

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Morita H, Rehm HL, Menesses A, et al. Shared genetic causes of cardiac hypertrophy in children and adults. *N Engl J Med* 2008;358:1899-908. DOI: 10.1056/NEJMoa075463.

**Supplemental Table 1. Race and Ethnicity Categories**

<b>Subject ID:</b>		
<b>Please Check <u>all</u> Categories that Describe Your Race &amp; Ethnicity</b>		
<b>Ethnicity</b>	<b>Yes</b>	<b>No</b>
Hispanic or Latino		
<b>Race</b>	<b>Yes</b>	<b>No</b>
American Indian/Alaska Native		
Asian		
Native Hawaiian or Other Pacific Islander		
Black or African American		
White		

**Ethnic Categories:**

Hispanic or Latino: A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race.

**Racial Categories:**

American Indian or Alaska Native: A person having origins in any of the original peoples of North, Central, or South America, and who maintains tribal affiliation or community attachment.

Asian: A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam.

Black or African American: A person having origins in any of the black racial groups of Africa.

Native Hawaiian or Other Pacific Islander: A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific Islands.

White: A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

**Supplemental Table 2. Primer sets and restriction enzymes**

Gene	Mutation	Forward primer (5'-3')	Reverse primer (5'-3')	Restriction enzyme
<i>MYH7</i>	Lys146Asn	ATCTTTCTCTAACTCCCAAATCA	ACTCACGTGATCAGGATGGACTGG	-Ear I
<i>MYH7</i>	Val606Met	ATAACTGTACTCAGAGCTGAGCCTA	TCCATCCCCTGAGTCTGTAAACCT	+Nla III
<i>MYH7</i>	Arg663His	CATCTCTGTGACTTCTCGAATTCT	CACTGTGGTGGTAGGTAGGGAGAT	-Hha I
<i>MYH7</i>	Arg719Gln	ACAAAGCCAGGATCAGAACCCAGA	GTCCAGAGTCACCCATGCTCTGCA	-Msp I
<i>MYH7</i>	Val763Met	TCCCTCGTACCCCTCCCTAGTCATGGCCAACA CACACCTTGCACGCAG	CCAGCCTGGGCCTCAGAGAAGCGG	-Ale I
<i>MYH7</i>	Arg787Cys	TAGGCTGTTACCCTTCCTAAGGTA	GCCTCTGACCCTGTGACTGCAGTG	+Pml I
<i>MYH7</i>	Leu908Val	AGAGGAAGAGGTCCAGATGAAGAT	TGTTTCTCCTTCTCCACTTTGGCC	-Pvu II
<i>MYH7</i>	Glu924Lys	TCAGCTGGAGGCTAAGGTGAAGGAGATGCTC	CCTTACCTTGTTCTCTGTTGCGTG	-Xho I
<i>MYH7</i>	Leu1414Met	CAGAGGGTGCCTGGGTCTCCACGC	AGGGCTGCAGCAGCAGCATTGGA	+Taq $\alpha$ I
<i>MYBPC3</i>	Gly278Glu	TGGGAAAGGCTGGGAAGGTGAGAT	GCAGGGGTACCTGATCCGCCGACGACgT	-Aat II
<i>MYBPC3</i>	Gly490Arg	ACCCACCCGGCTAGGCCCTAGGAC	AGAAGGATGAGGTTTAGGCT	+EcoN I
<i>MYBPC3</i>	Arg495Gly	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Sma I
<i>MYBPC3</i>	Arg502Trp	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Age I
<i>MYBPC3</i>	Arg502Gln	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Age I
<i>MYBPC3</i>	Asp605Asn	AGAATACCAACAAGCCAGGACAAG	GCCCCAGGACCCCAATTTTGAT	-Aat II
<i>MYBPC3</i>	Arg943Stop	TCAGAGGAGTGGGCAGTGGGAGTG	TCCTGTCTCTGCCAGCGTTCT	+Dde I
<i>MYBPC3</i>	Thr1028Ser	GCGAGGAGGTGAGCATCCGCAACAGCCCCAC AGACT	CAGCCCAGCCCAGGGAAGGGAAAC	-Hinf I
<i>MYBPC3</i>	IVS31+2t→g	TGACTGACGCCTGGGGTCTTAATGT	CAGTGAAGGGTAGCTGCGGCCTGG	-Hph I
<i>MYBPC3</i>	Gly1248Arg	TTGGTTCCATGTTTGTTCAGCCT	TGGAAGCTATTGCCATCTGGGCGTG	+EcoN I
<i>TNNT2</i>	Arg92Gln	AGCACAGCCAGGCTGGGTGCCCAT	AGGCGCCGAGGAAGGCTGTCTGGA	-Msp I
<i>TNNT2</i>	Glu96del	GGTCCACCCACAGGACATCCACCGGAAGCGC cTG	GGAGGTGGGGCCTCACAAAAGGGA	-Bpm I
<i>TNNI3</i>	Lys178del	ACCCTGCGGAGAGTGAGGATCTC	CATTTCTGAGGACCCCTTACT	-Mbo II
<i>ACTC</i>	His90Tyr	TTCTCATAGGGAGTTATGGTGGGT	CAACTGGGGGATCTGATTCACAGC	-Ban I
<i>ACTC</i>	Arg97Cys	GAGAAGATCTGGCACCACACCTTCTACAATGA GGCC	CAACTGGGGGATCTGATTCACAGC	+Stu I
<i>PRKAG2</i>	His530Arg	GAGATGATGTCTCACTGCTATTCC	TCTATACACTTTCCTCACTCACG	+Hpy99 I

