana	No	Yes	miR-16
Ding et al[39]	Citrate	PRP	2 cycles of centrifugation	TRIzol	Yes	Yes	miR-16
Jäger et al[40]	EDTA	Plasma	NA	miRNeasy	No	Yes	miR-39
Kaudewitz et al[41]	Citrate	Plasma	2 cycles of centrifugation	miRNeasy	No	Yes	U6
Li et al[31]	EDTA	Blood cells	NA	TRIzol	No	Yes	U6
Li et al[34]	EDTA	PRP	3 cycles of centrifugation	miRNeasy	No	Yes	R43
Peng et al[43]	Citrate	PRP	Anti-CD45 beads	mirVana	No	Yes	U6
Shi et al[42]	Citrate	PRP	Anti-CD45 beads	TRIzol + mirVana	No	Yes	U6
Tang et al[44]	EDTA	Plasma	1 cycle of centrifugation	mirVana	Yes	Yes	miR-39
Xie et al[32]	NA	Leukocytes	NA	TRIzol + miRNeasy	No	Yes	U6
Zhang et al[45]	Citrate	Plasma	NA	RNAiso	No	Yes	Human-5s-rRNA
Zufferey et al[46]	EDTA	PRP	1 cycle of centrifugation	mirVana	Yes	Yes	Internal controls

Abbreviations: EDTA, Ethylenediaminetetraacetic acid; NA, not available; PPP, platelet-poor plasma; PRP, platelet-rich plasma; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction.
[a]Centrifugation until leucocyte to platelet ratio <0.4%.

of aspirin and clopidogrel preceded by loading dose (►**Table 2**).[33,34,38,39,42,43,45] In the studies on patients with coronary artery disease (CAD), the antiplatelet treatment consisted of either aspirin monotherapy or dual antiplatelet therapy with aspirin plus adenosine diphosphate (ADP) inhibitor (►**Table 3**).[32,33,36,37,40,41,44] In the two studies examining patients with intermittent claudication[35] or patients with symptomatic atherothrombotic diseases,[46] all patients were treated with aspirin monotherapy (►**Table 4**).

Study Characteristics

The included studies were divided into three groups based on patient characteristics. ►**Table 2** presents studies investigating ACS patients ($n = 6$).[34,38,39,42,43,45] ►**Table 3** presents studies investigating stable CAD patients ($n = 7$).[31,32,36,37,40,41,44] ►**Table 4** presents studies investigating other patient groups including both ACS and CAD patients ($n = 1$),[33] patients with intermittent claudication ($n = 1$)[35] and patients with symptomatic atherothrombotic diseases defined as CAD, ischemic cerebrovascular disease and/or peripheral artery disease ($n = 1$).[46] In the studies using sequencing to investigate a variety of miRs,[33,39,44,46] only those miRs presented with corresponding association with platelet function or used in further analyses are presented in the tables. Seven studies reported miRs as the miR family without further specifications.[34,38,40–43,45] Nine studies further specified the investigated miR, that is 3p- or 5p arm of the particular miR.[31–33,35–37,39,44,46] Therefore, if a particular miR was reported in various ways, the tables present the overall miR family. A comprehensive overview of miRs including the number of studies and the corresponding patient groups is available in the ►**Supplementary Table S1** (available in the online version). The qualitative assessment of the included studies is also indicated in ►**Tables 2,3,4**. Six studies were rated good,[31,39,42–45]10 studies fair[32–38,40,41,46] and no studies were rated poor. A detailed review of the ratings including the related questions is available in the ►**Supplementary Table S2** (available in the online version).

Expression Levels of miRs and Platelet Function and Maturity

Out of 58 investigated miRs, 45 miRs correlated significantly with platelet function or maturity (rho ranging from –0.68 to 0.38, all $p < 0.05$) or differed significantly between HPR patients and LPR patients (p-values ranging from 0.0001 to 0.05), whereas 13 miRs showed no association with platelet function or maturity (►**Table 5**).

Of the miRs significantly associated with platelet function, 10 of 45 were investigated in more than one study (►**Fig. 2**). The most frequently investigated miRs were miR-223 ($n = 9$),[32,34,37,38,40–43,45] miR-126 ($n = 5$),[33,34,40,41,44] miR-21 ($n = 5$)[33,34,40,43,44] and miR-150 ($n = 4$),[34,40,41,46] whereas

Table 2 Studies investigating microRNA (miRs) and platelet function or maturity in patients with acute coronary syndromes ($n = 6$)

