SUPPLEMENTAL MATERIAL

Supplementary Tables

Supplementary Table 1. All genes with shared rare mutations between WES affecteds and not shared by WES unaffecteds. Bold, implicated LVNC gene. Underline, known cardiomyopathy gene.

Family	Genes
A	CANT1, CHRNE, ERCC8, EVPL, EXOC7, FOXA2, GPR98, KIAA1324, MRPS15, MTG1, *MYH6*, NBPF3, PDE4DIP, RSPH4A, SLC25A23, TMC1, TOR1B, **TPM1**, TRIP10, USP8, ZNF280D, ZNF644
B	ANKRD36, CCDC150, CDC27, CNTNAP4, DCHS2, DNAH10, DRG1, MAL2, **MYH7**, OR11G2, PSAPL1, RP1L1, TRIL
C	A4GNT, AGBL5, ALPK2, BHMT2, C2orf16, COL5A3, CR2, FBXO4, FCRL2, FLG, FLG, FRRS1, GSDMC, MAP1S, MMP12, **NNT***, OR52W1, PNN, PSAP, RSPH3, TOR1AIP1, VPS41, ZNF180, ZNF181, ZNF229
D	ACOT4, AHI1, ANAPC2, ASXL3, ATP4B, BBX, BMP1, BTBD10, *CACNG2*, CADM4, CASZ1, CCDC158, CCDC66, CHID1, COL6A1, CPE, DCX, DMXL2, DNM1, DUPD1, EPPK1, FBRSL1, FCHO2, GFM2, GPC1, GPR176, GREB1, HAL, HCCS, HOXB2, HSP90B1, INPP5D, KIF26A, LAMA1, LSG1, MAP2, MAST3, MED16, MEX3A, MRPL39, MTHFD1, MTHFD1L, **MYH7**, N4BP2, NAP1L1, NAV1, NEDD4L, NOTCH3, OR52I2, OSMR, PARP1, PEPD, PLXNA4, PPIP5K2, PPP1R12C, PTH2R, SBF1, SCN11A, SCN7A, SEMA3G, SI, SLC16A3, SLC22A5, SLC45A2, SLC45A4, SMPD4, SPEF2, STRAP, TCEA1, TIMM44, TMEM180, TOP2A, TRIM59, UMOD, UNKL, WDFY4, WDR4, ZNF354B, ZNF451, ZNF646
E	ARSB, BRCA2, FAM38A, FRRS1, MAGI3, **MYH7**, NOVA1, RNF6, SLAIN1, TEP1

Supplementary Table 2. LVNC cohort data. LVNC cohort used for *NNT* sequencing. All cohort members were diagnosed with LVNC. Gender and additional phenotype information is listed. Samples used for *MYH7* sequencing are indicated, with any discovered *MYH7* mutations and any previous descriptions.

Patient ID#	Gender	Additional Phenotype	Used in *MYH7* Sequencing Study	*MYH7* Mutation(s)	Previous Description
L051	F	DISEASE ONSET-BIRTH. POST DELIVERY, AVA PRESENTED WITH PERSISTENT HYPOXIA AND RESPIRATORY DISTRESS, PULMONARY HYPERTENSION, HYPOTENSION AND WAS DIAGNOSED WITH LVNC AND VSD'S. DEVELOPED THROMBOSIS IN LEFT ATRIUM AND LEFT VENTRICLE.	1	N/A	
L100	F	LVNC, VENTRICULAR SEPTAL DEFECT, SMALL NORMAL LV SIZE, LVEF 47% BY MRI	1	p.Q163P	
L012	M	Present at 15 days age-cyanosis	1	N/A	
L030	F	Genetic Analysis for alpha- dystrophin	1	p.R1045H, p.T547A	Frisso et al. CLIN GENET 2009
L034	M	AGE OF DISEASE ONSET: 6 YEARS, Dyspnea, sporadic palpitations. NYHA class II. Echocardiogram was compatible with non-compaction cardiomyopathy, mitral stenosis and mild mitral regurgitation.	1	N/A	
L040	F	presented with cardiac arrest on day 7 of life. >25 of possible arrest with ischmicencepholopathy. Initial LV SF<15%-3 days later= he fxn but poss/prob noncompaction seen on	1	N/A	