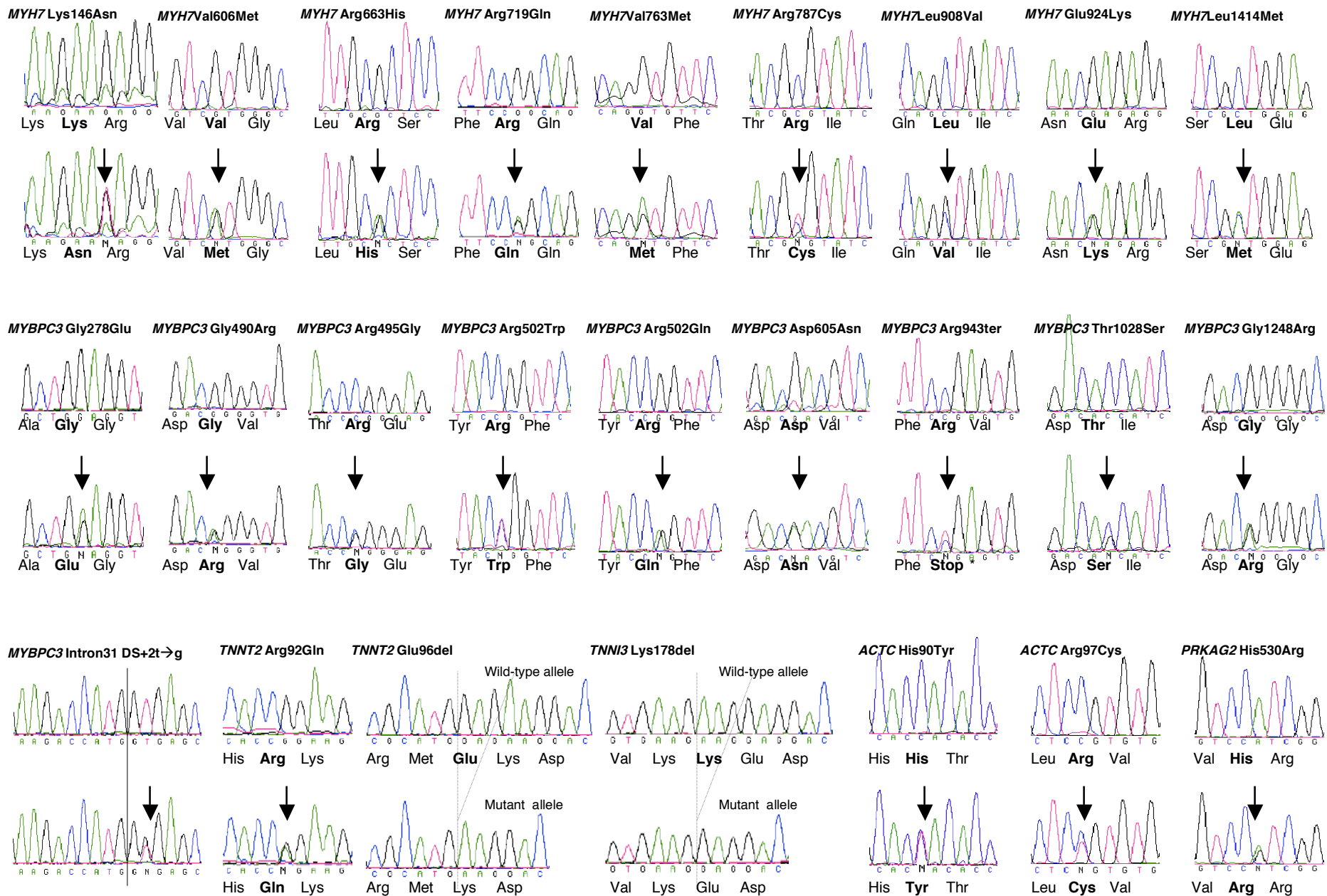
Lower letters indicate the mismatch bases; +, mutation creates the restriction site; -, mutation abolishes the restriction site.

