Supplementary Materials for

DNA methylation of a *PLPP3* MIR transposon-based enhancer promotes an osteogenic program in calcific aortic valve disease

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Whole genome gene expression dataset

A total of twelve CAVD valves and twelve normal aortic valves were selected for microarray analysis. The description of the population as well as the detailed methodology of the microarray have been previously published[1]. Briefly, to reduce heterogeneity, only tricuspid valves from Caucasian male subjects without diabetes were selected. The two valve groups were also matched for patient's age, body mass index, and the absence of renal failure. Expression studies were performed using the HumanHT-12 v4 Expression BeadChip (Illumina, CA, USA). QC was performed as described by Chow and coworkers [2]. The raw data were quantile normalized after log2-transformation with the lumi package in R[3]. Genes differentially expressed by microarray were identified using the significant analysis of microarrays (SAM) method[4] with the false discovery rate set at 5% and fold change threshold set at 1.2.

Valve interstitial cell isolation

Aortic leaflets were incubated in 1mg/ml type I collagenase at 37°C for 30 minutes, then washed in HEPES1X, cut into pieces and incubated in 4.5mg/ml type I collagenase at 37°C for 30 minutes. Tissues were then washed in normal medium 3 times and seeded. Cells were used between passages 3 to 7.

Luciferase reporter sequences

DNA sequence minimal promoter:

CTGAGACTCCTTCACCCAGAAAAAAGGTAATTGCTAACCCTCACAGAGCACTTCCTA
TGTACTGGGCACTGTTCTAGGAGCTTATACGGATTAACAAATTAAGTCTTCACAACC
ACCCTATATGAAAAGGAACAGTTATCTCTAGTTACATACGGGGAAACTGAGGCACAG
AGTCACAGGATTGAAATCCAGGAGATGTGGTTCCACAGTCGTGCAGTCTTTTTCATTT
TAGTATTCTCAAAATCTAGCGTAGTACCCGGTGTTGGATGTTAAGATCTTAAACAAA
CAAACAACTTGTTTTCCTTAAAAATTATATTTTCAGGGTAATACAATTCAAGTCACAA
TAAAGACCTGAAAGCCTCAGGTTTGTAGGTGAGGAAAAATGCATTTTAGCTGTACAG
CCCTAACTTTTACTTGACCCAAGGCCAGCAAGAAAATGTTTCTTTTTATTTATATATA
TTTTTTCTTTGGTGACAATTTGGTGTTTCTGAACGCTAGGGGCTCCCGGTTTCTCTGGT
TTGAGAGTAACTTTTCCTTTTAGGACTTTTTTTTTTTTTTTT

DNA sequence enhancer:

ACAAGTACTGTGCGTTTTCCATTACACCATGATTCGCTTTAAAAAAAAAAAAATCCT
AGGCTGACTTCAACCTTGGAAGTCCTGTTCATTCTTGTGAAGCGCTTTCTCCCTCCCC
TACCGAGTTCATAAATCCTCCTCGCCCTCCCACTCCAATTCCTTTAACTCCCACCGCT
CTTCCAGCTGTGGCTTCCAACGACTTTGGAAAACAGAAGTGGACCATCGCCTCAACT
TTCTGGGGGCGCAATTAAATTTGTGGTCCCCAAACAAGATCGCAGCTGGCTTTCCCA
GTGCCTCAATGTGCCCATCAGGGAAATGGGGCTCATCACCCCTGCTGCACCGAGTCA
GAGAGTGGGCGAGCTAAAACTGATGCCCCTGAAGCCGCCTACCACCTGGCACACAG
TAGGCACTCAAGTGATTGCGAAGCCGTGCCCAGCACCAAGCCCGGGGAAAGCACCT
ACAGGAGAGGAGCAAGTGAAAAGGCCGGCTAGCCAGTCTGGCGAGCCTGGGCAGCA

Supplementary table 1: Clinical characteristics of patients for microarray analysis

	Control valves (n=12)	**CAVD (n=12)**	**p-value**
Age	58 ± 2	64 ± 1	0.01
Male (%)	100	100	--
Smoking (%)	0	0	--
Hypertention (%)	25	75	0.03
Diabetes (%)	0	0	--
Coronary heart disease (%)	33	50	0.68
Bicuspid aortic valves (%)	--	0	--
BMI (kg/m^2)	28 ± 1	29 ± 2	0.39
Statins (%)	42	92	0.02
Aortic valve area (cm^2)	--	0.84 ± 0.08	--
Aortic peak gradient (mmHg)	--	58 ± 6	--
Aortic mean gradient (mmHg)	--	36 ± 4	--
Triglycerides (mmol/L)	1.27 ± 0.22	1.51 ± 0.33	0.55
LDL (mmol/L)	2.00 ± 0.24	2.01 ± 0.11	0.98
HDL (mmol/L)	1.09 ± 0.09	1.15 ± 0.06	0.58
Creatinine (µmol/L)	103 ± 6	91 ± 6	0.13
Creatinine clearance (ml/min)	79 ± 6	84 ± 6	0.58

Values are mean±SEM or %; P values: Student t test or Fisher exact test; BMI: body mass index; LDL: low-density lipoprotein cholesterol; HDL: high-density lipoprotein cholesterol; and SEM, standard error of the mean.

Reference List

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