		echo.			
L044	F	asymptomatic, identified by echocardiogram	1	N/A	
L046	F	age 13 so born in 1991;vheart failure	1	N/A	
L054	M	NON COMPACTION CM, HOCM	1	N/A	
L058	F		1	p.M531R	Kaneda et al. CS(L), 2008
L060	F		1	N/A	
L061	M		1	N/A	
L131	F	CARDIAC US AT AGES 1 YEAR AND 6 YEARS - NORMAL; AT AGE OF 8, SYSTOLIC MURMUR WAS HEARD AND LEFT ATRIUM WAS MILDLY ENLARGED; 6/2005, CATHETHRISATION AND IN THE APEX OF LEFT VENTRICLE TRABECULATIONS WERE SEEN, HAS ANTICOAGULANT MEDICATION	1	N/A	
L069	M		1	N/A	
L119	M	patient is proband	1	N/A	
L086	F	pronounced biventricular involvement. Died in 2002.	1	N/A	
L025	F	21 MONTHS, severe left ventricular dysfunction; isolated left ventricular non-compaction.	1	N/A	
L092	F	Symptoms include: heart failure, Dx of ventricular noncompaction	1	N/A	
L094	F	LV non-compaction ascertained 2003 (age 34). Neonatal VSD, unrepaired.	1	N/A	
L095	F	VSD, LV DYSFUNCTION	1	N/A	
L098	M	WPW, DCM, LVNC	1	N/A	

L099	M		1	N/A	
L102	M		0	N/A	
L104	F	DX LVNC, NO SYMPTOMS	0	N/A	
L109	M	Mitral Valve Prolapse	1	N/A	
L110	F	LVNC, HCM	1	N/A	
L111	M	LVNC, HCM	1	N/A	
L112	F		1	N/A	
L117			1	p.R904H	Waldmüller et al. EJHF , 2011
L122			1	N/A	
L123	M	DX LVNC, NO SYMPTOMS	1	N/A	
L127	M		1	p.E1350K	
L132	F		1	N/A	
L134	M	Brother is affected with DCM, parents are first cousins	1	N/A	
L135	M	mother died at age 37 of cardiomyopathy	1	N/A	
L137	F	Both siblings and mother affected with LVNC and HCM	1	p.L915P	
L139	F	Sister died at day 17 with LVNC	1	N/A	
L141	M		0	N/A	
L143	M		0	N/A	
L008	M	Dilated Cardiomyopathy and hypotonia	1	N/A	
L009	M	Present in infancy with cyanosis as a result of congenital heart disease.	1	N/A	
L010	M	Present in 1997 (6 years old) with acute myocarditis associated with bradycardia due to varying heart block.	1	N/A	
L011	M	Diagnosed in infancy with cyanotic congenital heart disease	1	N/A	
L014	M	Present at age 9 (July 01) with congestive failure	1	N/A	
L020	M		0	N/A	

L022	F	POST-PART. CM DX1990. NON-COMPACTION OF LV DX 1996.	1	p.L961P	L>R has been reported http://genepath.med.harvard.edu/seidman/cg3/muts/MYH7_mutations_TOC.html
L023	M	CHF. DCM WITH SPONG MYOCARDIUM AND VACTERL.	1	N/A	
L026	M	ISOLATED LVNC WITH HLHS	1	N/A	
L031	F	Diagnosed with LVNC following a sudden cardiac arrest and her daughters have been diagnosed with an asymptomatic form on echocardiogram	1	p.R243H	http://genepath.med.harvard.edu/seidman/cg3/muts/MYH7_mutations_TOC.html
L050	F		0	N/A	
L079	F	LVNC. 3 BEAT RUN OF VT	1	N/A	
L084	M		0	N/A	
L085	F	mother and daughter both have LVNC, found to have same pathology	1	N/A	
L087	F	CONGESTIVE HEART FAILURE AT 3 MONTHS OLD. PRESENTED WITH PULMONARY EDEMA.	1	N/A	
L121	M		0	N/A	
L129	M	INCREASED DYSPNEA AND LVH ON ECHOCARDIOGRAM. MRI SHOWS LVNC	1	N/A	
L130	M	Early puberty, Pediatrician heard cardiac click sent for echo, LV non-compaction found; subsequently dad had echo showing DCM	1	N/A	

Supplementary Table 3. Genes and rare variants identified in LVNC Family C that are shared between WES affecteds, but not shared by WES unaffected.

