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Supplemental Data

An Excess of Deleterious Variants in VEGF-A Pathway Genes in Down Syndrome-Associated Atrioventricular Septal Defects

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Table S1. Candidate-Gene List

Gene	Rationale for Inclusion	Function in Heart Development
ACVR1 (ALK2)	AVSD in mouse model,[1] genetic association with AVSD in humans.[2; 3]	Loss of ACVR1 prevents EMT in the AV endocardial cushions[1]
BMP2	AVSD in mouse model.[4] Required for SHF proliferation.[5; 6]	Modulates SHH-induced proliferation of the SHF.[5; 7] Control of AV cushion morphogenesis by regulating TBX2 and TBX3.[8]
BMP4	AVSD in mouse model.[9] Required for SHF proliferation.[10]	Loss of BMP4 prevents EMT in the AV endocardial cushions.[9] BMP4 signaling is critical to SHF specification upstream of Isl1.[11] BMP4 interacts with BMP2 in AV valve formation.[12; 13]
BMP5	Required for AV cushion formation.[14]	Expressed in the dorsal mesenchyme.[15]
CITED2	Reduced cell density in AV cushions.[16]	Transcriptional coactivator required for initiation of Nodal, controlling left-right patterning.[17]
COL18A1	Expressed in AV cushions.[18]	Facilitates EMT and mesenchymal cell migration in the AV endocardial cushions.[18]
COL6A1	Expressed in AV cushions. [19; 20]gene association studies[21-24]	Type VI collagen is a major component of the matrix in endocardial cushions.[19; 20]
COL6A2	Expressed in AV cushions. (Kitten, 1996; Klewer, 1998); Gene association studies[21-24]	Type VI collagen is a major component of the matrix in endocardial cushions.[19; 20]
COL6A3	Expressed in AV cushions.[19; 20]Gene association studies.[21-24]	Type VI collagen is a major component of the matrix in

		endocardial cushions.[19; 20]
CRELD1	Mutations in sporadic AVSD,[25] and previous study of AVSD in DS.[26]	Modulates VEGF-A signaling during AV cushion morphogenesis(C.L.M., unpublished data)
CRELD2	Association with *CRELD1*[27]	Possible antagonistic of CRELD1[27]
CTGF (CCN2)	Expressed in AV cushions.[28]	Functional redundancy with CYR61.[28]
CYR61(CCN1)	Haploinsufficiency of CYR61 results in AVSD in mouse model. *CYR61* maps to the human *AVSD1* locus on chromosome 1.[28]	Haploinsufficiency of CCN1 results in apoptosis in the AV junction at the stage of fusion between the endocardial cushion tissue and the atrial and ventricular septa.[28]
FBLN2	Maps to the *AVSD2* locus on human chromosome 3.[29]	Marker of EMT in AV cushions.[30]
FGF2	Involved in AV cushion remodeling.[31]	FGF2 stimulates growth and inhibits apoptosis in endocardial cushions.[31]
FRZB	Marker of EMT in AV cushions.[32]	FRZB is a modulator of Wnt-9A-medicated β-catenin signaling during AV endocardial cushion development.[33]
GATA4	Mutations in familial AVSD.[34]	Gata4 and Tbx5 interact during heart development, with complete AVSD in 100% of double heterozygous mice.[35]
GATA5	Expressed in AV cushions.[36]	GATA5 is a transcriptional regulator of AV valve morphogenesis.[36]
HEY2	Septal defects in mouse model.[37]	HEY2 controls developmental patterning of the AV canal by regulating BMP2.[38]
ROCK1	Needed for mesenchymal cell migration into AV cushions.[39]	Activates CTGF and TGFβ2, facilitating mesenchymal cell migration.[40]
SH3BGR	Expressed in AV cushions.[41] Association mapping in human AVSD.[42]	Unknown
SHH	Required for SHF,[43; 44] and development of DMP[45]	SHH signaling is required to specify atrial septal progenitor fate.[45]
TBX1	Required for proper alignment of AV canal,[46] and SHF proliferation.[47]	Regulates proliferation and differentiation of heart progenitors through the GATA4>MEF2c pathway.[48]
TBX20	Regulates AV endocardial cushion development.[5; 49]	Stage-specific effects on cardiomyocyte proliferation.[50]
VTN	Cardiac valve and septa development.[51]	Cell adhesion molecule. Binds CYR61, anchoring it to the ECM.[52]
WNT9A	Expressed in AV cushions[33]	Interacts with FRZB[33]