Study, *rating*	Participants	Antiplatelet treatment	Platelet analysis	miRs	Results reported
Ding et al,[39] *good*	3 cohorts: (1) *Sequencing*: 5 HPR and 5 LPR patients (2) *Validation I*: 5 HPR and 5 LPR patients 3) *Validation II*: 24 HPR and 30 LPR patients	Dual treatment with 100-mg OD aspirin and 75-mg clopidogrel OD Loading dose with 300-mg clopidogrel and 300-mg aspirin	Platelet aggregation, (LTA with ADP as agonist) Mean/median as cut-off between HPR and LPR	*Sequencing cohort*: miR-204–5p, miR-501–3p, miR-584–5p, miR-1273h-3p, miR-1908–5p, miR-nov-chr1, miR-nov-chr8, miR-nov-chr22 *Validation cohort I*: miR-204–5p, miR-584–5p *Validation cohort II*: miR-204–5p	*Sequencing cohort:* ↑miR-204–5p HPR vs. LPR patients ($p = 0.004$) ↓miR-1908–5p HPR vs. LPR patients ($p = 0.04$) →miR-501–3p, miR-584–5p, miR-1273h-3p, miR-nov-chr1, miR-nov-chr8, miR-nov-chr22 HPR vs. LPR patients (all $p > 0.05$) *Validation cohort I:* ↑miR-204–5p HPR vs. LPR patients ($p = 0.001$) →miR-584–5p HPR vs. LPR patients ($p > 0.05$) *Validation cohort II:* ↑miR-204–5p HPR vs. LPR patients ($p = 0.023$)
Peng et al,[43] *good*	39 patients: 18 HPR and 21 LPR patients	Dual treatment with 100-mg OD aspirin and 75-mg clopidogrel OD Loading dose with 300-mg clopidogrel	Platelet aggregation, (LTA with ADP as agonist) Relative platelet inhibition = ([pre-treatment-post-treatment]/pre-treatment) × 100% as cut-off, >84% = HPR and <10% = LPR	miR-21, miR-221, miR-223	↓miR-21 HPR vs. LPR patients ($p = 0.01$) ↓miR-221 and miR-223 HPR vs. LPR patients ($p = 0.004$) ↓miR-223 HPR vs. LPR patients ($p = 0.02$)
Li et al,[34] *fair*	15 patients: HPR and LPR	Loading dose with 600-mg clopidogrel and 300-mg aspirin	PRU: 235 as cut-off between HPR and LPR PRI: mean/median as cut-off between HPR and LPR	miR-1, miR-21, miR-126, miR-150, miR-223	→any miRs HPR vs. LPR patients (all $p > 0.05$)
Chyrchel et al,[38] *fair*	21 patients	Dual treatment with 75- to 100-mg aspirin OD plus either 75-mg clopidogrel OD or 10-mg prasugrel OD or 90-mg ticagrelor TD Loading dose with either 300- to 600-mg clopidogrel or 60-mg prasugrel or 180-mg ticagrelor	Platelet aggregation, (MEA, ADP as agonist)	miR-223	Correlation: miR-223 and platelet aggregation (rhoS = −0.52; $p = 0.015$)
Zhang et al,[45] *good*	62 patients: 31 HPR and 31 LPR patients	Dual treatment with 100-mg OD aspirin and 75-mg clopidogrel OD for 5 d or a loading dose of 300-mg clopidogrel and 300-mg aspirin	PRI: mean/median as cut-off between HPR and LPR	miR-223	Correlation: miR-223 and PRI (rhoS = −0.38, $p = 0.002$) ↓miR-223 HPR vs. LPR patients ($p = 0.007$)
Shi et al,[42] *good*	33 patients: 16 HPR and 17 LPR patients	Dual treatment with 100-mg OD aspirin and 75-mg clopidogrel OD for 5 d or a loading dose of 300-mg clopidogrel and 300-mg aspirin	Platelet aggregation (LTA with ADP as agonist): mean/median as cut-off between HPR and LPR PRI: Mean/median as cut-off between HPR and LPR	miR-96, miR-223	Correlation: miR-223 and PRI (rhoS = −0.40, $p = 0.02$) ↓miR-223 PRI determined HPR vs. LPR patients ($p = 0.037$) →miR-96 PRI determined HPR vs. LPR patients ($p > 0.05$) →miR-96 platelet aggregation determined HPR vs. LPR patients ($p > 0.05$) →miR-223 platelet aggregation determined HPR vs. LPR patients ($p > 0.05$)

Abbreviations: ADP, adenosine diphosphate; HPR, high platelet reactivity; LPR, low platelet reactivity; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; OD, once-daily; rhoS, Spearman's rho; PRI, platelet reactivity index, based on flow cytometric vasodilator-stimulated phosphoprotein (VASP) measurement; TD, twice-daily; PRU, P2Y12 reaction unit, measured by VerifyNow assay using ADP as agonist; ↑, higher value; ↓, lower value; →, no difference or no correlation.

Table 3 Studies investigating microRNA (miRs) and platelet function or maturity in patients with coronary artery disease ($n = 7$)