Gene name	Nucleotide: Amino acid Variant (heterozygous)	Nucleotide sequence	Mutation type	Human disease association (OMIM)	Mouse mutant phenotypes (MGI)
A4GNT	c.G803T: p.W268L	NM_016161	nonsynonymous		cardiovascular (increased angiogenesis in the gastric mucosa), cellular, digestive/alimentary, immune, tumorigenesis
AGBL5	c.791_794del: p.264_265del	NM_0218314	frameshift deletion		
ALPK2	c.C772T: p.R258C	NM_052947	nonsynonymous		
BHMT2	c.C565T: p.P189S	NM_017614	nonsynonymous		
C2orf16	c.C730G: p.R244G	NM_032266	nonsynonymous		
COL5A3	c.C1648T: p.R550C	NM_015719	nonsynonymous		adipose, cellular, endocrine/exocrine, growth/size, homeostasis, integument, muscle
CR2	c.G641A: p.R214H	NM_001877	nonsynonymous	Immunodeficiency, common variable, 7 [MIM614699]	hematopoietic, immune, mortality/aging
FBXO4	c.A676G: p.N226D	NM_012176	nonsynonymous		cellular, hematopoietic, immune, mortality/aging, tumorigenesis
FCRL2	c.G952T: p.E318X	NM_030764	nonsense		hematopoietic, immune
FLG	c.G4533C: p.R1511S	NM_002016	nonsynonymous	Ichthyosis vulgaris [MIM 146700]	cellular, craniofacial, growth/size, hearing/vestibular/ear, hematopoietic, homeostasis, immune, integument, mortality/aging
FLG	c.G2280C: p.Q760H	NM_002016	nonsynonymous		
FRRS1	c.T1292C: p.M431T	NM_001013660	nonsynonymous		mortality/aging
GSDMC	c.G590A: p.S197N	NM_031415	nonsynonymous		behavior, craniofacial, digestive/alimentary, growth/size, hearing/vestibular/ear, homeostasis, integument, mortality/aging, respiratory

MAP1S	c.C128G: p.S43C	NM_018174	nonsynonymous		cardiovascular (cardiomyocytes in neonates and adult exhibit a 3-fold increase in dysfunctional mitochondria compared with wild-type mice), cellular, muscle
MMP12	c.G697T: p.A233S,	uc001phk.3	nonsynonymous		hematopoietic, homeostasis, immune, nervous system, reproductive
NNT*	c.638_639insT: p.R213fs	NM_012343	frameshift deletion	Glucocorticoid deficiency 4 [MIM614736]	cellular, endocrine/exocrine, homeostasis, cardiovascular (lower LV shortening and increased LV weight, higher aortic ejection time)
OR52W1	c.G923A: p.R308Q	NM_00100517	nonsynonymous		
PNN	c.A1264G: p.S422G	NM_002687	nonsynonymous		embryogenesis, mortality/aging, vision/eye
PSAP	c.C409G: p.L137V	NM_001042465V	nonsynonymous	Combined SAP deficiency [MIM611721] Gaucher disease, atypical [610539] Krabbe disease, atypical [MIM611722] Metachromatic leukodystrophy due to SAP-b deficiency [MIM249900]	behavior, growth/size, hematopoietic, homeostasis, immune, mortality/aging, nervous system, reproductive, skeleton
RSPH3	c.C1126T: p.R376W	NM_031924	nonsynonymous		
TOR1AIP1	c.T983G: p.I328S	NM_015602	nonsynonymous		cellular, mortality/aging
VPS41	c.C881T: p.T294M	NM_014396	nonsynonymous		cellular, embryogenesis, mortality/aging
ZNF180	c.A1513G: p.S505G	NM_013256	nonsynonymous		
ZNF181	c.A352G: p.K118E	NM_001029997	nonsynonymous		

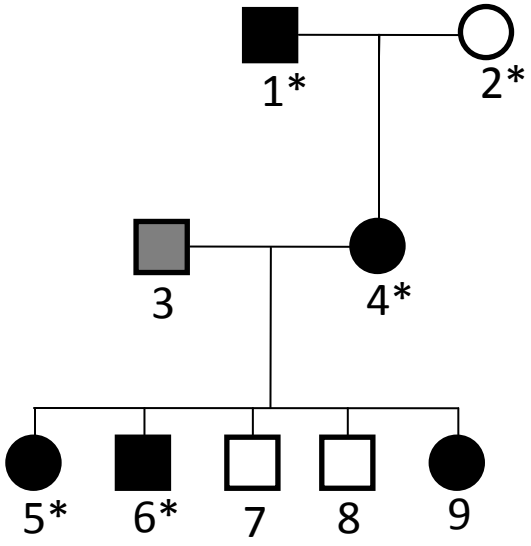
ZNF229	c.G1537C: p.G513R	NM_014518	nonsynonymous		

Supplementary Table 4. Morpholinos used for *in vivo* functional assays. Relationship of the variants identified in LVNC family C to the zebrafish morpholinos (MO)s used for assays of physiological relevance and variant pathogenicity. *Variants in *HACL1, NAV2, RAB27A,* and *RBM28* are present in <0.01% of controls (Exome Variant Server), shared by the affected individuals but do not segregate fully with disease in the pedigree. ***notch1a* MO was published previously[1].

