

## **SUPPLEMENTARY DATA**

## **SUPPLEMENTARY METHODS**

### **Echocardiographic studies**

Transthoracic echocardiograms were performed in all patients with General Electric Vivid E9 equipment (GE Healthcare Vingmed, Trodheim) as part of the work-up to exclude potential causes of stroke. All images were stored for subsequent off-line analysis in the EchoPAC v201 workstation. Echocardiographic measurements were acquired and analyzed following international recommendations.<sup>1</sup> Echocardiographic evaluation included measurement of standard parameters regarding LV dimensions and function, and, specifically, anatomical and functional assessment of the left atrium (LA), which included evaluation of LA anteroposterior diameter, LA biplanar area, LA biplanar volume (calculated from the 4-chamber and 2-chamber apical axes using the area-length method), and left atrial ejection fraction according to the formula: maximum LA volume – minimum LA volume / maximum LA volume x 100.<sup>1</sup>

### **Insertable cardiac monitor implantation and atrial fibrillation definition**

Insertable cardiac monitor devices (Biomonitor and Biomonitor-2, Biotronik) were implanted subcutaneously under local anesthesia in the left chest region and were programmed with the specific algorithm for AF detection, defined by an RR interval variability > 12.5% lasting > 1 minute, as previously described.<sup>2</sup> RR intervals with cycle lengths below 200 ms were considered noise. All patients were included in a remote monitoring system (Biotronik Home Monitoring). Recordings automatically detected as potential AF episodes were reviewed by a specialized cardiologist. AF was defined by the presence of a confirmatory revised insertable cardiac monitor recording.

### **MicroRNA extraction and profiling**

MiRNAs were isolated from plasma using the miRNeasy Serum/Plasma kit (Qiagen), and retrotranscribed into cDNA with the TaqMan Advanced miRNA cDNA Synthesis Kit (Life Technologies). In the discovery phase, a TaqMan OpenArray Human Advanced MicroRNA Panel (ThermoFisher) was carried out to screen 754 of the most commonly expressed human miRNAs. Briefly, cDNA was mixed 1:80 with the TaqMan master mix and cycled on a QuantStudio 12K Flex. Expression levels of all miRNAs were analyzed with the Cloud software (ThermoFisher), specifically designed for high-throughput gene expression studies. Mean global normalization was used to normalize samples. Those miRNAs found to have a significant differential expression among groups ( $P < .05$  and fold change  $> 2$  or  $< 0.5$ ) and/or were considered of potential relevance with regards to AF were selected for replication. MiRNA determination in the replication phase was performed by qPCR using the Fast Advanced master mix (ThermoFisher) with specific TaqMan miRNA probes (Life Technologies), and cycled in a 7500 ThermoCycler (Applied Biosystems). Results were analyzed using the Expression Suite software (Life Technologies). MiR-16, miR-92a and miR-486 showed high stability between groups and were therefore used as controls for normalization.

**Table 1 of the supplementary data.** Characteristics of Set I and Set II populations

|  | Set I (discovery phase, matched for age and sex) |                |                | Set II (replication phase, unmatched) |                 |      |
|--|--|----------------|----------------|---------------------------------------|-----------------|------|
|  | CrS-SR (n = 9)                                   | CrS-AF (n = 9) | CES-AF (n = 9) | CrS-SR (n = 27)                       | CrS-AF (n = 19) | P    |
| Age, y                                 | 75.6 ± 8.2                                       | 75.2 ± 7.6     | 74.7 ± 8.0     | 72.2 ± 12.8                           | 77.4 ± 10.0     | .145 |
| Female sex                             | 4  | 4              | 4              | 14 (51.9)                             | 7 (36.8)        | .377 |
| BMI, kg/m <sup>2</sup>                 | 28.1 ± 3.3                                       | 29.6 ± 6.4     | 27.8 ± 3.7     | 27.1 ± 4.1                            | 26.4 ± 4.5      | .624 |
| Hypertension                           | 7  | 5              | 7              | 21 (77.8)                             | 12 (63.2)       | .331 |
| Diabetes mellitus                      | 2  | 1              | 3              | 7 (25.9)                              | 3 (15.8)        | .488 |
| Hyperlipidemia                         | 5  | 5              | 5              | 16 (59.3)                             | 9 (47.4)        | .550 |
| Current smoking                        | 1  | 1              | 2              | 4 (14.8)                              | 2 (10.5)        | .999 |
| Peripheral artery disease              | 1  | 2              | 1              | 3 (11.1)                              | 5 (26.3)        | .246 |
| Coronary artery disease                | 0  | 0              | 1              | 1 (3.7)                               | 2 (10.5)        | .561 |
| Prior stroke/TIA                       | 2  | 3              | 1              | 3 (11.1)                              | 3 (15.8)        | .680 |
| CHA <sub>2</sub> DS <sub>2</sub> -VASc | 3.3 ± 2.2  | 3.3 ± 1.9      | 3.4 ± 1.3      | 3.1 ± 1.6                             | 3.1 ± 1.9       | .991 |
| TIA/stroke*                            | 1/8  | 0/9            | 1/8            | 6 (22.2)                              | 4 (21.1)        | .999 |

AR, atrial fibrillation; BMI, body mass index; CES, cardioembolic stroke; CrS, cryptogenic stroke; SD,

standard deviation; SR, sinus rhythm; TIA, transient ischemic attack.