Study, *rating*	Participants	Antiplatelet treatment	Platelet analysis	miRs	Results reported
Li et al,[31] *good*	81 patients	Not reported	MPV	miR-202–3p	Correlation: miR-202–3p and MPV (rP = –0.29, $p = 0.01$)
Tang et al,[44] *good*	115 patients: HPR and LPR patients	Loading dose with 300-mg clopidogrel and 100-mg aspirin	ARU: 550 as cut-off between HPR and LPR PRU: 208 as cut-off between HPR and LPR	miR-21, miR-27a-3p, miR-106a, miR-126, miR-130a-5p, miR-142–5p	Correlation: PRU and miR-21 (rhoS = 0.23, $p = 0.01$), miR-27a-3p (rhoS = 0.30, $p = 0.001$), miR-126 (rhoS = 0.38, $p < 0.0001$), miR-130a-3p (rhoS = 0.30, $p = 0.001$), miR-142–5p (rhoS = 0.33, $p = 0.0004$) →correlation: miR-106a and PRU ($p > 0.05$) ↑miR-27a-3p ($p < 0.001$), ↑miR-106a- 5p ($p < 0.05$), ↑miR-126 ($p < 0.0001$), ↑miR-130a-5p ($p < 0.0001$) and ↑miR- 142–5p ($p < 0.001$) PRU determined HPR vs. LPR patients →miR-21–5p PRU determined HPR vs. LPR patients ($p > 0.05$) ↑miR-27a-3p ($p < 0.05$), ↑miR-126 ($p < 0.05$) and ↑miR-130a-5p ($p < 0.05$) in ARU determined LPR + PRU determined HPR vs. ARU + PRU determined LPR patients ↓miR-21 ($p < 0.05$) in ARU determined LPR + PRU determined HPR vs. ARU + PRU determined LPR patients →miR-106a-5p and →miR-142–5p in ARU determined LPR + PRU determined HPR vs. ARU + PRU determined LPR patients ($p > 0.05$) ↑miR-21–5p ($p < 0.05$), ↑miR-27a-3p ($p < 0.05$), ↑miR-126 ($p < 0.01$), ↑miR-130a-5p ($p < 0.01$) and ↑miR-142–5p ($p < 0.001$) in ARU + PRU determined HPR than ARU determined HPR + PRU determined LPR patients →miR-106a-5p in ARU + PRU determined HPR vs. ARU determined HPR + PRU determined LPR patients ($p > 0.05$)
Jäger et al,[40] *fair*	62 patients	Dual treatment with 100-mg aspirin OD plus either 75-mg clopidogrel OD or 10-mg prasugrel OD or 90-mg ticagrelor TD	Platelet aggregation, (MEA, ADP as agonist)	miR-21, miR-126, miR-150, miR-223	Baseline (last day of P2Y12 inhibitor intake): →correlation: any miRs and platelet aggregation (all $p > 0.05$) 10 d: correlation: miR-150 and platelet aggregation (rhoS = 0.269, $p = 0.049$) Correlation: miR-223 and platelet aggregation (rhoS = 0.282, $p = 0.039$) 30 d: →correlation: Any miRs and platelet aggregation (all $p > 0.05$) 6 mo: →correlation: any miRs and platelet aggregation (all $p > 0.05$)
Chen et al,[37] *fair*	< 155 patients: HPR and LPR patients	Dual antiplatelet treatment with 100-mg aspirin OD plus either 75-mg clopidogrel OD or 75-mg clopidogrel OD and 100-mg cilostazol TD or 90-mg ticagrelor TD Loading dose with 300-mg aspirin and either 300-mg clopidogrel or 180-mg ticagrelor or no loading dose of cilostazol	PRU: >208 = HPR and <85 = LPR as cut-off	miR-15a-5p, miR-96, miR-107, miR-223, miR-339–3p, miR-365–3p, miR-495–3p	Baseline (24 h after treatment start): ↑miR-365–3p HPR vs. LPR patients ($p < 0.001$) →miR-15a-5p, →miR-96, →miR- 107, →miR-223, →miR-339–3p and →miR-495–3p HPR vs. LPR patients ($p > 0.05$) 7 d: ↑miR-365–3p HPR vs. LPR patients ($p < 0.001$) →miR-15a-5p, →miR-96, →miR-107, →miR-223, →miR-339–3p and →miR-495–3p HPR vs. LPR patients ($p > 0.05$)
Xie et al,[32] *fair*	60 patients: 36 HPR and 24 LPR patients	Dual treatment with 100-mg OD aspirin and 75-mg clopidogrel OD	Platelet aggregation (MEA, ADP as agonist) > 10Ω = HPR and <1Ω = LPR as cut-off	miR-223	→miR-223 HPR vs. LPR patients ($p > 0.05$) →correlation: miR-223 and platelet aggregation (r = NA, $p > 0.05$)
Chen et al,[36] *fair*	43 patients: 17 HPR and 26 LPR patients	300-mg clopidogrel loading dose, followed by 75-mg OD	PRI: mean/median as cut-off between HPR and LPR	miR-23a, miR-26a, miR-199	↑platelet miR-26a HPR vs. LPR patients ($p < 0.05$) →serum miR-26a HPR vs. LPR patients ($p > 0.05$) →miR-199 HPR vs. LPR patients ($p > 0.05$) →miR-23a HPR vs. LPR patients ($p > 0.05$)

Table 3 (*Continued*)

Study, *rating*	Participants	Antiplatelet treatment	Platelet analysis	miRs	Results reported
Kaudewitz et a,l[41] *Fair*	125 patients	Either 75-mg aspirin OD monotherapy or dual treatment with 75-mg aspirin OD plus either 75-mg clopidogrel OD or 10-mg prasugrel OD or 90-mg ticagrelor TD	Platelet aggregation, (LTA with ADP and AA as agonists) PRU PRI	miR-20b, miR-24, miR-30b, miR-93, miR-106a, miR-126, miR-146b, miR-150, miR-191, miR-197, miR-223	→correlation: any miRs and platelet aggregation (all $p > 0.05$) Correlation: miR-126 and PRU (rP = 0.35, $p = 0.033$) →correlation: any other miRs and PRU (all $p > 0.05$) Correlation: PRI and miR-20b (rP = 0.22, $p = 0.014$), miR-24 (rP = 0.25, $p = 0.006$), miR-30b (rP = 0.23, $p = 0.012$), miR-126 (rP = 0.22, $p = 0.016$), miR-191 (rP = 0.24, $p = 0.009$), miR-197 (rP = 0.23, $p = 0.012$) and miR-223 (rP = 0.28, $p = 0.002$). →correlation miR-93, miR-106a, miR-146b, miR-150 and PRI (all $p > 0.05$)