Human gene	Variant in LVNC Family C*	Zebrafish gene	MO sequence
NNT	c.638_639insT p.R213fs	*nnt-a*	ACCATTGTCAGAGGACTTACCCAGC
		nnt-b	GTAAGTGTGGATGTTTCACCTTCGC
HACL1	c.G1036T p.E346X	*hacl1*	GTTGAAATATTCATACCGTCCAGTC
NAV2	c.C3071G p.S1024W	*nav2*	TTTTGAAGTGTTTTTACCTTCAGCT
RAB27A	c.C518G p.A173G	*rab27a*	AAGGATTTTCTTACCCGTATTTCTC
RBM28	c.G1488C p.K496N	*rbm28*	ACTTGGTCAAACTTTTACCTGGTTT
NOTCH1	Not applicable	*notch1a***	GAAACGGTTCATAACTCCGCCTCGG

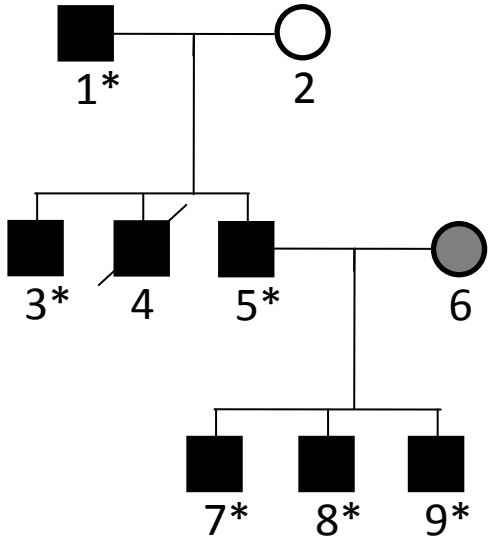
Supplementary Figures

Supplementary Figure 1A. Pedigree, sequencing platform and sequence coverage for each family **A.** LVNC status for each individual is given: Black (affected), white (unaffected), gray (unknown status), * indicates subject was capture sequenced.



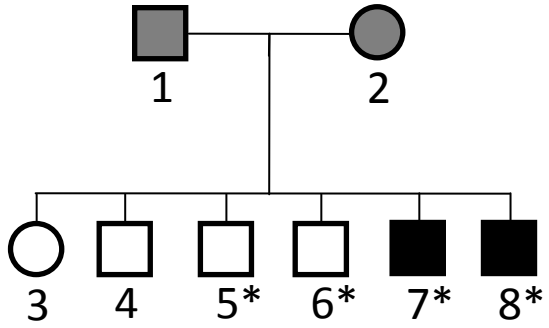
ID	Platform	Gbp	Avg Cov	% 10x
1	SOLiDv4	6.9	26	64.4
2	SOLiDv4	6.8	26	64.4
4	SOLiDv4	7.1	26	64.5
5	SOLiDv4	6.6	24	61.4
6	SOLiDv4	6.7	25	64.0

Supplementary Figure 1B. Pedigree, sequencing platform and sequence coverage for family **B.**



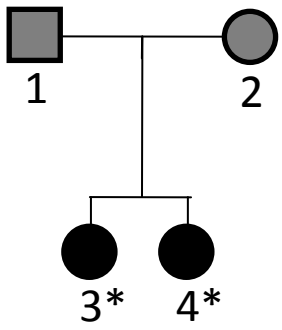
ID	Platform	Gbp	Avg Cov	% 10x
1	Illumina	16.3	195	96.2
3	Illumina	10.2	128	95.0
7	Illumina	16.3	184	94.1
8	Illumina	12.0	153	95.7
9	Illumina	11.3	142	95.8

Supplementary Figure 1C. Pedigree, sequencing platform and sequence coverage for family **C.**