The data are expressed as absolute number, No. (%), or mean ± standard deviation.

\* In population Set II, the numbers of TIA are presented.

**Table 2 of the supplementary data.** Results of the open array study

| Differential miRNA expression<br>CrS-AF vs CrS-SR |         |       | Differential miRNA expression<br>CES-AF vs CrS-SR |         |       |
|---|---------|-------|---|---------|-------|
| miRNA   | Rq      | P     | miRNA   | Rq      | P     |
| hsa-miR-320a*                                     | 362.020 | <.001 | hsa-miR-320a*                                     | 344.390 | <.001 |
| hsa-miR-362-5p*                                   | 0.388   | .005  | <b>hsa-miR-19b-3p</b>                             | 0.017   | .003  |
| <b>hsa-miR-744-5p</b>                             | 10.594  | .007  | hsa-miR-190a-<br>5p*                              | 2.889   | .003  |
| hsa-miR-664a-<br>3p*                              | 0.389   | .009  | hsa-miR-362-5p*                                   | 0.330   | .005  |
| <b>hsa-miR-1-3p</b>                               | 8.644   | .014  | <b>hsa-miR-744-5p</b>                             | 10.547  | .011  |
| <b>hsa-miR-377-3p</b>                             | 4.136   | .015  | hsa-miR-214-3p*                                   | 0.418   | .013  |
| <b>hsa-miR-181b-5p</b>                            | 4.686   | .017  | hsa-miR-181d-5p                                   | 4.514   | .014  |
| hsa-miR-190a-<br>5p*                              | 2.227   | .021  | hsa-miR-1180-<br>3p*                              | 0.395   | .017  |
| <b>hsa-miR-19a-3p</b>                             | 0.152   | .022  | <b>hsa-miR-1-3p</b>                               | 7.932   | .018  |
| hsa-miR-483-5p*                                   | 2.439   | .024  | hsa-miR-31-5p*                                    | 0.411   | .019  |
| <b>hsa-miR-425-5p</b>                             | 3.262   | .026  | hsa-miR-34a-3p*                                   | 0.411   | .019  |
| hsa-miR-16-2-3p*                                  | 0.324   | .028  | hsa-miR-455-3p*                                   | 0.411   | .019  |
| hsa-miR-625-3p*                                   | 0.413   | .031  | hsa-miR-499a-<br>5p*                              | 0.411   | .019  |
| hsa-miR-502-3p*                                   | 1.862   | .031  | hsa-miR-570-3p*                                   | 0.411   | .019  |
| <b>hsa-miR-191-5p</b>                             | 4.758   | .039  | hsa-miR-29b-2-<br>5p*                             | 0.435   | .021  |
| <b>hsa-miR-19b-3p</b>                             | 0.128   | .049  | hsa-miR-154-3p*                                   | 0.434   | .021  |
|   |         |       | hsa-miR-548e-<br>3p*                              | 0.458   | .022  |
|   |         |       | hsa-miR-548j-5p*                                  | 0.454   | .024  |
|   |         |       | hsa-miR-100-5p*                                   | 0.500   | .025  |
|   |         |       | hsa-miR-188-5p*                                   | 0.465   | .025  |
|   |         |       | hsa-miR-452-5p*                                   | 0.453   | .025  |
|   |         |       | hsa-miR-23b-5p*                                   | 0.461   | .027  |
|   |         |       | hsa-miR-493-3p*                                   | 0.518   | .029  |
|   |         |       | hsa-miR-17-5p                                     | 6.432   | .031  |
|   |         |       | hsa-miR-99b-5p                                    | 4.144   | .032  |
|   |         |       | hsa-miR-500a-<br>5p*                              | 0.479   | .036  |
|   |         |       | <b>hsa-miR-425-5p</b>                             | 2.970   | .037  |
|   |         |       | hsa-miR-29c-3p                                    | 13.762  | .037  |
|   |         |       | hsa-miR-380-3p*                                   | 2.070   | .038  |
|   |         |       | hsa-miR-941*                                      | 0.420   | .038  |
|   |         |       | hsa-miR-532-3p                                    | 2.780   | .039  |
|   |         |       | hsa-miR-186-5p                                    | 4.605   | .039  |
|   |         |       | <b>hsa-miR-377-3p</b>                             | 4.277   | .041  |

AR, atrial fibrillation; CES, cardioembolic stroke; CrS, cryptogenic stroke; SR, sinus rhythm.

\*Inconsistent values of Ct among samples (high standard deviation), or very low (Ct > 36)

expression in both groups. miRNAs selected for replication are highlighted in bold.