SUPPLEMENTAL MATERIAL

Supplemental Methods

First source population (A)

The first source population, used for the discovery and validation phase, consisted of patients who underwent a percutaneous coronary intervention (PCI) at the Academic Medical Center – University of Amsterdam. Patients were included between February 1993 and October 2010. Our institution is a high-volume tertiary referral hospital with on-site cardiac surgery. PCI was performed according to the standard PCI guidelines by highly experienced operators. All patients received a loading dose aspirin and clopidogrel, prasugrel or ticagrelor and unfractioned heparin at the start of the procedure. Atherectomy and thrombectomy was performed at the choice of the operator. The use of glycoprotein IIb/IIIa inhibitor was at the discretion of the operator. The diagnosis acute coronary syndrome or stable angina pectoris was made by the cardiologist who referred the patient for PCI according to the European Society of Cardiology definition [1, 2].

Second source population (B)

The second source population, consisted of serum samples from a biobank of families with premature atherosclerosis. This population consisted of patients with premature atherosclerosis and their first or second degree family members without a history of CAD or complaints. All individuals from this source population visited the outpatient clinic for cardiovascular risk assessment of the Academic Medical Center in Amsterdam between July 2009 and July 2014. Serum was collected and all individuals without CAD underwent coronary computerized tomography (CT) -scanning as described in detail below to assess their atherosclerotic burden.

Discovery cohort (a)

All patients were selected from the first source population and matched for age (within 2 years) and gender. This cohort consisted of 2 groups: 1. patients of whom thrombectomy material was

collected, during PCI for a ST-elevated myocardial infarction (UCAD group; n=25; Aa) and 2. patients of whom atherectomy material was collected, during PCI for stable angina pectoris (SCAD group; n=14; Aa).

First validation cohort (b)

The UCAD group and the SCAD group were selected from the first source population (A) whereas, the control group was selected from the second source population (B). This cohort consisted of 3 groups: 1. Patients whom underwent urgent PCI for an acute coronary syndrome (UCAD group; n=64; Ab); 2. patients whom underwent elective PCI because of stable angina (SCAD group; n=139; Ab) and 3. control subjects (Control; n=192; Bb). Controls did not have any detectable atherosclerosis as indicated by an Agatston coronary artery calcium (CAC) score of 0 determined by coronary CT-scanning and had to be over the age of 35 years.

Second validation Cohort (c)

The UCAD group was selected from the first source population whereas the SCAD group, the SubA group and control group were selected from the second source population. This cohort consisted of 4 distinct groups: 1. patients whom underwent acute PCI for an ST-elevated myocardial infarction (UCAD group; n=250; Ac); 2. patients with a history of coronary artery disease, without cardiac complains at the moment of the visit (SCAD group; n=250; Bc); 3. patients with subclinical atherosclerosis, as indicated by a CAC score of ≥1, without cardiac complains at the moment of the visit (SubA group; n=250; Bc); 4. Control subjects without coronary atherosclerosis as indicated by a CAC score of 0 and age 35 years or older (control; n=250; Bc).

Blood withdrawal

Concerning the first source population, serum samples were drawn before the administration of heparin, at the start of the PCI procedure (directly after insertion of the sheath). Concerning the second source population, serum samples were drawn during the patient's visit to the outpatient clinic at our hospital. Blood was centrifuged for 5 minutes at 1900g at 18 °C to obtain serum. All serum samples were stored in cryovials at –80 °C.

Selection of candidates for the first validation phase

After measuring the tissue-derived miRNAs from the discovery cohort, the miRNAs were ranked based on the mean difference in normalized expression level between UCAD and SCAD. From this ranking we created 2 lists of miRNAs: 1) miRNAs that showed a high expression in SCAD patients as compared to UCAD patients and 2) miRNAs that showed a high expression in UCAD patients as compared to SCAD patients. From each list, the top 3 were chosen for the first validation, taking into account that the 3p or 5p strand originate from the same miRNA, in which case we chose to use the strand with the highest expression level difference.

Tissue collection and blood withdrawal from the first source populations

Atherectomy specimens were obtained using directional coronary atherectomy (Simpson AtheroCath, Devices for Vascular Intervention), placed in formalin and fixed for 24 hours. Thrombectomy specimens were obtained with the 7-F Rescue catheter (Boston Scientific/Scimed, Inc., Maple Grove, Minnesota), which became available in August 2001 and was mainly used until the end of 2004; the 6-F Export aspiration catheter (Medtronic Vascular Inc., Santa Rosa, California), which became available in August 2004; and the 6-/7-F Proxis embolic protection device (St. Jude Medical, St. Paul, Minnesota), which became available in February 2004 and combines aspiration with distal embolic protection. Immediately after thrombosuction, the filter of the device was placed in formalin and the aspirated material was fixed for 24 hours.

Blood was collected in 5 mL BD Vacutainer® SST II Plus plastic serum tubes (Becton, Dickinson and Company, New Jersey, USA). In the first source population, blood was drawn at the start of

the PCI procedure after inserting the sheath and before unfractioned heparin was administered. The blood was allowed to clot and was centrifuged for 10 minutes at 2000g at 18 °C to obtain serum.