Abbreviations: AA, arachidonic acid; ADP, adenosine diphosphate; ARU, aspirin reaction unit measured by VerifyNow assay using AA as agonist; HPR, high platelet reactivity; LPR, low platelet reactivity; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; MPV, mean platelet volume; NA, not available; OD, once-daily; rhoS, Spearman's rho; rP, Pearson's r; PRI, platelet reactivity index, based on flow cytometric VASP measurement; TD, twice-daily; PRU, P2Y12 reaction unit, measured by VerifyNow assay using ADP as agonist; ↑, higher value; ↓, Lower value; →, no difference or no correlation.
Note: In Chen et al,[37] the number of patients in each subgroup are not reported; hence, *N* is indicated as <155 (total number of patients).

miR-1,[34,46] miR-93,[33,41] miR-96,[37,42] miR-106a,[41,44] miR-197[33,41] and miR-204–5p[39,46] were investigated in two studies only. Only one study reported on platelet maturity by measurements of MPV.[31]

miR-223

Significant negative associations were found between miR-223 and platelet function in four of the five studies including ACS patients,[38,42,43,45] whereas Li et al reported no association between miR-223 and platelet function in ACS patients.[34] On the contrary, among four studies including only CAD patients, two studies reported positive correlations between miR-223 and platelet function,[40,41] whereas two studies found no association.[32,37]

miR-126

Five studies investigated miR-126 and platelet function. In a study including ACS patients only, no association was found.[34] In patients with CAD only, two studies reported positive correlations between miR-126 and platelet function,[41,44] whereas one study demonstrated no association.[40] Becker et al included both ACS and CAD patients and found that miR-126 correlated negatively with platelet function in the target cohort consisting of ACS patients.[33] However, in the validating cohorts of CAD patients, a positive correlation with platelet function was found.[33]

miR-21

In the two studies of miR-21 in ACS patients, Peng et al reported lower miR-21 in HPR patients than LPR patients,[43] whereas Li et al found no association between miR-21 and platelet function.[34] In studies of CAD patients, one demonstrated a positive correlation between miR-21 and platelet function,[44] whereas another study reported no association.[40] Becker et al included both ACS and CAD patients and found that miR-21 negatively correlated with platelet function in the sequencing cohort of ACS patients, but not in the target cohort of ACS patients or in the validating cohorts consisting of CAD patients.[33]

miR-150

miR-150 was investigated in four studies.[34,40,41,46] No association was found in ACS patients.[34] In CAD patients, Jäger et al found a positive correlation between miR-150 and platelet function,[40] whereas Kaudewitz et al found no association.[41] In patients with symptomatic atherothrombotic diseases, miR-150 was lower in HPR patients than in LPR patients.[46]

miRs Investigated in Two Studies

miR-1 was only investigated in one study of ACS patients where no association with platelet function was found.[34] In patients with symptomatic atherothrombotic diseases, miR-1 was found to be higher in HPR patients than in LPR patients.[46]

In two studies of CAD patients, Tang et al reported miR-106a to be higher in HPR patients than LPR patients,[44] whereas Kaudewitz et al found no association between miR-106a and platelet function.[41]

Table 4 Studies investigating microRNA (miRs) and platelet function or maturity in patients with other cardiovascular diseases ($n = 3$)