**Supplemental Table 3. Gene Mutations in Childhood-onset Cases with Familial LVH**

<b>Gene</b>	<b>Designation</b>	<b>Consequence</b>	<b>Charge</b>	<b>Reported</b>
<i>MYH7</i>	Arg403Gln*	Missense	-1	Y
<i>MYH7</i>	Arg453Cys	Missense	-1	Y
<i>MYH7</i>	Gly716Arg	Missense	+1	Y
<i>MYH7</i>	Gly741Trp	Missense	0	Y
<i>MYH7</i>	Met852Thr	Missense	0	Y
<i>MYH7</i>	Glu903Gly	Missense	+1	N
<i>MYH7</i>	Leu908Val*	Missense	0	Y
<i>MYH7</i>	Arg1712Gln	Missense	-1	Y
<i>MYH7</i>	Ser1836Leu	Missense	0	N
<i>MYBPC3</i>	Ile154Thr	Missense	0	N
<i>MYBPC3</i>	Glu258Lys	Missense	+2	Y
<i>MYBPC3</i>	IVS14-2a→g	Splice →Truncation	..	Y
<i>MYBPC3</i>	Arg495Gln	Missense	-1	Y
<i>MYBPC3</i>	Arg502Trp	Missense	-1	Y
<i>MYBPC3</i>	Asp605del	Deletion of codon 605	+1	N
<i>MYBPC3</i>	Ser858Asn	Missense	0	N
<i>TNNT2</i>	Arg141Trp	Missense	-1	Y
<i>TNNT2</i>	Glu163del	Deletion of codon 163	+1	Y
<i>TNNI3</i>	Arg141Gln	Missense	-1	Y
<i>TPM1</i>	Asp175Asn	Missense	+1	Y
<i>TPM1</i>	Ser215Leu	Missense	0	N
<i>MYL3</i>	Met173Val	Missense	0	N

Designation, mutations are denoted by normally encoded amino acid, residue number, substituted amino acid or termination signal (ter) or altered splice signal (IVS); Consequence, mutational effects on protein; Charge, altered charge by mutant amino acid change; Reported. HCM mutations previously described-yes (Y) or no (N) . Mutations identified in 2 (\*) unrelated cases. Three cases had compound mutations: *MYBPC3*Arg502Trp / *MYBPC3*Ser858Asn, *MYBPC3*Arg495Gln / *TNNI3*Arg141Gln, *MYBPC3*Ile154Thr / *MYBPC3*Asp605del.