Discovery methods

RNA isolation and quantification of miRNAs by RT-qPCR.

Artherectomy and thrombectomy tissue were sectioned, dissolved in 1 ml of Trizol LS reagent (Invitrogen Corp., Carlsbad, CA) and incubated for 10 minutes at room temperature followed by addition of 200 µl chloroform. The mixture was centrifuged at 12,000 g for 10 minutes, and the aqueous layer was transferred to a new tube. RNA was precipitated by isopropanol and washed with 75% ethanol. The RNA pellet was collected in 30 µl RNAse free water. Nucleic acid quantification could not be performed due to the low concentration of RNA. To obtain cDNA, 8 µl of RNA was reverse transcribed using the miRCURY LNA™ Universal RT miRNA PCR, Polyadenylation and cDNA synthesis kit (Exiqon, Vedbaek, Denmark). CDNA was diluted 50x and in total, 742 miRNAs (human panel I and II) and negative controls were measured by qPCR according to the protocol for miRCURY LNA™ Universal RT miRNA PCR (Exiqon, Vedbaek, Denmark) on the LightCycler® 480 Real-Time PCR System (Roche, Basel, Switzerland). Amplification curves were analyzed using the Roche LC software, both for determination of C_q (by the 2nd derivative method) and for melting curve analysis. Amplification efficiency was calculated using algorithms of the LinRegPCR software.

RT-qPCR data handling and normalization

RT-qPCR results with a $C_q \geq 37$ or a C_q within 5 cycles from the negative control were excluded from the analysis. NormFinder[3] found the average of assays (average – assay C_q) in all samples to be the best available normalizer, which was therefore used for normalization. MiRNAs were only included in the analysis when at least 14 of 25 in the UCAD group and 14 of 14 expression values in the SCAD group were available as we determined 14 values per group the minimum for a reliable statistical analysis.

First validation phase

RNA isolation

RNA was extracted from 250 µl serum using 750 µl TRIzol LS reagent (Invitrogen Corp., Carlsbad, CA), incubated for 10 minutes at room temperature followed by addition of 200 µl chloroform. The mixture was centrifuged at 12,000 g for 10 minutes, and the aqueous layer was transferred to a new tube. RNA was precipitated by isopropanol and washed with 75% ETOH. RNA pellet was collected in 40 µl RNAse free water. Nucleic acid quantification could not be performed due to the low concentration of RNA in serum. DNAse and RNAse treatments were omitted since previous experiments showed no difference of miRNA expression in serum with or without these treatments (data not shown).

Complementary DNA synthesis and quantitative polymerase chain reaction (qPCR)

In the first validation cohort, complementary DNA was synthesized using the Taqman MiRNA Reverse Transcription Kit (Applied Biosystems, Gent, Belgium) and miRNA-specific stem-loop primers using the manufacturer's instructions. Each reaction contained 2.5 µL of RNA, 1.5 µL reverse transcriptase primers and 3.5 µL of master mix. The reaction mixture was incubated for 30 minutes at 16 °C, 30 minutes at 42 °C, 5 minutes at 85 °C, 5 minutes at 15 °C and finally at 4 °C for 10 minutes. CDNA was diluted in a 1:15 ratio using nuclease-free water. MiRNA expression levels were quantified in triplicate by RT-qPCR using Taqman miRNA assays (Applied Biosystems) according to the manufacturer's instructions. Each reaction consisted of 5 µL 480 probe master, 0.5 µL Taqman primers, 1.33 µL diluted cDNA and 3.17 µL nuclease free water. On a LightCycler 480 system II (Roche, Basel, Switzerland), the reaction mixture was incubated for 10 minutes at 95 °C followed by 50 cycles of 15 seconds of 95 °C and 60 seconds at 60 °C. PCR efficiencies per cycle were between 1.87 and 2.01 for all miRNAs. Data were analysed using LinRegPCR quantitative PCR data analysis software, version 11[4].

RT-qPCR data handling and normalization

Circulating miRNA experiments in plasma or serum are sensitive to false or inaccurate signals, which is largely explained by the often low concentrations of miRNAs in plasma and serum[5]. Therefore, a strict quality assessment pipeline was used to ensure the validity of each measurement and increase accuracy of the results. This pipeline is described elsewhere [6]. In brief, we distinguished 3 groups of measurements: 'valid', 'invalid', and 'undetectable' (less than 10 copies of material in sample). In case of undetectable, the sample was set to a low value, which was based on the qPCR experiment parameters. If the measurement did not pass the quality controls of the pipeline, it was marked as 'invalid'. Invalid measurements were taken into the analysis as missing at random and imputed using multiple imputations. If the measurement passed all the quality checks, it was marked as 'valid' and the mean of the replicates was used in the analysis. MiRNA expression was normalised to the geometric mean of an established miRNA normalisation panel for serum samples consisting of miR-1260, miR-1280 and miR-484, as previously described in Kok et al. [7].