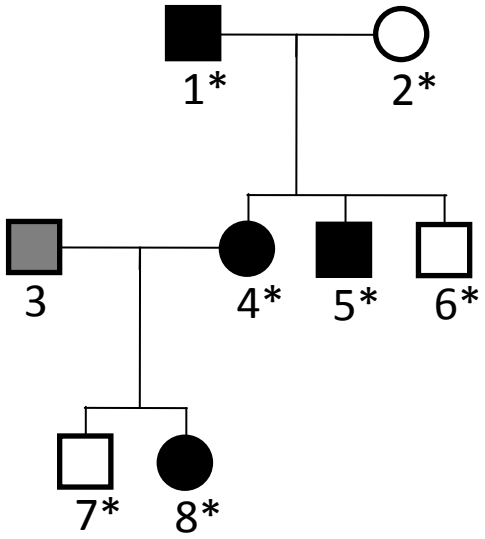
ID	Platform	Gbp	Avg Cov	% 10x
5	Illumina	8.3	103	84.0
6	Illumina	8.4	105	88.3
7	Illumina	6.0	70	74.3
8	Illumina	7.1	84	78.0

Supplementary Figure 1D. Pedigree, sequencing platform and sequence coverage for family **D.**



ID	Platform	Gbp	Avg Cov	% 10x
3	SOLiDv4	7.3	28	67.6
4	SOLiDv4	7.6	27	66.9

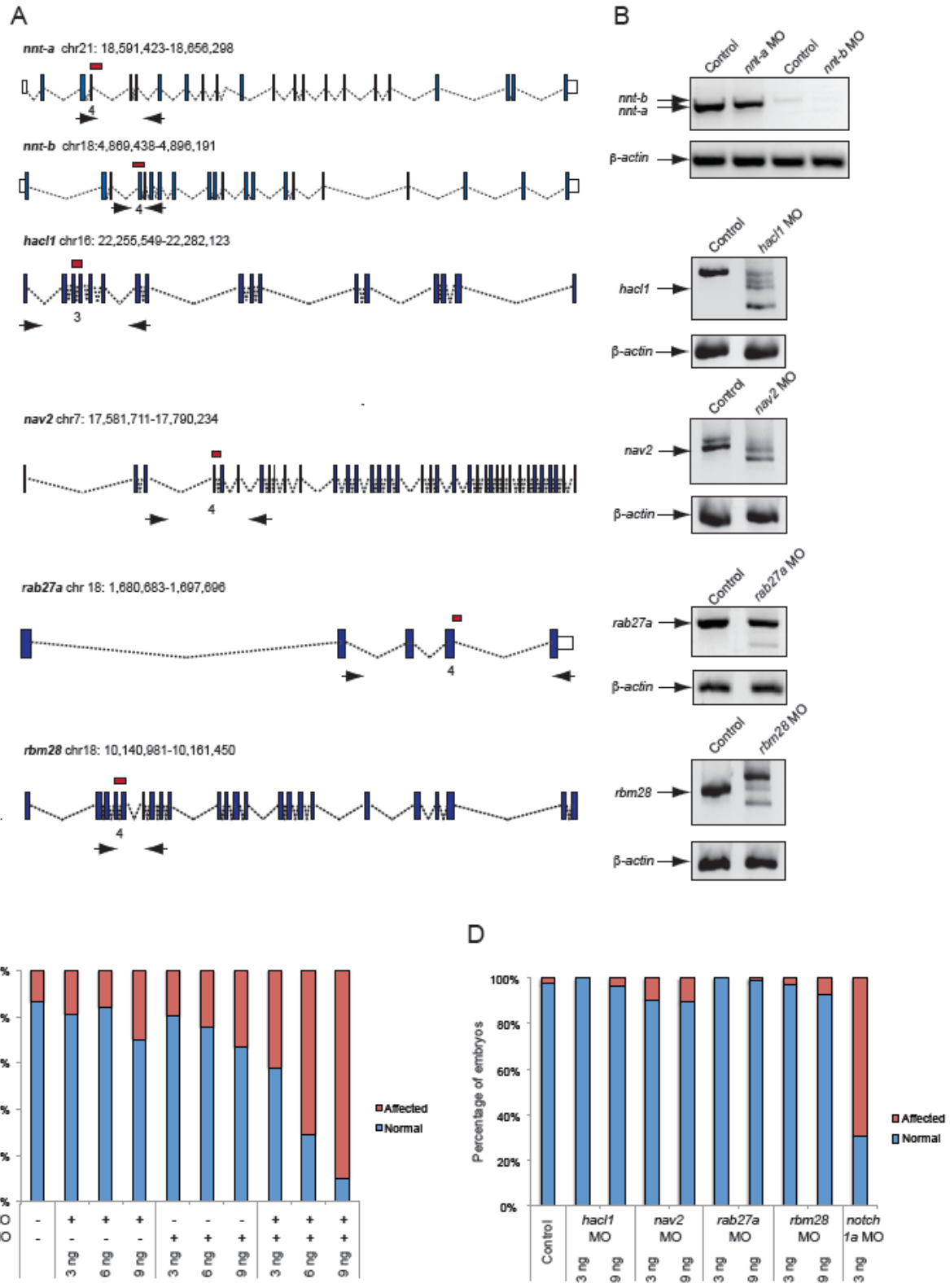
Supplementary Figure 1D. Pedigree, sequencing platform and sequence coverage for family **D.**