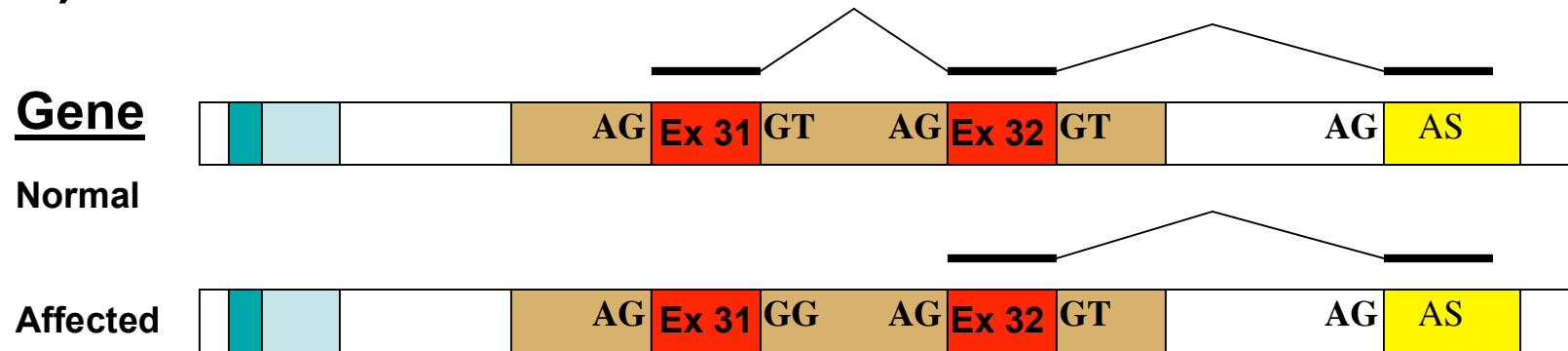
# Supplemental Figure 1



**Supplemental Figure 1 Legend.** Sequence analyses of genomic DNA from 25 variants;  $\beta$ -cardiac myosin heavy chain (*MYH7*) Lys146Asn, Val606Met, Arg663His, Arg719Gln, Val763Met, Arg787Cys, Leu908Val, Glu924Lys, Leu1414Met; cardiac myosin binding protein-C (*MYBPC3*) Gly278Glu, Gly490Arg, Arg495Gly, Arg502Trp/Gln, Asp605Asn, Arg943Stop, Thr1028Ser, Gly1248Arg, IVS31+2t→g; cardiac troponin T (*TNNT2*) Arg92Gln, Glu96del; cardiac troponin I (*TNNI3*) Lys178del; cardiac actin (*ACTC*) His90Tyr, Arg97Cys and  $\gamma$ 2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*) His530Arg. *TNNT2* Glu96del and *TNNI3* Lys178del were detected after wildtype allele and mutant alleles were subcloned and sequenced. Arrows indicate substituted nucleotides; altered residues are bolded.