Second validation phase

RNA isolation

RNA was extracted from 200 µl serum using 600 µl TRIzol LS reagent (Invitrogen Corp., Carlsbad, CA) and incubated for 10 minutes of incubation at room temperature. Then the sample was spiked with 10 µl of Cel-miR-39 (work dilution) to be able to monitor and correct for efficiencies in RNA isolation. Next, 160 µl of chloroform was added to each sample and the mixture was centrifuged at 12,000 g for 15 minutes. The aqueous layer was transferred to a new tube and RNA was precipitated by 400 µl isopropanol, centrifuged at 12,000 g for 10 minutes and washed with 800 µl 75% ETOH. RNA pellet was collected in 30 µl RNAse free water. Nucleic acid quantification could not be performed due to the low concentration of RNA in serum. DNAse and RNAse treatments were omitted since previous experiments showed no difference of miRNA expression in serum with or without these treatments (data not shown).

Complementary DNA synthesis and quantitative polymerase chain reaction (qPCR)

Complementary DNA was synthesized using the qScript© microRNA cDNA synthesis kit (Quanta Biosciences, Radnor, PA, USA). First a poly (A) tail was added. Each reaction contained 3 µl of RNA, 0.5 µl nuclease free water, 1 µl poly (A) polymerase, 2 µl poly (A) polymerase buffers and 3.5 µl Cel-miR-54 spike-in in a concentration of $1.6x10^6$ copies/µl. The reaction mixture was incubated for 60 minutes at 37 °C followed by 5 minutes at 70˚C. Next, cDNA was synthesized, in a reaction using 9µL of the poly (A) tailed RNA and 1 µL of qScript Reverse transcriptase. The reaction mixture was incubated for 20 minutes at 42˚C followed by 5 minutes at 85˚C. cDNA was diluted 25x.

RT-qPCR data handling and normalization

RT-qPCR data handling and normalization in the second validation phase was identical to that of the first validation phase. However, in the second validation cohort, some extra steps were taken to even further augment the precision and accuracy of the results. For normalization, we added the technical normalizers, Cel-miR-39 and Cel-miR-54 to the normalization panel of Kok et al.[7], and results were normalized to the geometric mean of this total of 5 normalizers. Additionally, to correct for unavoidable inter-plate differences we used a factor correction software program as recently described by Ruijter et al.[8]

Biochemical Parameters

Blood level for glucose, gamma glutamyl transferase (GGT), alanine aminotransferase (ALAT), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and high density lipoprotein cholesterol (HDL-C) were determined. Glucose, gammaGT, ALAT, total cholesterol, Triglycerides and HDL cholesterol were measured on a clinical chemistry analyzer (AU5800, Beckman Coulter, Brea CA) following standard procedures and protocols.

Coronary CT-scanning

Coronary artery CT-scanning was performed using a 64-slice multi-detector CT scanner (Brilliance 64, Philips Medical Systems, Best, the Netherlands). The CT acquisition protocol was as follows: tube voltage, 120 kV; tube current, 55 mAs; detector collimation, 40 × 0.625 mm;

gantry rotation, 420 ms. Images were reconstructed in a 220 mm field-of-view, 2.5 mm slice thickness, 2.5 mm increment, standard B filter, window lever 200 and window width 800. CAC score was evaluated according to Agatston et al [9].

SUPPLEMENTAL TABLES AND FIGURES

Supplemental table I. qPCR-based array candidates of UCAD versus SCAD candidates (A) and vice versa (B) .

A. UCAD candidates

miRNA	SCAD (mean ± SD)	UCAD (mean ± SD)	mean difference UCAD-SCAD	95% CI	p-value	B-H corrected p-value
miR-223-3p	4.69±1.15	7.23±0.55	2.55	1.86-3.24	6.31E-07	1.66E-06
miR-142-3p	3.52±1.02	5.76±0.55	2.24	1.62-2.86	6.16E-07	1.66E-06
miR-142-5p	-2.44±1.15	-0.26±0.72	2.18	1.47-2.89	3.72E-06	7.70E-06
miR-126-5p	0.16±1.21	1.93±1.18	1.77	0.94-2.59	1.57E-04	2.01E-04
miR-425-5p	-1.54±0.82	0.12±0.37	1.66	1.16-2.15	2.35E-06	5.23E-06
miR-766-3p	-3.78±0.87	-2.14±0.81	1.64	1.06-2.23	4.33E-06	8.56E-06
miR-144-3p	1.72±1.16	3.26±1.36	1.53	0.69-2.38	8.25E-04	9.20E-04
miR-144-5p	0.17±1.39	1.63±1.29	1.46	0.53-2.39	3.44E-03	3.48E-03
miR-126-3p	3.12±1.06	4.56±0.56	1.44	0.8-2.08	1.94E-04	2.44E-04
miR-191-5p	0.33±0.65	1.73±0.37	1.40	1.00-1.80	8.00E-07	1.99E-06
miR-17-5p	-2.35±0.68	-1.04±0.29	1.31	0.91-1.71	4.00E-06	8.09E-06
miR-190a	-3.58±0.76	-2.30±0.79	1.28	0.75-1.81	3.15E-05	4.72E-05
miR-15b-5p	1.66±0.49	2.94±0.33	1.28	0.97-1.59	3.88E-08	1.78E-07
miR-15b-3p	-3.85±0.86	-2.60±0.39	1.25	0.74-1.77	9.26E-05	1.26E-04
miR-106a-5p	1.75±0.64	2.99±0.31	1.24	0.86-1.63	3.60E-06	7.64E-06
miR-103a-3p	-1.99±0.80	-0.78±0.87	1.22	0.87-1.56	1.99E-07	1.79E-04
miR-32-5p	2.48±0.65	3.69±0.34	1.21	0.65-1.77	1.38E-04	1.06E-05
miR-20a-5p	1.95±1.39	3.15±0.70	1.21	0.81-1.60	5.99E-06	7.69E-03
miR-15a-5p	2.24±0.53	3.43±0.44	1.20	0.36-2.04	7.87E-03	8.41E-07
miR-107	1.12±0.50	2.26±0.44	1.14	0.81-1.47	2.06E-07	6.90E-07
miR-363-3p	-3.73±1.01	-2.60±1.10	1.13	0.42-1.84	2.98E-03	3.06E-03
miR-590-5p	-1.82±0.44	-0.73±0.24	1.09	0.82-1.35	9.87E-08	3.90E-07
miR-92a-3p	2.38±0.47	3.46±0.44	1.08	0.71-1.44	1.62E-06	6.44E-07
miR-486-5p	2.48±0.53	3.56±0.53	1.08	0.27-1.88	1.05E-02	3.80E-06