Study, *rating*	Participants	Antiplatelet treatment	Platelet analysis	miRs	Results reported
Becker et al,[33] *fair*	3 cohorts: (1) *TRILOGY:* ACS patients: 153 patients in sequencing cohort Up to 878 in target cohort (2) *Validation cohort I*: CAD patients: 24 HPR and 24 patients with LPR (3) *Validation cohort II*: CAD patients: 24 patients with HPR and 24 patients with LPR	*TRILOGY*: dual treatment with aspirin OD plus either 75-mg clopidogrel OD or 10-mg prasugrel OD or 90-mg ticagrelor TD *Validation cohorts*: dual treatment with aspirin OD plus and 75-mg clopidogrel OD No data on aspirin doses. Loading dose with 300-mg clopidogrel	*TRILOGY:* PRU *Validation cohort I*: PRI: mean/median as cut-off between HPR and LPR *Validation cohort II*: Platelet aggregation, (MEA, ADP as agonist) 46 AU as cut-off between HPR and LPR	Let-7d-3p miR-10a, miR-15b-5p, miR-21, miR-25–5p, miR-93, miR-126, miR-197 miR-324–5p, miR-345–5p, miR-574–3p, miR-589–5p, miR-671–3p, miR-939–5p, miR-1294, miR-3609, miR-4732–3p	*TRILOGY:* sequencing: correlation: PRU and miR-10a (FC = 0.21, $p = 0.05$), miR-15b-5p (FC = 0.13, $p = 0.03$), miR-21 (FC = –0.12, $p = 0.04$), miR-25 (FC = 0.23, $p = 0.04$), miR-93 (FC = 0.1, $p = 0.03$), miR-197 (FC = 0.16, $p = 0.01$), miR-324 (FC = –0.21, $p = 0.05$), miR-345–5p (FC = –0.11, $p = 0.005$), miR-589 (FC = –0.12, $p = 0.04$), miR-671–3p (FC = 0.18, $p = 0.05$), miR-939–5p (FC = 0.28, $p = 0.01$), miR-1294 (FC = 0.13, $p = 0.04$), miR-3609 (FC = 0.35, $p = 0.05$) and miR-4732–3p (FC = 0.11, $p = 0.05$) Target cohort: baseline (before P2Y12 inhibitor intake): →correlation: any miRs and PRU (all $p > 0.05$) 30 d: correlation: PRU and miR-126 (Beta = –0.22, $p = 0.013$) and miR-345–5p (Beta = –0.29, $p = 0.03$) 6 mo: correlation: PRU and miR-574–3p (Beta = –0.13, $p = 0.03$) and Let-7d-3p (Beta = –0.16, $p = 0.004$) *Validation cohorts:* correlation: PRI/platelet aggregation and miR-15b-5p (FC = 0.46), $p < 0.05$), miR-93 (FC = 0.23, $p < 0.01$) and miR-126 (FC = 0.33, $p < 0.01$) →correlation: miR-10a, miR-21, miR-25–5p, miR-197, miR-324–5p, miR-345–5p, miR-574–3p, miR-589–5p, miR-671–3p, miR-939–5p or Let-7d-3p and PRI/platelet aggregation
Zufferey et al,[46] *fair*	12 patients with documented symptomatic atherothrombotic diseases: 6 HPR and 6 LPR patients	100-mg aspirin OD	Platelet aggregation (LTA, epinephrine, AA, ADP and collagen as agonists) Extreme values used as cut-off based on platelet reactivity index calculated on the basis of results obtained from all agonists	miR-1, miR-133a, miR-135a-5p, miR-144–3p, miR-150, miR-204–5p, miR-410, miR-424–5p, miR-451a, miR-634, miR-656, miR-1283	Correlation: miR-135–5p and platelet aggregation (rhoS = –0.68, $p = 0.02$) Correlation: miR-204–5p and platelet aggregation (rhoS = –0.61, $p = 0.04$) ↑miR-1, ↑miR-133a, ↑miR-410 and ↑miR-656 HPR vs. LPR patients (all $p < 0.05$) ↓miR-135a-5p, ↓miR-144–3p, ↓miR- 150, ↓miR-204–5p, ↓miR-424–5p, ↓miR-451a, ↓miR-634 and ↓miR-1283 HPR vs. LPR patients (all $p < 0.05$)

Table 4 (*Continued*)

Study, *rating*	Participants	Antiplatelet treatment	Platelet analysis	miRs	Results reported
Binderup et al,[35] *fair*	50 patients with intermittent claudication: 10 HPR and 40 patients with LPR 50 healthy individuals	Either 75-, 100- or 150-mg aspirin OD	Platelet aggregation, (MEA, AA as agonist) 30 AU as cut-off between HPR and LPR	miR-92a	Correlation: miR-92a and platelet aggregation All participants: rhoS = 0.32, $p = 0.001$ Only patients: rhoS = 0.19, $p = 0.18$ ↑miR-92a HPR vs. LPR patients ($p = 0.016$)

Abbreviations: AA, arachidonic acid; ACS, acute coronary syndrome; ADP, adenosine diphosphate; AU, aggregation units; CAD, coronary artery disease; FC, log2 fold changes in miR per 1 SD PRU unit; HPR, high platelet reactivity; LPR, low platelet reactivity; LTA, light transmission aggregometry; MEA, multiple electrode aggregometry; OD, once-daily; rhoS: Spearman's rho; PRI, platelet reactivity index, based on flow cytometric VASP measurement; TD, twice-daily; PRU, P2Y12 reaction unit, measured by VerifyNow assay using ADP as agonist; ↑, higher value; ↓, lower value; →, no difference or no correlation.

In a study examining both ACS and CAD patients, miR-197 correlated positively with platelet function in the sequencing cohort of ACS patients, but neither in the target cohort of ACS patients nor in the validating cohorts consisting of CAD patients.[33] In CAD patients, miR-197 correlated positively with platelet function.[41]

miR-204–5p was higher in HPR patients than in LPR patients in a cohort of ACS patients,[39] whereas in patients with symptomatic atherothrombotic diseases, miR-204–5p was lower in HPR patients than in LPR patients and further correlated negatively with platelet function.[46]

Kaudewitz et al determined miR-93 in a cohort of CAD patients and reported no association with platelet function.[41] However, in the study by Becker et al, examining both ACS patients and CAD patients, miR-93 correlated positively with platelet function in both patient groups.[33]

With regard to miR-96, both the study of ACS patients and the study of CAD patients reported no association between miR-96 and platelet function.[37,42]

