Supplementary Material

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

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**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 | Log2expression in serummedian [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 to70079468 | 50 | 1 | 1 | 0.16 |
| miR-101-3p | 11.80 [10.57 - 13.21] | chr1: 65058434 to65058508 | 26 | - | - | - |
| miR-576-3p | 5.92 [3.34 - 7.46] | chr4: 109488698 to109488795 | 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 | - | - |