

Supplementary Data

Supplementary Table 1S - Cardiovascular Malformations in Study Group*

CVM	Count	Percent
ASD	84	31.2%
VSD	116	43.1%
Atrioventricular Canal Septal Defects	50	18.6%
Dextrocardia/Mesocardia	75	27.9%
Double Inlet LV	19	7.1%
Double Outlet RV	62	23.1%
d-TGA	95	35.3%
l-TGA	52	19.1%
PAPVR	17	6.1%
TAPVR	34	12.6%
Pulmonary Atresia	53	19.7%
Pulmonic Stenosis	97	36.1%
Hypoplastic Left Heart/Left Obstructive Defects	64	23.8%
Interrupted IVC	47	17.5%
Persistent Left SVC	52	19.3%

*malformations are counted independently and more than one malformation typically was found in each subject.

ASD, atrial septal defect; d-TGA, dextro-transposition of the great arteries; IVC, inferior vena cava; l-TGA, levo-transposition of the great arteries; LV, left ventricle; PAPVR, partial anomalous pulmonary venous return; RV, right ventricle; SVC, superior vena cava; TAPVR, total anomalous pulmonary venous return.

Supplementary Table 2S - Synonymous nucleotide substitutions and common variants detected in affected cases

Amplicon	Nucleotide	Amino acid	Affecteds	Controls
2b	c.494G>A	R165H	165/269	ND
2c	c.588C>G	L196L	2/269	0/298
2c	c.612G>A	Q204Q	1/269	0/298
3	c.924C>T	V308V	1/269	ND

ND, not determined

Supplementary Table 3S - Primer pairs and PCR Conditions for Amplification of Human *NODAL*

Exon	5' Primer	3' Primer	Product size (BP)	A.T.	Additional reagents
1	CAGGATATAAGGGCTGGA	ACAGCACTTCCCGAGTCC	325	53 °C	BETAINE
2A	GAGCTATGGTGGTTGTGACT	GAAAAGGTGACCTGGGACAA	329	53 °C	NONE
2B	CTTGCCATTGAGATTTCCA	CGAGAGGTTGGAGTAGAGCATAA	235	51 °C	NONE
2C	ATGGTTTTGGAGGTGACCAG	CCATCCGATCAGGTTGAAGT	333	56 °C	NONE
2D	CAACTGTGTCGGAAGGTCAA	CCTGGTACATTGGAGGTGCT	285	60 °C	NONE
3	TTGCACTCAGGAAGTACTTTAAC	TCATGTCTTCCAGGAGTTCC	332	52 °C	BETAINE

A.T., recommended annealing temperature

Supplementary Table 4S - Primers for site-directed mutagenesis of NODAL

Mutation	cDNA change	Primers
E203K	607A>G	5' CTCCAACCTCTCGCAG A AGCAGAGGCAGCTGGG3' 5' CCCAGCTGCCTCTGCT T CTGCGAGAGGTTGGAG3'
G260R	778G>A	5' GACTTCAACCTGATC A AGATGGGGCTCCTGGAT3' 5' ATCCAGGAGCCCCATC T GATCAGGTTGAAGTC3'
R275C	823C>T	5' GCAGTACAACGCCTAT T GCTGTGAGGGCGAGTG3' 5' CACTCGCCCTCACAGC A AATAGGCGTTGTACTGC3'
V284F	850G>T	5' GCGAGTGTCTAATCCT T TTGGGGAGGAGTTTCATCC3' 5' GGATGAAACTCCTCCCC A AAGGATTAGGACACTCGC3'
24bp Del	700-723	5' GGGAGTGGGGCAAGGACAGAAGTCAACTGT3' 5' ACAGTTGACTTCTGTCCTTGCCCCACTCCC5'
9bp Ins	700-708	5' GGGAGTGGGGCAAG TTGACTTCC GACAGAAGTCAACTGT3' 5' ACAGTTGACTTCTGTC GGAAGTCAA CTTGCCCCACTCCC3'

Bold typeset indicates alterations introduced via site directed mutagenesis