Supplementary Material

Small-RNA sequencing identifies serum microRNAs associated with abnormal electrocardiography findings in patients with Chagas disease

Michael Mueller, Alice Blandino, Dominique Scherer, Inés Zulantay, Werner Apt, Nelson M. Varela, Marcelo Llancaqueo, Lineth Garcia, Lourdes Ortiz, Emanuele Nicastri, Maria Letizia Giancola, Andrea Angheben, Simona Gabrielli, Trine B. Rounge, Hilde Langseth, Melanie Waldenberger, Pamela Salinas-Alvarezand Justo Lorenzo Bermejo

Correspondence: Justo Lorenzo Bermejo, Email: [lorenzo@imbi.uni.heidelberg.de](mailto:lorenzo@imbi.uni.heidelberg.de)

**Table S1** Association between right/left bundle-branch block and the expression in whole blood samples from the Framingham Heart Study of the miRNAs associated with abnormal ECG in Bolivian and Chilean Chagas patients investigated in the present study

|  |  |  |  |
| --- | --- | --- | --- |
| miRNA | Expression difference  abs vs. pre | 95% CI | p-value |
| *Right bundle-branch block* |  |  |  |
| miR-576-3p | 0.02 | -0.05 – 0.08 | 0.66 |
| miR-629-5p | 0.23 | -0.03 – 0.49 | 0.07 |
| *Left bundle-branch block* |  |  |  |
| miR-576-3p | 0.06 | -0.03 – 0.15 | 0.19 |
| miR-629-5p | 0.20 | -0.15 – 0.54 | 0.24 |

**Table S2** Expression of mi-R-629-5p, miR-576-3p and miR-101-3p stratified by Kuschnir score

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Kuschnir** |  | **Median**  **log2 expression** | **1st – 3rd quartile**  **log2 expression** | **Univariate logistic regression** | | | **Multiple logistic regression** | | |
| **Population** | **score** | **Patients** | **OR** | **95%-CI** | **Pval** | **OR** | **95%-CI** | **Pval** |
| ***miR-629-5p*** |  |  |  |  |  |  |  |  |  |  |
| All | 0 & 1 | 85 | 7.7 | 6.9 - 8.5 | Ref. |  | 0.032 | Ref. |  | 0.0022 |
|  | 2 & 3 | 40 | 7.5 | 6.5 - 8.0 | 0.666 | 0.458 - 0.967 |  | 0.657 | 0.433 - 0.998 |  |
| Women | 0 & 1 | 55 | 7.7 | 7.2 - 8.3 | Ref. |  | 0.078 | Ref. |  | 0.1 |
|  | 2 & 3 | 16 | 7.2 | 6.4 - 8.0 | 0.578 | 0.314 - 1.063 |  | 0.551 | 0.285 - 1.068 |  |
| Men | 0 & 1 | 30 | 7.7 | 6.6 - 8.6 | Ref. |  | 0.197 | Ref. |  | 0.054 |
|  | 2 & 3 | 24 | 7.6 | 6.6 - 8.1 | 0.728 | 0.449 - 1.180 |  | 0.722 | 0.422 - 1.237 |  |
| Less than 48 | 0 & 1 | 51 | 7.6 | 6.7 - 8.5 | Ref. |  | 0.112 | Ref. |  | 0.216 |
| years | 2 & 3 | 12 | 7.2 | 6.3 - 7.7 | 0.593 | 0.311 - 1.130 |  | 0.624 | 0.333 - 1.169 |  |
| 48 years or | 0 & 1 | 34 | 7.9 | 7.3 - 8.5 | Ref. |  | 0.090 | Ref. |  | 0.070 |
| more | 2 & 3 | 28 | 7.5 | 6.6 - 8.1 | 0.623 | 0.361 - 1.076 |  | 0.630 | 0.360 - 1.102 |  |
| ***miR-576-3p*** |  |  |  |  |  |  |  |  |  |  |
| All | 0 & 1 | 85 | 5.0 | 4.2 - 5.6 | Ref. |  | 0.1053 | Ref. |  | 0.00241 |
|  | 2 & 3 | 40 | 4.6 | 3.9 - 5.2 | 0.731 | 0.500 - 1.068 |  | 0.760 | 0.502 - 1.151 |  |
| Women | 0 & 1 | 55 | 5.1 | 4.3 - 5.4 | Ref. |  | 0.184 | Ref. |  | 0.1205 |
|  | 2 & 3 | 16 | 4.5 | 3.9 - 5.3 | 0.677 | 0.381 - 1.204 |  | 0.679 | 0.372 - 1.234 |  |
| Men | 0 & 1 | 30 | 4.8 | 4.2 - 5.7 | Ref. |  | 0.342 | Ref. |  | 0.076 |
|  | 2 & 3 | 24 | 4.6 | 4.0 - 5.1 | 0.774 | 0.457 - 1.313 |  | 0.852 | 0.482 - 1.508 |  |
| Less than 48 | 0 & 1 | 51 | 5.0 | 4.2 - 5.7 | Ref. |  | 0.734 | Ref. |  | 0.484 |
| Years | 2 & 3 | 12 | 4.9 | 4.4 - 5.3 | 0.900 | 0.491 - 1.651 |  | 0.905 | 0.496 - 1.653 |  |
| 48 years or | 0 & 1 | 34 | 5.1 | 4.2 - 5.4 | Ref. |  | 0.100 | Ref. |  | 0.057 |
| more | 2 & 3 | 28 | 4.5 | 3.8 - 5.1 | 0.625 | 0.358 - 1.093 |  | 0.611 | 0.343 - 1.087 |  |
| ***miR-101-3p*** |  |  |  |  |  |  |  |  |  |  |
| All | 0 & 1 | 85 | 10.3 | 10.0 - 10.9 | Ref. |  | 0.0027 | Ref. |  | 0.0005 |
|  | 2 & 3 | 40 | 9.8 | 9.4 - 10.6 | 0.418 | 0.236 - 0.740 |  | 0.341 | 0.176 - 0.660 |  |
| Women | 0 & 1 | 55 | 10.3 | 10.0 - 10.8 | Ref. |  | 0.017 | Ref. |  | 0.014 |
|  | 2 & 3 | 16 | 9.8 | 9.4 - 10.6 | 0.317 | 0.123 - 0.818 |  | 0.209 | 0.069 - 0.631 |  |
| Men | 0 & 1 | 30 | 10.2 | 9.6 - 11.3 | Ref. |  | 0.066 | Ref. |  | 0.035 |
|  | 2 & 3 | 24 | 9.8 | 9.4 - 10.5 | 0.524 | 0.263 - 1.043 |  | 0.427 | 0.184 - 0.989 |  |
| Less than 48 | 0 & 1 | 51 | 10.2 | 10.0 - 10.9 | Ref. |  | 0.010 | Ref. |  | 0.028 |
| Years | 2 & 3 | 12 | 9.7 | 8.9 - 10.1 | 0.176 | 0.047 - 0.663 |  | 0.176 | 0.046 - 0.677 |  |
| 48 years or | 0 & 1 | 34 | 10.4 | 9.9 - 11.0 | Ref. |  | 0.069 | Ref. |  | 0.059 |
| more | 2 & 3 | 28 | 9.9 | 9.5 - 10.6 | 0.517 | 0.253 - 1.053 |  | 0.528 | 0.255 - 1.092 |  |

