Table SI. Characteristics of samples used for genome-wide methylation microarray analysis to obtain a list of differentially methylated regions.

Variable	Controls	Isolated cardiac defects	Non-isolated cardiac defects
Number of subjects	4	2	3
Gestational age (weeks)	24.1±0.84	23.9±0.49	24.4±1.05
Maternal age (years)	29.2±6.71	31.7±3.01	30.8±5.81

Table SII. Phenotypes of samples with cardiac defects used for genome-wide methylation microarray analysis.

Categories	Subtypes of cardiac defects	Number of subjects
Isolated cardiac defects	PTA, VSD	1
	Overriding aorta, VSD, PS	1
Non-isolated cardiac defects	AVSD, DORV	1
	HRV, VSD, PS	1
	SA, SV	1

PTA, persistent truncus arteriosus; VSD, ventricle septum defect; PS, pulmonary stenosis; AVSD, atrioventricular septum defect; DORV, double outflow right ventricle; HRV, hypoplasia of right heart; SA, single atrium; SV, single ventricle.

Table SIII. Characteristics of samples used for genome-wide methylation microarray analysis to validate differentially methylated regions.

Variable	Controls	Isolated cardiac defects	Non-isolated cardiac defects
Number of subjects	18	15	11
Gestational age (weeks)	23.5±1.56	24.5±1.61	23.1±2.71
Maternal age (years)	29.2±6.71	31.7±3.01	30.8±5.81

Table SIV. Phenotypes of samples with cardiac defects used for MassARRAY analysis.

Categories	Subtypes of cardiac defects	Number of subjects
Isolated cardiac defects	Tetralogy of fallot	5
	PTA, VSD, severe PS, single outflow tract	1
	HLHS	1
	AVSD	1
	HRV, VSD, DORV	1
	AVSD, Overriding aorta, VSD, PS	1
	Overriding aorta, VSD, PS, right aortic arch	1
	Severe tricuspid dysplasia	1
	HRV, PS	1
	primary ASD	1
	HRV, AC, VSD, parallel aorta	1
Non-isolated cardiac defects	Overriding aorta, DORV, VSD	1
	AC	1
	aortic and pulmonary stenosis	1
	AVSD, DORV	1
	asymmetric four-chamber (no details)	1
	Single outflow tract	1
	SA, SV	1
	SV, PS, right aortic arch	1
	Tetralogy of fallot	1
	PTA, HLHS	1
	Overriding aorta, VSD, PS	1

PTA, persistent truncus arteriosus; VSD, ventricle septum defect; PS, pulmonary stenosis; HLHS, hypoplastic left heart; AVSD, atrioventricular septum defect; HRV, hypoplasia of right heart; DORV, double outflow of right ventricle; ASD, atrial septum defect; AC, coarctation of aorta; SA, single atrium; SV, single ventricle.

Table SV. Primers used for validation of methylation level of CpG sites adjacent to heart development-related genes by MassARRAY.

CpG site location	Forward 10-mer tag primer 1	Reverse T7 promoter tag 2	Product size (bp)	Assayed CGs (n)
Chr7:55379459-55380266	CTCCCCACTAAAACCC ATACCC	GAGAGTATTTATAGGGGAGG TTTAGG	301	24
Chr9:138514624-138515325	CCTCCCCCTAAACTACAAC TAAAAA	GGAGGGTAGGTGGGTATATA GGTAGT	396	21
Chr21:45877482-45878832	CCAACCTTCAAAACCCTA TAAACT	GGAAGTATTTTTTTGGTGTGGT	375	37

1,10-mer tag, cagtaatacgactcactatagggagaagg and 2;T7 promoter tag, aggaagagag were added.

Table SVI. Primers used for reverse transcription-quantitative PCR.

Gene	Primer	Sequence (5'-3')
GAPDH	Forward	CACCCACTCCTCCACC TTTG
	Reverse	ACCACCCTGTTGCT GTAGCC
EGFR	Forward	CAGGAGGTGGCTGGTTATGT
	Reverse	AGCTCCTTCAGTCCGGTTTT
SLC19A1	Forward	CGGCTACTACCTGGTGGTCT
	Reverse	GATGAGCAGCTTGGACCAG
NOTCH1	Forward	CGCGTCAATGACTTCCACT
	Reverse	GCAGGTACGAGCGTCATTCT

EGFR, epidermal growth factor receptor; SLC19A1, solute carrier family 19 member 1.