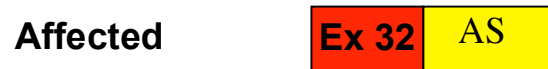
# Supplemental Figure 2

**A)**



**B)**

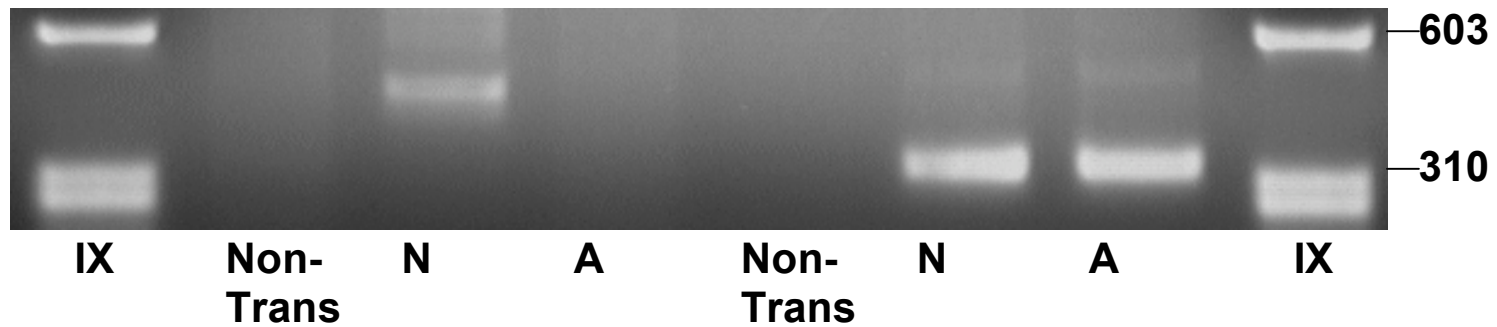
**RNA**



**C)**

**E31F & SA2**

**E32F & SA2**



**Supplemental Figure 2 Legend.** Assessment of splicing site mutations. Using the exon trapping vector pSPL3, the effect *MYBPC3* IVS31+2t>g variant on splicing was tested. Vectors containing the wildtype or mutant genomic fragment (A) were transfected in COS-7 cells. Splicing was assessed using forward PCR primers corresponding to *MYBPC3* exon 31 (E31F) and exon 32 (E32F), respectively (B). Using primers E32F and SA2, a 328 bp-PCR product, which included *MYBPC3* exon32, was amplified from cells transfected with cDNA derived from wildtype or mutant genomic fragments. Primers E31F and SA2 amplified a 467 bp-PCR product, which included *MYBPC3* exons 31 and 32, that were amplified only from cells transfected with cDNA derived from wildtype genomic fragments (C). Nucleotide sequences of each PCR product were confirmed by direct sequencing. Note that processed mRNA from cells transfected with cDNA derived from mutant genomic fragments did not contain the normal *MYBPC3* exon 31 nucleotide sequences, indicating that *MYBPC3* IVS31+2t>g variant caused splicing abnormalities.