EMT, epithelial to mesenchymal transformation; SHF, secondary heart field; SHH, sonic hedgehog; ECM, extracellular matrix

Table S2. Comparison of Minor Allele Frequencies of 5′ UTR Variants in Cases and Controls

Gene	cDNA Position	Number of Cases	Number of Controls	*p* value
AVCR1	c.–53C>T	6	3	0.50
CITED2	c. –91G>A	4	2	0.68
CITED2	c. –52G>C	6	5	1.00
COL6A1	c. –3C>G	55	61	0.55
CTGF	c. –92C>G	6	7	1.00
FBLN2	c. –6T>C	41	28	0.10
FGF2	c. –71G>AC	1	3	0.62
FRZB	c. –117G>A	18	21	0.73
ROCK1	c. –895C>T	16	15	1.00
ROCK1	c. –428C>TG	2	2	1.00
ROCK1	c. –427C>T	1	3	0.62
ROCK1	c. –365C>G	1	1	1.00
ROCK1	c. –40G>A	2	1	1.00
SHH	c. –116G>A	13	12	1.00
TBX1	c. –85C>G	66	62	0.72

Table S3. Case-Specific Variants with Actionable Hypotheses—Whites Only

Gene	Variant	Frequency [a]	General Score (Confidence Level)[c]	Structure/Function Hypotheses (Probability scores)
COL6A1[c]	p.Val117Ala	2/141	0.776 (VC)	Loss of helix (p=0.0076), gain of loop (p=0.0079), loss of stability (p=0.0292), gain of disorder (p=0.0353), gain of ubiquitination at K121 (p=0.04441)
	p.Gln768His	1/141	0.537 (A)	Gain of sheet (p=0.0016), loss of helix (p=0.0017), gain of loop (p=0.024)
COL6A2[c]	p.Arg853Gln	1/141	0.869 (C)	Gain of ubiquitination at K851 (p=0.0354)
	p.Glu106Lys	5/141	0.759 (C)	Gain of methylation at E106 (p=0.0122)
CRELD1	p.Arg329Cys	2/135	0.860 (NP; validated)	NP; Biochemical analysis shows misfolding
	p.Glu414Lys	1/135	0.798 (VC)	Gain of methylation (p=0.016), Gain of MoRF binding (p=4e-04)
FBLN2	p.Ile1039Thr	1/141	0.696 (A)	Loss of stability (p=0.0211)
FRZB	p.Phe100Ser	1/141	0.543 (A)	Gain of disorder (p=0.0078)
GATA5	p.Gln3Arg	2/141	0.712 (C; validated)	Gain of MoRF[d] binding (p=8e-04), gain of methylation (p=0.0283). Transcription assay shows gain of function.
	p.Tyr142His	1/141	0.743 (A)	Gain of disorder (p=0.0409)

[a] Frequency is the number of individuals in which each variant was identified over the number of total cases resequenced for that gene.

[b] Confidence level: A, actionable hypotheses; C, confident hypotheses; VC, very confident hypotheses; NP, none predicted.

[c] gene is located on chromosome 21 (trisomic for this population)

[d] Gain of MoRF binding is gain of molecular recognition factor binding (interaction with other molecules enhanced).

Table S4. Control-Specific Variants with Actionable Hypotheses—Whites Only

Gene	Protein Variant	Frequency [a]	General Score (Confidence Level)[b]	Structure/Function Hypotheses (Probability scores)
COL6A3	p.Tyr727Ser	1/141	0.769 (C)	Gain of disorder (p=0.0248)
	p.Ala932Ser	1/141	0.515 (A)	Gain of disorder (p=0.0218)
COL18A1[c]	p.Pro1213Trp	1/141	0.610 (A)	Loss of methylation at R1213 (p=0.007)

[a] Frequency is the number of individuals in which each variant was identified over the number of total cases resequenced for that gene.

[b] Confidence level: A, actionable hypotheses; C, confident hypotheses; VC, very confident hypotheses; NP, none predicted.

[c] gene is located on chromosome 21 (trisomic for this population).

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