miR-93-5p	1.49±1.17	2.57±1.18	1.07	0.69-1.45	9.38E-06	9.93E-03
miR-103a-3p-2	1.38±0.60	2.45±0.42	1.05	0.74-1.37	2.83E-07	1.57E-05
miR-151a-3p	-1.45±0.76	-0.40±0.79	1.05	0.52-1.58	3.45E-04	4.11E-04
miR-25-3p	-0.08±0.80	0.96±0.85	1.04	0.48-1.60	7.14E-04	8.07E-04
miR-151a-5p	-0.41±0.48	0.58±0.64	0.99	0.62-1.36	4.51E-06	8.61E-06
miR-19b-3p	2.91±0.63	3.90±0.62	0.99	0.57-1.42	5.64E-05	7.92E-05
miR-185-5p	0.47±0.89	1.46±0.42	0.99	0.46-1.52	1.17E-03	1.28E-03
miR-484	-0.01±0.41	0.97±0.43	0.98	0.69-1.27	1.18E-07	4.41E-07
miR-374a-5p	-1.38±0.34	-0.43±0.52	0.95	0.67-1.23	4.84E-08	2.11E-07
miR-221-3p	-0.18±0.73	0.74±0.81	0.92	0.40-1.44	1.09E-03	1.20E-03
miR-451a	6.83±1.25	7.74±1.28	0.92	0.05-1.78	3.86E-02	3.32E-02
let-7d-5p	-1.6±0.61	-0.71±0.56	0.89	0.49-1.30	1.22E-04	1.61E-04
miR-374b-5p	-0.54±0.6	0.28±0.54	0.82	0.42-1.21	2.80E-04	3.38E-04
miR-16-5p	4.6±0.70	5.39±0.76	0.79	0.29-1.28	2.89E-03	3.00E-03
let-7f-5p	0.22±0.50	1.00±0.41	0.78	0.45-1.10	5.37E-05	7.66E-05
miR-192-5p	-2.88±0.73	-2.14±0.93	0.74	0.19-1.28	9.64E-03	9.22E-03
miR-130a-3p	-2.78±0.73	-2.08±0.76	0.71	0.20-1.21	8.09E-03	7.82E-03
miR-423-3p	-0.59±0.28	0.10±0.58	0.69	0.41-0.97	1.31E-05	2.15E-05
miR-146a-5p	-1.81±0.73	-1.14±0.87	0.67	0.14-1.20	1.53E-02	1.43E-02
miR-26b-5p	0.77±0.28	1.36±0.49	0.59	0.34-0.84	2.59E-05	3.95E-05
miR-30e-5p	-2.76±0.39	-2.22±0.36	0.54	0.28-0.80	2.51E-04	3.08E-04
miR-197-3p	-0.64±0.66	-0.17±0.64	0.47	0.02-0.92	3.97E-02	3.39E-02
miR-423-5p-2	1.73±0.54	2.12±0.34	0.41	0.03-0.79	3.41E-02	2.17E-02
miR-30c-5p	3.32±0.64	3.66±0.72	0.39	0.06-0.72	2.39E-02	1.16E-01
miR-26a-5p	-2.89±0.69	-2.57±0.67	0.34	-0.12-0.79	1.42E-01	1.30E-01
let-7g-5p	1.77±0.39	2.09±0.49	0.32	0.03-0.61	2.95E-02	2.61E-02
miR-101-3p	1.95±0.37	2.26±0.47	0.31	0.04-0.59	2.80E-02	2.51E-02
miR-331-3p	-2.13±0.58	-1.85±0.53	0.28	-0.10-0.67	1.45E-01	1.18E-01
miR-30b-5p	1.62±0.44	1.90±0.30	0.28	0.00-0.56	4.68E-02	3.95E-02
miR-328	-2.98±0.48	-2.80±0.76	0.15	-0.20-0.50	3.85E-01	2.82E-01
let-7a-5p	-2.77±0.43	-2.62±0.64	0.15	-0.32-0.62	5.18E-01	2.91E-01
miR-181a-5p	0.01±0.76	0.16±0.51	0.13	-0.25-0.51	4.77E-01	3.82E-01
miR-148a-3p	0.47±0.62	0.60±0.35	0.12	-0.50-0.75	6.79E-01	3.55E-01
let-7d-3p	-1.90±1.03	-1.77±0.52	0.12	-0.28-0.52	5.39E-01	4.83E-01
miR-30d-5p	-1.28±0.60	-1.16±0.56	0.12	-0.22-0.45	4.73E-01	3.94E-01
miR-598	-1.98±0.49	-1.87±0.48	0.10	-0.53-0.74	7.37E-01	3.55E-01
miR-423-5p	-4.18±1.02	-4.07±0.69	0.10	-0.39-0.59	6.79E-01	5.17E-01
let-7i-5p	0.00±0.55	0.08±0.64	0.09	-0.31-0.48	6.62E-01	4.76E-01
miR-30e-3p	-1.92±0.66	-1.89±0.47	0.03	-0.38-0.45	8.69E-01	6.05E-01