# Supplemental Figure 3

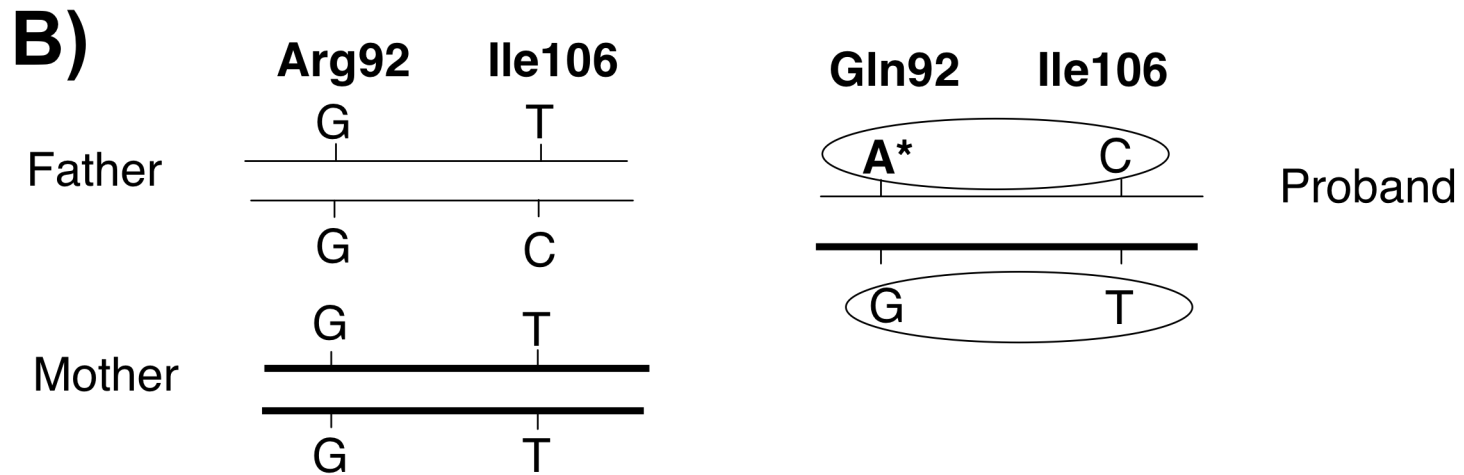
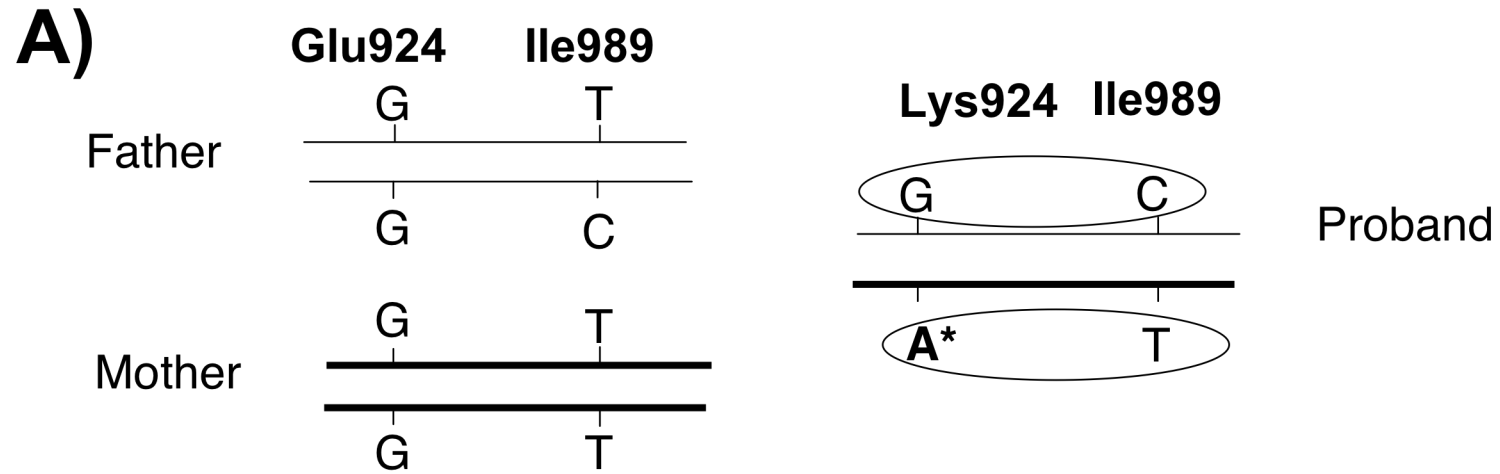
	146	606	663	719	763	787	908	924	1414
Subject	***N***	***M***	***H***	***Q***	***M***	***C***	***V***	***K***	***M***
Human <i>MYH7</i>	YRGGKRSEA	NETVVGLYQ	MTNLRSTHP	YGDFRQRYR	GHTKVFFKA	RI ITR IQAQ	RCDQL IKNK	KEMNERLED	KCSSLEKTK
Rabbit Cardiac	-----	***A***	*****	*****	*****	*****	*****	*****	-----
$\beta$ Human Cardiac	*****	*****	***T***	*****	*****	*****	*****	*****	*****
$\alpha$ Rat Cardiac $\alpha$	*****	*****	***T***	*****	*****	*****	*****	***T***	*****
Mouse Cardiac $\alpha$	*****	*****	***KT***	*****	*****	*****	*****	***T***	*****
Chicken atrial	*****T*V	*****	*A*KT**	*****	*****	L*****	*****	***T***E	*****
Human Skeletal IIa	*****Q**	*****	*****	*A*K**K	*****	QL**T**R	*****T*	**VT**A**	**A*****
Mouse Skeletal 2A	*****Q**	*****	*****	*A*K**K	*****	QL**T**M	*****T*	**VT**A**	**A*****
Chicken Sk. Fast	*****Q**	***I***	*A*****	*A*K**K	*****	QL**T**R	*****T*	**VT**A**	**A*****
Xenopus Skeletal	*****Q**	*D*I**F*	*S**T***	***K**K	*****	HV**T**M	*****S*	*L*****	**A*****
Human Perinatal	*****Q**	*D*****	*****	***K**K	*****	Q**T**V	**E*****	**VT**A**E	**A*****
Mouse Perinatal	*****Q**	*D*****	*****	***K**K	*****	Q**T**V	**E*****	**VT**A**	**A*****