**Table S3**. Identified and validated cis-miRNA-eQTLs for the three identified miRNAs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| miRNA | Log2  expression  in serum  median  [95% CI] | Chromo-somal  location  (GRCh38) | N. of  candidates  cis-miRNA-eQTLs  based on  miRNA-eQTL  database | N. of  validated  cis-miRNA  -eQTLs | N. of  cis-miRNA  -eQTLs  used  as  predictors | Adjusted  r-squared best  model for prediction |
| miR-629-5p | 8.45  [3.82 - 10.13] | chr15:  70079372 to  70079468 | 50 | 1 | 1 | 0.16 |
| miR-101-3p | 11.80  [10.57 - 13.21] | chr1:  65058434 to  65058508 | 26 | - | - | - |
| miR-576-3p | 5.92  [3.34 - 7.46] | chr4:  109488698 to  109488795 | 0 | - | - | - |

**Table S4**. Identified cis-miRNA-eQTLs for miR-629-5p

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SNP ID | Chromosomal location  (GRCh38) | MAF | Penetrance  model | β1 coefficient | p-value β1 | β2 coefficient | p-value β2 |
| *rs11630316* | chr15:  70077039:T:C | 0.49 | Additive | -0.3751 | 0.0149 | - | - |
| Three-genotypes | -0.6131 | 0.0248 | -0.7568 | 0.0118 |

**Table S5**. Median genotype-based log2 expression of miR-629-5p in serum samples from Chagas disease patients with normal ECG findings, and association between genotype-based log2 miR-629-5p expression and abnormal ECG

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| miRNA | Median genotype-based  log2 expression (normal ECG) | OR | 95% CI | p-value |
| *miR-629-5p* | 8.24 | 0.71 | 0.30 - 1.72 | 0.45 |

**Table S6**. Results from association analysis between *CCL5* and rs2107538

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| SNP ID | Chromosomal location  (GRCh38) | MAF | Penetrance  model | β1 coefficient | p-value β1 | β2 coefficient | p-value β2 |
| *rs2107538* | chr17:  35880776:C:T | 0.22 | Three-genotypes | 2.2665 | 2x10-12 | 2.2481 | 9x10-13 |
| Dominant | 2.2599 | 3x10-15 | - | - |