B. SCAD candidates

miRNA	SCAD (mean ± SD)	UCAD (mean ± SD)	mean difference SCAD-UCAD	95% CI	p-value	B-H corrected p-value
miR-125b-5p	4.30±1.01	-1.64±1.77	5.94	5.04-6.85	9.97E-16	8.68E-14
miR-455-3p	-1.34±0.95	-7.00±1.98	5.66	4.66-6.67	6.36E-13	1.38E-11
miR-193b-3p	-0.80±0.93	-6.46±1.74	5.66	4.78-6.54	3.78E-15	1.64E-13
miR-455-5p	-4.19±0.97	-9.73±1.97	5.54	4.35-6.73	3.63E-09	1.98E-08

miR-145-3p	-2.68±1.40	-7.94±1.52	5.26	4.24-6.29	1.74E-11	1.89E-10
miR-145-5p	4.84±1.30	-0.30±1.58	5.14	4.18-6.10	2.93E-12	4.25E-11
miR-34a-5p	-0.01±1.11	-5.12±1.17	5.11	4.32-5.90	7.93E-14	2.30E-12
miR-195-5p	1.49±1.34	-3.40±1.37	4.90	3.97-5.82	2.00E-11	1.93E-10
miR-143-5p	-4.75±1.55	-9.61±1.47	4.86	3.71-6.01	3.13E-09	1.82E-08
miR-217	-4.98±1.16	-9.64±1.53	4.66	3.65-5.67	3.84E-10	3.03E-09
miR-95	-3.52±1.08	-7.85±1.63	4.33	3.44-5.23	1.25E-11	1.56E-10
miR-365b-3p	-1.13±1.20	-4.89±1.06	3.76	2.96-4.55	6.71E-10	4.87E-09
miR-30a-5p	-2.74±0.91	-6.38±0.93	3.63	3.00-4.27	2.43E-12	4.23E-11
miR-133b	-2.08±1.49	-5.14±1.65	3.05	1.99-4.12	2.18E-06	5.00E-06
miR-133a	-3.31±1.46	-6.30±1.67	2.99	1.94-4.05	2.48E-06	5.39E-06
miR-99a-5p	-0.34±1.73	-3.19±1.08	2.85	1.78-3.92	2.29E-05	3.56E-05
miR-30a-3p	-2.86±1.19	-5.63±0.74	2.77	2.03-3.51	2.19E-07	7.01E-07
let-7b-3p	-2.49±0.87	-5.03±0.77	2.54	1.96-3.11	2.48E-09	1.54E-08
miR-940	-1.81±1.13	-4.17±0.94	2.36	1.60-3.12	1.16E-06	2.80E-06
miR-127-3p	-2.33±1.13	-4.57±1.34	2.23	1.40-3.06	5.33E-06	9.87E-06
miR-92b-3p	-3.82±0.89	-5.92±0.70	2.10	1.53-2.68	1.22E-07	4.41E-07
miR-152	-0.35±0.63	-2.41±0.58	2.06	1.64-2.48	2.30E-10	2.01E-09
miR-574-3p	0.21±0.77	-1.83±0.65	2.04	1.54-2.55	1.74E-08	8.41E-08
miR-21-5p	6.54±0.66	4.53±0.51	2.01	1.59-2.44	1.94E-09	1.30E-08
miR-23b-3p	3.21±0.99	1.35±0.82	1.86	1.21-2.50	4.55E-06	8.61E-06
miR-27b-3p	3.10±0.68	1.3±0.60	1.79	1.35-2.24	1.54E-08	7.87E-08
miR-99b-5p	0.40±0.75	-1.36±0.92	1.76	1.21-2.32	2.90E-07	8.41E-07
miR-140-5p	0.47±0.94	-1.27±0.52	1.75	1.17-2.32	5.45E-06	9.89E-06
miR-140-3p	1.66±0.93	0.01±0.73	1.65	1.05-2.25	9.41E-06	1.57E-05
miR-29a-3p	-0.37±1.13	-2.00±0.49	1.63	0.96-2.30	1.01E-04	1.35E-04
miR-199b-3p	0.45±0.98	-1.14±1.10	1.58	0.89-2.28	6.64E-05	9.16E-05
miR-29c-3p	0.38±1.12	-1.02±0.64	1.39	0.71-2.08	4.47E-04	5.12E-04
let-7b-5p	3.48±0.57	2.19±0.72	1.29	0.87-1.72	6.26E-07	1.66E-06