	278	490	495	502	605	1028	1248
Subject	***E***	***R***	***G***	*W****	***N***	***S***	***R***
Human <i>MYBPC3</i>	TSLAGG GRR	WLKDGVELTREETFK	YRFKGD	LTIDDVTPA	SPTDTILFI	CPFDGGIYV	***Y***V**
Mouse Cardiac	***A***	*****	*****	*****	*****	*****	*****
Chicken Cardiac	***A***	*E*****	*****	***E**G	*S*****	T****F*T	*****
Xenopus Cardiac	**V*AAK**	*E*****	*****	***V*Q	*EA*S***	S*****V*T	*****
Human Sk. Fast	-----	*M*****DS*AR*****	*****	*V***R*E	*DF**VF*V	S***A*T*T	*****
Mouse Sk. Fast	-----	*M***M**DSY*AR*****	*****	*V***R*E	*DF**VF*V	S***A*T*S	*****
Chicken Sk. Fast	-----	*D**V**DDA**	*****	*V***R*E	*DV*SVF**	G****T*G	*****

	92	96	Subject	178	Subject	90	97	Subject	530
Subject	***Q*** $\Delta$ ***	*** $\Delta$ ***	Subject	*** $\Delta$ ***	Subject	***Y***	***C***	Subject	***R***
Human <i>TNNT2</i>	DDIHRKRM $\Delta$ EKDLN	KQV	Human <i>TNNI3</i>	KKEDTE	Human <i>ACTC</i>	KIWHHTFYNELRVAPE	*****	Human <i>PRKAG2</i>	RAEVHRLVV
Rabbit Cardiac	*****	***	Rabbit Cardiac	*****	Rat Cardiac	*****	*****	Mouse Prkag2	*****
Rat Cardiac	*****	***I*	Rat Cardiac	***I*	Rat Cardiac	*****	*****	Human PRKAG1	E*****
Mouse Cardiac	*****V*****	***I*	Mouse Cardiac	***I*	Chicken Cardiac	*****	*****	Bovine Prkag1	E*****
Chicken Cardiac	*****	R**	Chicken Cardiac	R*D*A*	Xenopus Cardiac	*****	*****	Mouse Prkag1	E*****
Xenopus Cardiac	*****T	***KQT**D*AD	Xenopus Cardiac	***KQT**D*AD	Zebrafish Cardiac	*****	*****	Human PRKAG3	*EQ*****L
Human Sk. Fast	**QK**QN**M	***	Human Sk. Fast	***	Human Skeletal	*****	*****	Pig Prkag3	*EQ*****L
Mouse Sk. Fast	**QK**QN**M	***	Mouse Sk. Fast	***	Mouse Skeletal	*****	*****	Drosophila Snf4	*****
Chicken Sk. Fast	**QK**QN**I	***	Chicken Sk. Fast	***	Xenopus Skeletal	*****	*****	S. cerevisiae Snf4	K*R**FF*
Human Sk. Slow	*****L	***S*	Human Sk. Slow	*S*	Zebrafish Skeletal	*****	*****		
Mouse Sk. Slow	*****L	***S*	Mouse Sk. Slow	*S*	Human Smooth Muscle	*****S	*****		
Chicken Sk. Slow	*****L	***S*	Chicken Sk. Slow	*S*	Mouse Smooth Muscle	*****S	*****		

