**Precision Cardiovascular Medicine: Artificial Intelligence and Epigenetics for the Pathogenesis and Prediction of Coarctation in Neonates**

**SUPPLEMENTAL MATERIAL**

**Supplemental Table S1:** Clinical demographics of CoA cases versus controls

|  |  |  |
| --- | --- | --- |
| **Demographics** | **Mean (SD)** | **p-value** |
| **Controls** | **CoA** |
| Number | 16 | 24 | - |
| Mother Age (years) | 27.062 (4.697) | 29.333 (5.998) | 0.2103(T) |
| Gender |
| Male | 9 | 16 | 0.521 (W) |
| Female | 7 | 8 |
| Ethnicity  |
| White | 11 | 16 | 0.9062 (W) |
| African American | 3 | 8 |
| Other | 2 | 0 |
| Gestational Age (Weeks) | 38.875 (1.147) | 37.792(3.120) | 0.599 (W) |

T- Student t-test

W- Wilcoxon Mann Whitney test

**Supplemental Table S2:** Coarctation prediction using Artificial Intelligence (stringent p-values\*)

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | SVM | GLM | PAM | RF | LDA | DL |
| AUC95% CI | 0.92(0.73-1) | 0.93(0.73-1) | 0.96(0.73-1) | 0.93(0.72-1) | 0.91(0.72-1) | 0.97(0.80-1) |
| Sensitivity | 0.90 | 0.90 | 0.90 | 0.85 | 0.83 | 0.95 |
| Specificity | 0.85 | 0.90 | 0.87 | 0.85 | 0.90 | 0.98 |

\*Individual CpG locus p< 5x10-8 for defining methylation change

Most important CpG biomarkers listed in decreasing order of contribution - per AI platform used:

**SVM**: cg17259183, cg12071328, cg15578311, cg05867499, cg04708753

**GLM**: cg17259183, cg05867499, cg04708753, cg02484732, cg14825413

**PAM**: cg17259183, cg12071328, cg15578311, cg05867499, cg02484732

**RF**: cg12071328, cg17259183, cg05867499, cg15578311, cg14825413

**LDA**: cg17259183, cg12071328, cg15578311, cg02484732, cg04043455

**DL**: cg23490161, cg15578311, cg17259183, cg04043455, cg02484732

**Supplemental Table S3:** Coarctation prediction using Artificial Intelligence\*

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | SVM | GLM | PAM | RF | LDA | DL |
| AUC95% CI | 0.89(0.72-1) | 0.91(0.72-1) | 0.91(0.73-1) | 0.88(0.71-1) | 0.76(0.56-0.96) | 0.94(0.82-1) |
| Sensitivity | 0.82 | 0.85 | 0.87 | 0.75 | 0.70 | 0.95 |
| Specificity | 0.75 | 0.85 | 0.75 | 0.75 | 0.75 | 0.93 |

\*Individual CpG locus significant methylation difference (CoA vs controls) defined as FDR p-value <0.05

Most important CpG biomarkers listed in decreasing order of contribution - per AI platform used:

**SVM**: cg17259183, cg14150199, cg27047371, cg12071328, cg20739510

**GLM**: cg09428571, cg05867499, cg05042705, cg12193484, cg04708753

**PAM**: cg17259183, cg12071328, cg27047371, cg20739510, cg25910466

**RF**: cg22753548, cg25910466, cg27149073, cg12071328, cg26394619

**LDA**: cg17259183, cg20739510, cg27047371, cg12071328, cg14150199

**DL**: cg17259183, cg09428571, cg15578311, cg02484732, cg18017761

**Supplemental Table S4:** Prediction of Coarctation- Logistic Regression Analysis

|  |  |  |  |
| --- | --- | --- | --- |
| **Group** | **AUC (95% CI)** | **Specificity** | **Specificity** |
| Training/Discovery | 0.969 (0.952 ~ 0.986) | 0.926 (0.891 ~ 0.961) | 0.882 (0.829 ~ 0.935) |
| 10-fold Cross-Validation | 0.883 (0.766 ~ 1.000) | 0.917 (0.917 ~ 1.000) | 0.875 (0.713 ~ 1.000) |

Regression equation: logit (P) = log(P / (1 - P)) = -12.62 + 33.584 cg17259183
 + 95.598 cg12071328 + 15.737 cg05867499

**Supplemental Table S5:** Epigenetically Modified Genes in Coarctation: Role in Heart Development and Function

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene Symbol** | **Gene Name** | **Role and effect of mutations in Heart/ CVS** | **References** |
| *TGFβ1*  | Transforming growth factor, beta 1 | Cardiac fibrosis, ventricular remodeling, valvular pathogenesis | (1) |
| *SMAD1* | Small Mother against decapentaplegic homolog-1 | Endothelial proliferation, angiogenesis, left ventricle remodeling and cardiac hypertrophy | (2) |
| *FOSL2* | Fos-Like antigen | Epithelial mesenchymal transformation (EMT), gives rise to all heart cell types, Mutation leads to CHD | (3) |
| *THBS1* | Thrombospondin 1 | Affects tissues modeling: heart disease, heart failure | (4) |
| *MYO5A* | Myosin 5A | Regulates potassium ion channels:myocyte repolarization, cardiac rhythm | (5) |
| *TNNI3* | Troponin I | Familial disease Left ventricular dysfunction and abnormal morphology of cardiovascular system | (6) |

**Supplemental Table S6:** Epigenetically Dysregulated Molecular Pathways in isolated CoA

|  |  |  |  |
| --- | --- | --- | --- |
| **Molecular** **Pathways** | **Function** | **Role in Heart/ CVS** | **Reference** |
| **IntegrinSignaling** | Vasculogenesis, angiogenesis (e.g. VEGFR) | Heart and vessel remodeling and development, cardiac growth | (7) |
| **TGFβsignaling** | Family of growth and differentiation factors | Critical for CVS development and heart modeling | (8) |
| **RAR (retinoid acid receptor)** | RA binds to RAR controls mammalia developmental processes | Multifaceted role in heart-regulate damage and repair in remodeling | (9) |
| **Glucocorticoid receptorsignaling** | Glucocorticoid actions mediated by GR | GR signaling in myocardiocytes: cardiac development and function | (10) |

**Supplemental Table S7:** Gene enrichment statistics under disease pathways associated with CoA.

|  |  |
| --- | --- |
| **Disease pathway** | **Significance** |
| Abnormal morphology of cardiovascular system | 0.0037 |
| Left ventricular dysfunction | 0.0013 |
| Heart conduction disorder | 0.0024 |
| Thrombus | 0.0083 |
| Coronary artery disease | 0.0041 |

**Supplemental Figure S1:** Three dimensional PLS-DA overfitting model of CoA cases vs normal controls followed by VIP scores of the significant CpG markers. The data are color coded according to their grouping analysis (Red nodes represent cases and green nodes represent controls).



**References**

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