**Table 1: Baseline Characteristics of the Study Population**

|  |  |  |
| --- | --- | --- |
|  | **TAV (n=16)** | **BAV (n=16)** |
| Age (years, mean ± SD) | 63 ± 7 | 53 ± 14 |
| Sex (% female [N]) | 19 (3) | 19 (3) |
| Race (% white) | 75 (12) | 94 (15) |
| BMI (m2) | 27 ± 6 | 27 ± 5 |
| History of tobacco use (% [N]) | 44 (7) | 44 (7) |
| Hypertension (% [N]) | 81 (13) | 50 (8) |
| Diabetes mellitus (% [N]) | 0 (0) | 0 (0) |
| Statin use (% [N]) | 25 (4) | 31 (5) |
| ACEi or ARB use (% [N]) | 44 (7) | 25 (4) |
| Ejection fraction (%, mean ± SD) | 53 ± 4 | 53 ± 7 |
| Maximum aortic diameter (cm, mean ± SD) | 6.1 ± 1.4 | 5.4 ± 0.7 |
| Aortic stenosis > moderate | 0 (0) | 25 (4) |
| Aortic regurgitation > 2+ | 31 (5) | 31 (5) |

Data are presented as mean ± standard deviation for continuous variables and as percentage (raw number) for categorical variables. TAV-tricuspid aortic valve; BAV-bicuspid aortic valve; BMI-body mass index; ACEi-angiotensin converting enzyme inhibitor; ARB-angiotensin II receptor blocker

**Table 2: Top Differentially Methylated and Expressed Genes**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Gene** | **Gene Function** | **CpGs\*** | **Delta Beta^** | **TAV Expression†** | **BAV Expression†** | **Expression P** |
| **PTPN22** | T-cell signaling | 4 | +15.4 to +15.8 | 7.9 | 5.1 | 2.39 x 10-5 |
| **RIPK1** | Apoptosis, TNF signaling | 3 | -10.3 to -11.5 | 10.7 | 11.3 | 2.71 x 10-5 |
| **LIMS2** | Protein-protein interactions in extracellular matrix | 3 | -10.0 to -14.0 | 11.1 | 12.0 | 5.53 x 10-5 |
| **ZBP1** | Innate immunity, interferon production | 2 | +10.5 to +11.4 | 8.4 | 4.9 | 4.24 x 10-4 |
| **MYO18A** | Epithelial cell migration | 2 | -11.0 to -17.7 | 7.7 | 9.7 | 4.44 x 10-4 |
| **GLRX** | Antioxidant defense system | 3 | +14.5 to +17.6 | 13.0 | 12.5 | 4.63 x 10-4 |
| **ERGIC1** | Transport between endoplasmic reticulum and Golgi | 6 | -10.0 to -12.4 | 7.9 | 9.0 | 5.06 x 10-4 |
| **GAPT** | B-cell regulation | 2 | +10.0 to +12.9 | 9.6 | 6.6 | 5.32 x 10-4 |
| **KLHL6** | B-cell signaling | 2 | +10.8 to +12.6 | 8.8 | 4.9 | 5.40 x 10-4 |

\* number of CpGs showing differential methylation

† log 2 expression

^ percent

**Table 3: Top Differentially Methylated Genes Related to Cardiovascular Development and Function that were not Differentially Expressed**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Gene** | **CpGs** | **Delta Beta^** | **Gene Function** | **Mouse Knockout** |
| **TBX5** | 15 | +10.1 to +13.2 | Mesoderm differentiation | Heart morphology, function |
| **PRDM16** | 12 | +10.1 to +16.4 (5 probes)  -10.1 to -19.3  (7 probes) | Development of brown adipocytes | Ventricular hypoplasia, abnormal morphology |
| **GATA4** | 9 | +10.0 to +16.1 | Embryogenesis, myocardial differential and function | Abnormal ventricle morphology |
| **NKX2-6** | 7 | +12.2 to +18.1 | Pharyngeal and cardiac embryonic development | Abnormal atrial morphology |
| **TCF21** | 7 | +10.1 to +12.3 | Differentiation of epicardial cells | Abnormal vascular morphology |
| **ACTA2** | 4 | -10.0 to -22.9 | Cell motility, structural integrity. Familial thoracic aortic aneurysm and dissection | Abnormal aorta morphology |
| **CACNB2** | 3 | -10.4 to -12.8 | Voltage dependent calcium channel protein | Abnormal myocardial fiber physiology, |
| **MGAT1** | 3 | +10.5 to +11.1 | Diet induced obesity | Abnormal vascular development |

^ percent