miRs Investigated in One Study Only

Of the 35 miRs only investigated in one study, 2 miRs were investigated in ACS patients,[39,43] 12 miRs in CAD patients,[36,37,41,44] 12 in both ACS and CAD patients,[33] 2 in patients with intermittent claudication and healthy individuals,[35] and 9 in patients with symptomatic atherothrombotic diseases.[46] In ACS patients, miR-221 and miR-1908–5p were lower in HPR patients than in LPR patients.[33,39,43] In CAD patients, miR-20b, miR-24, miR-27a-3p, miR-30b, miR-130a-5p, miR-142–5p and miR-191 demonstrated a positive correlations with platelet function, and miR-26a, miR-27a-3p, miR-130a-5p, miR-142–5p and miR-365–3p were higher in HPR than in LPR patients.[36,37,41,44] In the study by Becker et al, examining both ACS and CAD patients, let-7d-3p, miR-324–5p, miR-345–5p, miR-574–3p and miR-589–5p correlated negatively, whereas miR-10a, miR-15b-5p, miR-25–5p, miR-671–3p, miR-939–5p, miR-1294, miR-3609 and miR-4732–3p correlated positively with platelet function in ACS patients but not in CAD patients.[33] In patients with intermittent claudication, miR-92a correlated positively with platelet function when pooling patients and healthy individuals, but not exclusively in patients.[35] In addition, miR-92a was higher in HPR patients than in LPR patients.[35] In patients with symptomatic atherothrombotic diseases, miR-135a-5p correlated negatively with platelet function, miR-133a, miR-410 and miR-656 were higher in HPR patients than in LPR patients, and miR-135a-5p, miR-144–3p, miR-424–5p, miR-451a, miR-634 and miR-1283 were lower in HPR patients than in LPR patients.[46] Only one study investigated platelet maturity and found that miR-202–3p correlated negatively with platelet maturity.[31]

miRs without Association to Platelet Function and Maturity

miR-15a-5p,[37] miR-23a,[36] miR-146b,[41] miR-107,[37] miR-199,[36] miR-339–3p,[37] miR-495–3p,[37] miR-501–3p,[39] miR-584–5p,[39] miR-1273h-3p,[39] miR-nov-chr1,[39] miR-nov-chr8[39] and miR-nov-chr22[39] did not show any association

Table 5 Investigated microRNAs (miRs) and the corresponding association with platelet function and maturity across patient groups

miRs	No. of studies[refs.]	No. of patients	Findings
Let-7d-3p	1[32]	974	↓correlation: ACS patients ($n=427$) →: ACS patients ($n=451$) →: CAD patients ($n=96$)
miR-1	2[33,45]	27	→: CAD patients ($n=15$) ↑: SAD patients ($n=12$)
miR-10a	1[32]	249	↑correlation: ACS patients ($n=153$) →: CAD patients ($n=96$)
miR-15a-5p	1[36]	<155[a]	→: CAD patients
miR-15b-5p	1[32]	1,127	↑correlation: ACS patients ($n=153$) ↑correlation: CAD patients ($n=96$) →: ACS patients ($n=878$)
miR-20b	1[40]	125	↑correlation: CAD patients
miR-21	5[32,33,39,42,43]	1,358	↓: ACS patients ($n=39$) ↓correlation: ACS patients ($n=153$) →: ACS patients ($n=893$) ↑: CAD patients ($n=115$) →: CAD patients ($n=158$)
miR-23a	1[35]	43	→: CAD patients
miR-24	1[40]	125	↑correlation: CAD patients
miR-25–5p	1[32]	1,127	↑correlation: ACS patients ($n=153$) →: ACS patients ($n=878$) →: CAD patients ($n=96$)
miR-26a	1[35]	43	↑: CAD patients
miR-27a-3p	1[43]	115	↑correlation: CAD patients ↑: CAD patients
miR-30b	1[40]	125	↑correlation: CAD patients
miR-92a	1[34]	50	→: Claudication patients ↑correlation: Claudication patients ($n=50$) + healthy individual ($n=50$)
miR-93	2[32,40]	374	↑correlation: ACS patients ($n=153$) ↑correlation: CAD patients ($n=96$) →: CAD patients ($n=125$)
miR-96	2[36,41]	<188[a]	→: ACS patients ($n=33$) →: CAD patients ($n<155$)
miR-106a	2[40,43]	240	↑: CAD patients ($n=115$) →: CAD patients ($n=125$)
miR-107	1[36]	<155[a]	→: CAD patients
miR-126	5[32,33,39,40,43]	1,291	↓correlation: ACS patients ($n=464$) →: ACS patients ($n=429$) ↑correlation: CAD patients ($n=336$) →: CAD patients ($n=187$)
miR-130a-5p	1[43]	115	↑correlation: CAD patients ↑: CAD patients
miR-133a	1[45]	12	↑: SAD patients
miR-135a-5p	1[45]	12	↓correlation: SAD patients ↓: SAD patients
miR-142–5p	1[43]	115	↑correlation: CAD patients ↑: CAD patients
miR-144–3p	1[45]	12	↓: SAD patients
miR-146b	1[40]	125	→: CAD patients
miR-150	4[33,39,40,45]	214	

Table 5 (*Continued*)