**Supplemental Figure 3 Legend.** Conservation of amino acid residues altered by missense or deletion mutations in presumed sporadic cases of childhood-onset LVH.  $\beta$ -cardiac myosin heavy chain (*MYH7*) Lys146Asn, Val606Met, Arg663His, Arg719Gln, Val763Met, Arg787Cys, Leu908Val, Glu924Lys, Leu1414Met; cardiac myosin binding protein-C (*MYBPC3*) Gly278Glu, Gly490Arg, Arg495Gly, Arg502Trp/Gln, Asp605Asn, Thr1028Ser, Gly1248Arg; cardiac troponin T (*TNNT2*) Arg92Gln, Glu96del; cardiac troponin I (*TNNI3*) Lys178del; cardiac actin (*ACTC*) His90Tyr, Arg97Cys and  $\gamma$ 2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*) His530Arg. The altered residue and eight flanking amino acid residues are displayed in single letter code.

# Supplemental Figure 4



**Supplemental Figure 4 Legend.** Parental origin of the chromosomes containing *de novo* mutations. (A) The region flanking nucleotides corresponding to Glu924 and Ile989 (rs 7157716) in *MYH7* was amplified followed by subcloning using TOPO TA cloning kit (Invitrogen). The *de novo* mutation *MYH7* Glu924Lys (starred) was found the chromosome of maternal origin. (B) The region flanking nucleotides corresponding to Arg92 and Ile106 (rs 3729547) in *TNNT2* was amplified followed by subcloning using TOPO TA cloning kit (Invitrogen). The *de novo* mutation *TNNT2* Arg92Gln (starred) was found on the chromosome of the paternal origin.

# Supplemental Figure 5

	903	1836
Subject	***G***	***L***
Human <i>MYH7</i>	ADAEERCDDQ	RNAESVKGM
Human Cardiac	N*****	*****
$\alpha$ Rat Cardiac $\alpha$	*****	*****
Mouse Cardiac $\alpha$	N*****	*****
Chicken atrial	*****	***I**L
Human Skeletal IIa	*****	***A**L
Mouse Skeletal 2A	*****	***A**L
Chicken Sk. Fast	*****	*S**A**V
Xenopus Skeletal	T*****	*ST**A**V
Human Perinatal	*****E*	***A**L
Mouse Perinatal	*****E*	***A**L

	154	605	858
Subject	***T***	***A***	***N***
Human <i>MYBPC3</i>	PDDP IGLFV	LTIDDVTPA	MSRPS PASQ
Mouse Cardiac	*****L	*****	*****
Chicken Cardiac	*V*****	***E***G	*****
Xenopus Cardiac	*K*E***L	*****V*Q	**I**QH**
Human Sk. Fast	-----	*V***R*E	V*Q**MNTK
Mouse Sk. Fast	-----	*V***R*E	V*Q**MNTK
Chicken Sk. Fast	-----	*V***R*E	V*Q**LNT*

	215
Subject	***L***
Human <i>TPMI</i>	AEKYSQKED
Rat $\alpha$	*****
Mouse $\alpha$	*****
Chicken $\alpha$	*****
Xenopus $\alpha$	*****

	173
Subject	***V***
Human <i>MYL3</i>	VEKLMAGQE
Rat Ventricular	*****
Mouse Ventricular	*****
Chicken Ventricular	*D*****
Human Atrial/Fetal	**Q*L***
Rat Atrial/Fetal	**Q*LT**
Mouse Atrial/Fetal	**Q*LS**
Chicken Atrial/Embryonic	**Q***L*

**Supplemental Figure 5 Legend.** Conservation of amino acid residues altered by novel missense or deletion mutations in familial cases of childhood-onset LVH.  $\beta$ -cardiac myosin heavy chain (*MYH7*) Glu903Gly, Ser1836Leu; cardiac myosin binding protein-C (*MYBPC3*) Ile154Thr, Asp605del, Ser858Asn,  $\alpha$ -tropomyosin (*TPMI*) Ser215Leu, essential light chain (*MYL3*) Met173Val.