let-7e-5p	-0.78±0.98	-2.03±0.58	1.25	0.65-1.85	3.70E-04	4.29E-04
miR-210	-2.10±0.84	-3.34±0.91	1.24	0.64-1.83	2.07E-04	2.58E-04
miR-146b-5p	-2.69±0.71	-3.91±0.60	1.22	0.76-1.69	1.47E-05	2.37E-05
miR-342-3p	0.70±0.51	-0.51±0.58	1.21	0.84-1.58	2.25E-07	7.01E-07
miR-24-3p	4.51±0.50	3.31±0.44	1.21	0.87-1.54	9.73E-08	3.90E-07
miR-28-3p	-1.24±0.56	-2.43±0.52	1.20	0.82-1.57	6.71E-07	1.72E-06
miR-29a-5p	-2.66±0.70	-3.81±0.53	1.15	0.71-1.60	2.25E-05	3.55E-05
miR-155-5p	-1.59±1.00	-2.68±0.80	1.09	0.45-1.74	1.96E-03	2.08E-03
miR-29b-1-5p	-4.51±1.42	-5.57±0.87	1.06	0.18-1.94	2.03E-02	1.86E-02
miR-29b-3p	-1.34±1.06	-2.36±0.69	1.02	0.36-1.68	4.23E-03	4.23E-03
miR-502-3p	-4.10±0.51	-5.07±0.62	0.98	0.60-1.35	9.33E-06	1.57E-05
miR-27a-3p	3.84±0.54	2.95±0.56	0.89	0.51-1.27	4.24E-05	6.14E-05
miR-154-5p	4.50±0.62	3.64±0.70	0.78	0.14-1.42	1.81E-02	4.29E-04
miR-222-3p	-3.48±0.83	-4.26±1.12	0.78	0.47-1.08	4.21E-05	1.67E-02
miR-22-5p	0.33±0.50	-0.45±0.27	0.74	0.07-1.41	3.27E-02	6.14E-05
miR-320a	-3.72±1.04	-4.46±0.82	0.54	0.21-0.86	2.07E-03	2.87E-02
miR-320b	2.43±0.47	1.89±0.47	0.53	0.22-0.84	1.54E-03	2.17E-03
miR-24-2-5p	2.14±0.45	1.60±0.47	0.49	-0.01-0.98	5.40E-02	1.65E-03
miR-532-3p	-2.81±0.77	-3.30±0.58	0.46	0.04-0.89	3.31E-02	4.51E-02
miR-28-5p	-2.58±0.60	-3.04±0.66	0.45	0.14-0.76	6.02E-03	2.88E-02

miR-150-5p	-1.12±0.40	-1.57±0.55	0.45	-0.20-1.09	1.69E-01	5.96E-03
let-7c	1.54±0.75	1.09±1.24	0.37	-0.18-0.92	1.75E-01	1.35E-01
miR-500a-5p	-2.30±0.92	-2.67±0.43	0.35	-0.11-0.82	1.30E-01	1.39E-01
miR-409-3p	-4.33±0.52	-4.68±0.89	0.33	-0.41-1.07	3.66E-01	1.08E-01
miR-532-5p	-2.87±1.08	-3.20±1.07	0.27	-0.18-0.73	2.22E-01	2.82E-01
miR-664a-3p	-3.26±0.68	-3.54±0.60	0.22	-0.15-0.58	2.35E-01	1.74E-01
miR-376a-3p	-3.48±0.54	-3.70±0.51	0.15	-0.50-0.81	6.37E-01	1.83E-01
miR-215	-3.23±0.82	-3.38±1.18	0.14	-0.60-0.88	6.97E-01	4.62E-01
miR-505-3p	-2.92±0.57	-2.99±0.41	0.07	-0.29-0.43	6.83E-01	4.83E-01

Shows the normalized expression and difference in expression from all miRNA measurements of the array experiment. Supp. table 1A shows the candidates in which expression was increased in the UCAD group and supp. table 1B shows those increased in the SCAD group. P-values were Benjamini-Hochberg (B-H) corrected for multiple testing.