miRs	No. of studies[refs.]	No. of patients	Findings
			→: ACS patients (*n* = 15) ↑correlation: CAD patients (*n* = 62) →: CAD patients (*n* = 125) ↓: SAD patients (*n* = 12)
miR-191	1[40]	125	↑correlation: CAD patients
miR-197	2[32,40]	1,252	↑correlation: ACS patients (*n* = 153) ↑correlation: CAD patients (*n* = 125) →: ACS patients (*n* = 878) →: CAD patients (*n* = 96)
miR-199	1[35]	43	→: CAD patients
miR-202–3p	1[30]	81	↓correlation: CAD patients
miR-204–5p	2[38,45]	86	↑: ACS patients (*n* = 74) ↓: SAD patients (*n* = 12) ↓correlation: SAD patients (*n* = 12)
miR-221	1[42]	39	↓: ACS patients
miR-223	9[31,33,36,37,39–42,44]	<572[a]	↓correlation: ACS patients (*n* = 116) ↓: ACS patients (*n* = 134) →: ACS patients (*n* = 15) ↑correlation: CAD patients (*n* = 187) →: CAD patients (n < 215)
miR-324–5p	1[32]	1,127	↓correlation: ACS patients (*n* = 153) →: ACS patients (*n* = 878) →: CAD patients (*n* = 96)
miR-339–3p	1[36]	<155[a]	→: CAD patients
miR-345–5p	1[32]	1,127	↓correlation: ACS patients (*n* = 615) →: ACS patients (*n* = 416) →: CAD patients (*n* = 96)
miR-365–3p	1[36]	<155[a]	↑: CAD patients
miR-410	1[45]	12	↑: SAD patients
miR-424–5p	1[45]	12	↓: SAD patients
miR-451a	1[45]	12	↓: SAD patients
miR-495–3p	1[36]	<155[a]	↓: CAD patients
miR-501–3p	1[38]	10	→: ACS patients
miR-574–3p	1[32]	974	↓correlation: ACS patients (*n* = 435) →: ACS patients (*n* = 443) →: CAD patients (*n* = 96)
miR-584–5p	1[38]	20	→: ACS patients
miR-589–5p	1[32]	249	↓correlation: ACS patients (*n* = 153) →correlation: CAD patients (*n* = 96)
miR-634	1[45]	12	↓: SAD patients
miR-656	1[45]	12	↑: SAD patients
miR-671–3p	1[32]	249	↑correlation: ACS patients (*n* = 153) →: CAD patients (*n* = 96)
miR-939–5p	1[32]	1,127	↑correlation: ACS patients (*n* = 153) →: ACS patients (*n* = 878) →: CAD patients (*n* = 96)
miR-1273h-3p	1[38]	10	→: ACS patients
miR-1283	1[45]	12	↓: SAD patients
miR-1294	1[32]	153	↑correlation: ACS patients
miR-1908–5p	1[38]	10	↓: ACS patients

(*Continued*)

Table 5 (*Continued*)

miRs	No. of studies[refs.]	No. of patients	Findings
miR-3609	1[32]	153	↑correlation: ACS patients
miR-4732–3p	1[32]	153	↑correlation: ACS patients
miR-nov1	1[38]	10	→: ACS patients
miR-nov8	1[38]	10	→: ACS patients
miR-nov22	1[38]	10	→: ACS patients

Abbreviations: ACS, acute coronary syndrome; CAD, coronary artery disease; refs, reference number; SAD, symptomatic atherothrombotic diseases; ↑, higher value in patients with high platelet reactivity (HPR) vs. patients with low platelet reactivity (LPR); ↓, lower value in HPR vs. LPR patients; ↑correlation, positive correlation; ↓correlation, negative correlation; →, no difference between HPR and LPR patients or no correlation.
Note: After each finding, the subgroup is indicated in which the findings are reported. The findings correspond to all examined patients unless otherwise indicated.
[a]The number of patients in each subgroup are not reported; hence, *n* is indicated as <155 (total number of patients in the study).

with platelet function or platelet maturity and were only investigated in one study (►**Table 5**).

Discussion

This systematic review included 16 studies examining the association between miRs and platelet function and maturity in patients with CVD, primarily ACS and stable CAD. We identified 44 miRs associated with platelet function and 1 miR associated with platelet maturity. Ten miRs were investigated in more than one study and of these, 4 miRs were investigated in more than two studies, namely miR-223, miR-126, miR-21 and miR-150.

miRs and Platelet Function

The expression level of miR-223 in relation to platelet function was investigated in 9 of the 16 included studies. Interestingly, different associations were reported across patient groups.[32,34,37,38,40–43,45] The function and target of miR-223 have been investigated in some studies.[27,48,49] Landry et al reported that the binding site of miR-223 is the 3′ untranslated region (3′ UTR) of the messenger RNA (mRNA) encoding for the P2Y12 receptor, known to be a mediator of granule secretion, platelet aggregation and thrombus formation.[27] Additionally, Landry et al reported that miR-223 regulates the *P2Y12* gene expression in both platelets and megakaryocytes.[27] Furthermore, it was demonstrated that miR-223-deficient mice had higher platelet aggregation, larger thrombus formation and delayed clot retraction compared with wild-type mice.[27] In addition, Fejes et al found that decreased miR-223 resulted in upregulated levels of P2Y12 mRNA.[48] Singh et al showed that the miR-223 level was higher in plaque material from the coronary arteries in unstable CAD patients than in stable CAD patients, and concluded that miR-223 may be a marker of plaque instability.[49] Pan et al reported that miR-223 affects endothelial cells causing cell apoptosis and endothelial dysfunction.[50] Kaur et al performed a recent review regarding the role of miRs to distinguish ACS patients from stable CAD patients.[51] The authors identified three studies examining miR-223 and found miR-223 to be inconclusive as studies reported both up- and down-regulation.[51] However, miR-223 may be a good marker of platelet function and ADP inhibitor monitoring due to its binding to the P2Y12 receptor.[27,52]

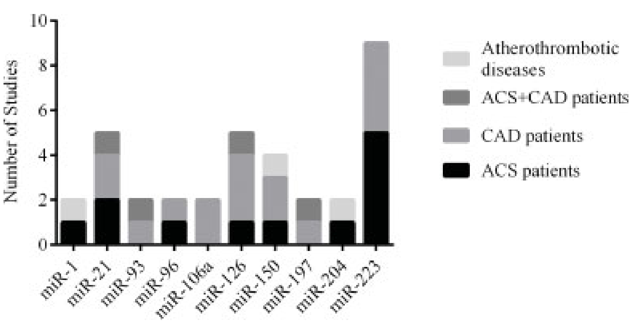

Fig. 2 Number of studies investigating microRNA (miRs). ACS, acute coronary syndrome; CADS, coronary artery disease.