ID	Platform	Gbp	Avg Cov	% 10x
1	SOLiDv4	13.4	82	83.6
2	SOLiDv4	12.8	83	84.3
4	SOLiDv4	11.7	43	72.3
5	SOLiDv4	12.7	78	82.3
6	SOLiDv4	12.1	74	81.2
7	SOLiDv4	13.3	79	78.8
8	SOLiDv4	7.3	40	74.7

Supplementary Figure 2. Knockdown efficiency of morpholinos

A. Schematic depicts the targeted *D. rerio* locus for each gene under investigation; white boxes, untranslated regions; blue boxes, coding exons; red box, morpholino (MO) target site; arrows indicate the position of RT-PCR primers (see B). B. Agarose gel images of *nnt-a*, *nnt-b, hacl1, nav2, rab27a,* and *rbm28* RT-PCR products show reduced (*nnt-a*, *nnt-b*) or alternatively spliced (*hacl1, nav2, rab27a, rbm28)* transcript in MO-injected embryos. *β-actin* is shown as a loading control. C. Quantification of the effects of single or double *nnt-a* and *nnt-b* MO injection at increasing concentrations; cardiac edema formation is used as a phenotypic readout for larval batches embryos scored at 3dpf. Comparisons of equivalent doses for each of *nnt-a* or *nnt-b* MOs alone are not significant (3ng, p=0.13; 6ng, p=0.17, 9ng, p=0.52). D. Quantification of control gene MO injection; cardiac edema formation is used as a phenotypic readout for larval batches scored at 5dpf. Negative control genes (harboring variants shared between the affected individuals in family C but not segregating with disease) show no significant phenotype; *notch1a*, implicated previously in cardiac valve development shows marked cardiac edema. n=50-100 embryos/injection batch, repeated twice with masked scoring.


A
nnt-a chr21: 18,591,423-18,656,298
nnt-b chr18:4,869,438-4,896,191
hacl1 chr16: 22,255,549-22,282,123
nav2 chr7: 17,581,711-17,790,234
rab27a chr 18: 1,680,683-1,697,696
rbm28 chr18: 10,140,981-10,161,450
B
Control
nnt-a MO
nnt-b MO
nnt-b
nnt-a
β-actin
hacl1 MO
hacl1
nav2 MO
nav2
rab27a MO
rab27a
rbm28 MO
rbm28
C
Percentage of embryos
100%
80%
60%
40%
20%
0%
nnt-a MO
nnt-b MO
3 ng
6 ng
9 ng
Affected
Normal
D
Control
hacl1 MO
nav2 MO
rab27a MO
rbm28 MO
notch 1a MO

Supplementary Movies

Supplementary Movie 1. Fluorescent video imaging of ventrally positioned 2 dpf control *cmlc2:GFP* larva. Imaging was acquired at 8.77 frames/second at 12x magnification; anterior structures, left; atrium, top chamber; ventricle, bottom chamber.

Supplementary Movie 2. Fluorescent video imaging of ventrally positioned 2 dpf *nnt-a/nnt-b* morphant *cmlc2:GFP* larva. Imaging was acquired at 8.77 frames/second at 12x magnification; anterior structures, left; atrium, top chamber; ventricle, bottom chamber.

References

1. Yeo S-Y, Kim M, Kim H-S, Huh T-L, Chitnis AB. Fluorescent protein expression driven by her4 regulatory elements reveals the spatiotemporal pattern of Notch signaling in the nervous system of zebrafish embryos. *Dev Biol*. 2007;301:555–567.