Supplemental table II. Classification of the miRNA measurements from the first validation cohort based on their quality.

miR-1280	undetectable n, (%)	16 (4)
	valid n, (%)	359 (91)
	invalid n, (%)	20 (5)
miR-223-3p	undetectable n, (%)	40 (10)
	valid n, (%)	343 (87)
	invalid n, (%)	12 (3)
miR-484	undetectable n, (%)	30 (8)
	valid n, (%)	364 (92)
	invalid n, (%)	1 (0)
miR-193b-3p	undetectable n, (%)	231 (58)
	valid n, (%)	163 (41)
	invalid n, (%)	1 (0)
miR-125b-5p	undetectable n, (%)	201 (51)
	valid n, (%)	189 (48)
	invalid n, (%)	5 (1)
miR-1260	undetectable n, (%)	39 (10)
	valid n, (%)	356 (90)
	invalid n, (%)	0
miR-142-3p	undetectable n, (%)	227 (57)
	valid n, (%)	165 (42)
	invalid n, (%)	3 (1)

Based on the quality of the measurement, miRNA measurements were classified as 'valid' (reliably measurable), 'invalid' (unreliable) or 'undetectable' (unmeasurably low).

Supplemental Table III. Odd ratios for the outcomes UCAD (A) and SCAD (B) in the first validation phase

	Outome	*A. UCAD*				*B. SCAD*			
	Reference	*SCAD*		*Control*		*UCAD*		*Control*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
miR-125b-5p	model1	0.62	0.41-0.94	1.34	0.91-1.99	1.61	1.07-2.42	2.08	1.53-2.82
	model2	0.61	0.40-0.92	1.96	0.96-3.99	1.64	1.09-2.48	2.09	1.30-3.36
miR-193b-3p	model1	0.89	0.66-1.19	1.50	1.12-2.00	1.12	0.84-1.50	1.68	1.33-2.11
	model2	0.87	0.64-1.17	1.19	0.72-1.96	1.15	0.85-1.56	1.41	1.00-2.00
miR-223-3p	model1	1.14	0.89-1.46	1.65	1.28-2.13	0.88	0.68-1.13	1.41	1.18-1.68
	model2	1.15	0.89-1.47	2.38	1.41-4.01	0.87	0.68-1.12	1.61	1.20-2.15
miR-142-3p	model1	0.94	0.72-1.24	1.26	1.01-1.56	1.06	0.81-1.40	1.37	1.14-1.66
	model2	0.94	0.71-1.24	1.62	1.12-2.35	1.06	0.81-1.40	1.38	1.05-1.82

Odds Ratios (OR) and 95% confidence intervals (CI) of the logistic regression analysis from the validation experiment. Model 1=univariate analysis; model 2=corrected for age and gender. UCAD = unstable coronary artery disease, SCAD = stable coronary artery disease.

Supplemental table IV. Classification of the miRNA measurements from the second validation cohort based on their quality

miR-122-5p	Undetectable n, (%)	41 (4)
	Valid n, (%)	946 (95)
	Invalid n, (%)	13 (1)
miR-125b-5p	Undetectable n, (%)	232 (23)
	Valid n, (%)	757 (76)
	Invalid n, (%)	11 (1)
miR-126-3p	Undetectable n, (%)	38 (4)
	Valid n, (%)	889 (89)
	Invalid n, (%)	73 (7)
miR-133a-3p	Undetectable n, (%)	56 (6)
	Valid n, (%)	558 (59)
	Invalid n, (%)	356 (53)
miR-142-3p	Undetectable n, (%)	119 (12)
	Valid n, (%)	843 (84)
	Invalid n, (%)	39 (4)
miR-146-3p	Undetectable n, (%)	28 (3)
	Valid n, (%)	730 (73)
	Invalid n, (%)	242 (24)
miR-155-5p	Undetectable n, (%)	550 (55)
	Valid n, (%)	175 (18)
	Invalid n, (%)	275 (28)
miR-193b-3p	Undetectable n, (%)	232 (23)
	Valid n, (%)	663 (66)
	Invalid n, (%)	105 (11)
miR-223-3p	Undetectable n, (%)	2 (0)
	Valid n, (%)	953 (95)
	Invalid n, (%)	45 (5)
miR-499-5p	Undetectable n, (%)	867 (87)
	Valid n, (%)	47 (5)
	Invalid n, (%)	86 (9)
miR-145-5p	Undetectable n, (%)	119 (12)
	Valid n, (%)	865 (87)
	Invalid n, (%)	15 (2)
Cel-miR-39	Undetectable n, (%)	8 (1)
	Valid n, (%)	981 (98)
	Invalid n, (%)	11 (1)
Cel-miR-54	Undetectable n, (%)	1 (0)
	Valid n, (%)	993 (99)
	Invalid n, (%)	6 (1)
miR-1260	Undetectable n, (%)	2 (0)
	Valid n, (%)	991 (99)
	Invalid n, (%)	7 (1)
miR-1280	Undetectable n, (%)	6 (1)
	Valid n, (%)	955 (96)
	Invalid n, (%)	39 (4)
miR-484	Undetectable n, (%)	39 (4)
	Valid n, (%)	924 (92)
	Invalid n, (%)	37 (4)

Based on the quality of the measurement, miRNA measurements were classified as 'valid' (reliably measurable), 'invalid' (unreliable) or 'undetectable' (unmeasurably low).