The association between miR-126 and platelet function differed across patient groups as one study found miR-126 to be negatively correlated with platelet function[33] in ACS patients, whereas three studies demonstrated a positive correlation in CAD patients.[33,41,44] miR-126 has been shown to be present in endothelial cells contributing to the regulation of cell proliferation and vascular inflammation.[53–55] In addition, miR-126 promotes the progression and function of endothelial progenitor cell.[56] Furthermore, miR-126 promotes endothelial repair and plays a key role for restoration of normal vascular haemostasis and limiting atherosclerosis after vessel injury.[57,58] These diverse roles may explain the different associations between miR-126 and platelet function reported across patient groups.

The findings regarding miR-21 and platelet function were inconclusive as two studies found miR-21 to be negatively associated with platelet function,[33,43] one study found a positive association[44] and three studies found no association.[33,34,40] However, Kaur et al identified five studies examining miR-21 to distinguish ACS patients from CAD patients, and reported that all studies found miR-21 to be upregulated in ACS patients compared with CAD patients.[51] Peng et al reported that P2Y12 mRNA was the predicted target site for miR-21.[43] Furthermore, miR-21 has been showed to have various effects regarding the regulation and progression of atherosclerosis and to be present in different cell types,[59–61] including endothelial cells[62] and smooth muscle cells.[63] In addition, the miR-21 expression was found to be highly up-regulated in atherosclerotic plaques.[60]

Four studies investigated miR-150 and platelet function with unconvincing results with findings of either a weak or no association.[33,34,41,46] Lu et al found that miR-150 affects the regulation and enhancement of megakaryocytopoiesis by influencing the megakaryocyte–erythrocyte progenitor cells towards the megakaryocyte line.[24] Tano et al suggested that miR-150 may also affect endothelial cells by regulation of endothelial repair process.[64]

miRs and Platelet Maturity

Only one of the included studies reported on platelet maturity in association with miR and found that miR-202–3p negatively correlated with MPV.[31] miR-202–3p might inhibit platelet production by modulating the expression of genes important for cell formation including the *ABCG4* gene.[31] In addition, *ABCG4* deficiency has been suggested to accelerate both atherosclerosis and thrombosis likely due to increased megakaryocyte proliferation and platelet production.[31,65]

Normalization and Sample Preparation

Comparisons across the included studies must be done with caution due to variation in the methods employed for miR examination. All studies used a normalization strategy to express miR levels, but different approaches were used. This may have influenced the results, as Faraldi et al have reported that different normalization strategies lead to different miR expression levels.[47] In addition, the expression of miRs was investigated in different sample materials. All types of blood cells contribute to the concentration of miRs in the plasma and PPP samples, whereas platelets predominantly contribute to miRs in PRP. However, the different types of sample material may still be used to investigate miRs, though maybe with different purposes. To investigate specific mechanisms of miRs in platelets, PRP may be an ideal choice, but when investigating miRs as potential biomarkers, plasma or whole blood samples may be preferable due to less pre-analytical sample processing. Furthermore, differences in sample preparation and anticoagulants may also affect the quantity and quality of miR levels.[66,67]

Most of the included studies predefined the included patient population based on platelet function test, thereby making platelet function the exposure of interest. Thus, the expression level of miR was the related outcome of interest. However, to further investigate the potential of miRs as biomarkers, future studies should explore miRs as exposure and determine corresponding outcomes, such as platelet function and maturity or clinical thromboembolic events. There is a great potential for miRs as biomarkers due to the easy availability and stability in the circulation. However, as the analysis of miRs is expensive and time-consuming as well as dependent on many pre-analytic factors, only few studies examining miRs as biomarkers and their clinical applications are currently available. In addition, other factors may affect the levels of miRs, for example, comorbidity and medication.[68–72] More studies are therefore warranted to investigate the significance of these variables and the potential of miRs as biomarkers in a clinical context.

Limitations

Some limitations of the present systematic review have to be considered. We defined patients as having either HPR or LPR to make studies comparable as different subgroup definitions were employed across the included studies. As some studies distinguished HPR patients and LPR patients by the median or 50% of a platelet function test, some patients may therefore have platelet function within the spectrum of healthy individuals. Furthermore, the studies used different platelet function tests, which may not be directly comparable as they correlate rather poorly.[15,73] The definitions of HPR and LPR differ between studies and no universal definition exists. This challenges comparison of results obtained across studies. In addition, the lack of standardization in the miR analyses may challenge the comparison between studies.

Conclusion

The present systematic review identified 45 miRs associated with platelet function or maturity in patients with CVD mainly suffering from ACS or stable CAD. However, the majority of miRs were only investigated in one study. Only miR-223, miR-126, miR-21 and miR-150 were investigated in more than two studies and among these, miR-223 and miR-126 showed the most consistent results across patient groups. Based on the findings of the present systematic review, more data are needed on the potential use of miRs as biomarkers for platelet function and maturity in CVD patients.

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Conflict of Interest
None declared.

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