Supplemental Table V. Odd ratios for the outcomes UCAD (A),S CAD (B) and SubA (C) in the second validation phase

	Outcome	A. UCAD						B. SCAD				C. SubA	
	Reference	*SCAD*		*SubA*		*Control*		*SubA*		*Control*		*Control*	
		OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
miR-122-5p	model1	7.84	5.37-11.46	11.61	7.49-18.00	11.79	7.64-18.21	1.49	1.17-1.91	1.52	1.17-1.99	0.96	0.77-1.21
	model2	7.79	5.17-11.73	10.85	6.88-17.11	9.32	5.60-15.50	1.38	1.07-1.77	1.45	1.09-1.93	1.05	0.81-1.36
miR-125b-5p	model1	0.98	0.83-1.15	1.13	0.97-1.33	1.03	0.87-1.21	1.22	1.01-1.47	1.07	0.89-1.29	0.87	0.72-1.05
	model2	1.09	0.91-1.31	1.24	1.04-1.49	1.22	0.97-1.54	1.19	0.98-1.44	1.06	0.85-1.33	0.92	0.75-1.13
miR-126-3p	model1	0.91	0.77-1.07	0.89	0.75-1.07	0.88	0.74-1.04	1.00	0.81-1.23	0.97	0.79-1.18	0.96	0.77-1.20
	model2	1.06	0.88-1.27	0.97	0.80-1.17	1.14	0.89-1.45	0.96	0.78-1.20	1.00	0.80-1.25	0.96	0.75-1.23
miR-133a-3p	model1	1.37	0.99-1.90	1.43	0.97-2.12	1.61	1.24-2.09	1.07	0.82-1.41	1.25	0.89-1.76	1.15	0.85-1.56
	model2	1.27	0.90-1.81	1.35	0.92-1.99	1.53	1.10-2.13	1.08	0.80-1.44	1.14	0.83-1.57	1.1	0.80-1.51
miR-142-3p	model1	0.81	0.71-0.92	0.82	0.72-0.94	0.82	0.72-0.93	1.03	0.88-1.21	1.02	0.87-1.19	0.99	0.84-1.16
	model2	0.87	0.76-1.01	0.83	0.72-0.96	0.90	0.75-1.07	1.01	0.85-1.19	1.06	0.88-1.26	1.01	0.85-1.21
miR-146-3p	model1	4.82	2.92-7.95	5.06	3.12-8.19	4.27	2.61-7.00	1.03	0.69-1.53	0.91	0.63-1.31	0.88	0.58-1.31
	model2	5.73	3.31-9.92	5.44	3.25-9.12	5.28	2.97-9.39	0.95	0.63-1.45	0.96	0.63-1.47	0.98	0.63-1.53
miR-155-5p	model1	4.48	3.18-6.32	3.77	2.71-5.25	3.52	2.66-4.66	0.85	0.53-1.36	0.81	0.55-1.19	0.96	0.70-1.30
	model2	4.29	3.01-6.13	3.85	2.80-5.28	3.67	2.64-5.11	0.83	0.52-1.33	0.80	0.57-1.12	1.01	0.73-1.42
miR-193b-3p	model1	1.07	0.86-1.32	0.94	0.74-1.19	0.99	0.79-1.25	0.87	0.69-1.11	0.93	0.74-1.16	1.07	0.83-1.38
	model2	1.22	0.95-1.56	1.01	0.77-1.32	1.17	0.85-1.61	0.83	0.64-1.06	0.94	0.71-1.23	1.14	0.86-1.52
miR-223-3p	model1	6.50	4.26-9.93	9.75	6.02-15.79	6.42	4.22-9.77	1.46	1.00-2.13	1.00	0.70-1.43	0.68	0.47-0.99
	model2	10.75	6.46-17.89	12.75	7.42-21.91	13.92	7.29-26.57	1.42	0.96-2.12	1.47	0.96-2.26	0.89	0.58-1.35
miR-499-5p	model1	25.59	14.22-46.05	104.63	46.16-237.16	28.34	15.23-52.74	1.21	0.72-2.02	0.92	0.62-1.37	0.71	0.42-1.20
	model2	20.63	11.16-38.15	96.10	40.13-230.14	15.73	7.80-31.72	1.20	0.70-2.06	0.73	0.46-1.17	0.65	0.37-1.16
miR-145-5p	model1	0.5	0.39-0.66	0.42	0.31-0.56	0.37	0.27-0.50	0.84	0.58-1.23	0.62	0.40-0.96	0.67	0.41-1.09
	model2	0.62	0.47-0.83	0.51	0.37-0.70	0.54	0.36-0.80	0.92	0.62-1.37	0.84	0.54-1.30	1.07	0.62-1.85

Odds Ratios (OR) and 95% confidence intervals (CI) of the logistic regression analysis from the validation experiment. Model 1=univariate analysis; model 2=corrected for age and gender. UCAD = unstable coronary artery disease, SCAD = stable coronary artery disease. SubA= subclinical atherosclerosis.

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