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

Subject ID:		
Please Check all Categories that Describe Your Race & Ethnicity		
Ethnicity	**Yes**	**No**
Hispanic or Latino		
Race	**Yes**	**No**
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
MYH7	Lys146Asn	ATCTTTCTCTAACTCCCAAAATCA	ACTCACGTGATCAGGATGGACTGG	-Ear I
MYH7	Val606Met	ATAACTGTACTCAGAGCTGAGCCTA	TCCATCCCACTGAGTCTGTAAACCT	+Nla III
MYH7	Arg663His	CATCTCTGTGACTTCTCGAATTCT	CACTGTGGTGGTAGGTAGGGAGAT	-Hha I
MYH7	Arg719Gln	ACAAAGCCAGGATCAGAACCCAGA	GTCCAGAGTCACCCATGCTCTGCA	-Msp I
MYH7	Val763Met	TCCCTCGTACCCCTCCCTAGTCATGGCCAACA CACACCTTGCACGCAG	CCAGCCTGGGCCTCAGAGAAGCGG	-Ale I
MYH7	Arg787Cys	TAGGCTGTTACCCTTCCTAAGGTA	GCCTCTGACCCTGTGACTGCAGTG	+Pml I
MYH7	Leu908Val	AGAGGAAGAGGTCCAGATGAAGAT	TGTTTCTCCTTCTCCACTTTGGCC	-Pvu II
MYH7	Glu924Lys	TCAGCTGGAGGCTAAGGTGAAGGAGATGCTC	CCTTACCTTGTTCTCTGTTGCGTG	-Xho I
MYH7	Leu1414Met	CAGAGGGTGCCTGGGTCTCCACGC	AGGGCTGCAGCAGCAGCATTGGA	+Taqα I
MYBPC3	Gly278Glu	TGGGAAAGGCTGGGAAGGTGAGAT	GCAGGGGTACCTGATCCGCCGACGACgT	-Aat II
MYBPC3	Gly490Arg	ACCCACCCGGCTAGGCCCTAGGAC	AGAAGGATGAGGTTTAGGCT	+EcoN I
MYBPC3	Arg495Gly	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Sma I
MYBPC3	Arg502Trp	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Age I
MYBPC3	Arg502Gln	GCAGCGGGTGGAGTTTGAGT	GGTCACCTCAGCATCGTCAT	-Age I
MYBPC3	Asp605Asn	AGAATACCAACAAGCCAGGACAAG	GCCCCAGGACCCCCAATTTTGAT	-Aat II
MYBPC3	Arg943Stop	TCAGAGGAGTGGGCAGTGGGAGTG	TCCTGTCTCTGCCCAGCGTTCT	+Dde I
MYBPC3	Thr1028Ser	GCGAGGAGGTGAGCATCCGCAACAGCCCCAC AGACT	CAGCCCAGCCCAGGGAAGGGAAAC	-Hinf I
MYBPC3	IVS31+2t→g	TGACTGACGCCTGGGGTCTTAATGT	CAGTGAAGGGTAGCTGCGGCCTGG	-Hph I
MYBPC3	Gly1248Arg	TTGGTTCCATGTTTGTTTCCAGCCT	TGGAAGCTATTGCCCATCTGGGCGTG	+EcoN I
TNNT2	Arg92Gln	AGCACAGCCAGGCTGGGTGCCCAT	AGGCGCCGAGGAAGGCTGTCTGGA	-Msp I
TNNT2	Glu96del	GGTCCACCCACAGGACATCCACCGGAAGCGCc TG	GGAGGTGGGGCCTCACAAAAGGGA	-Bpm I
TNNI3	Lys178del	ACCCTGCGGAGAGTGAGGATCTC	CATTTCTGAGGACCCCTTACT	-Mbo II
ACTC	His90Tyr	TTCTCATAGGGAGTTATGGTGGGT	CAACTGGGGGATCTGATTCACAGC	-Ban I
ACTC	Arg97Cys	GAGAAGATCTGGCACCACACCTTCTACAATGA GGCC	CAACTGGGGGATCTGATTCACAGC	+Stu I
PRKAG2	His530Arg	GAGATGATGTCTCACTGCTATTCC	TCTATACACTTTCCTCACTCACG	+Hpy99 I

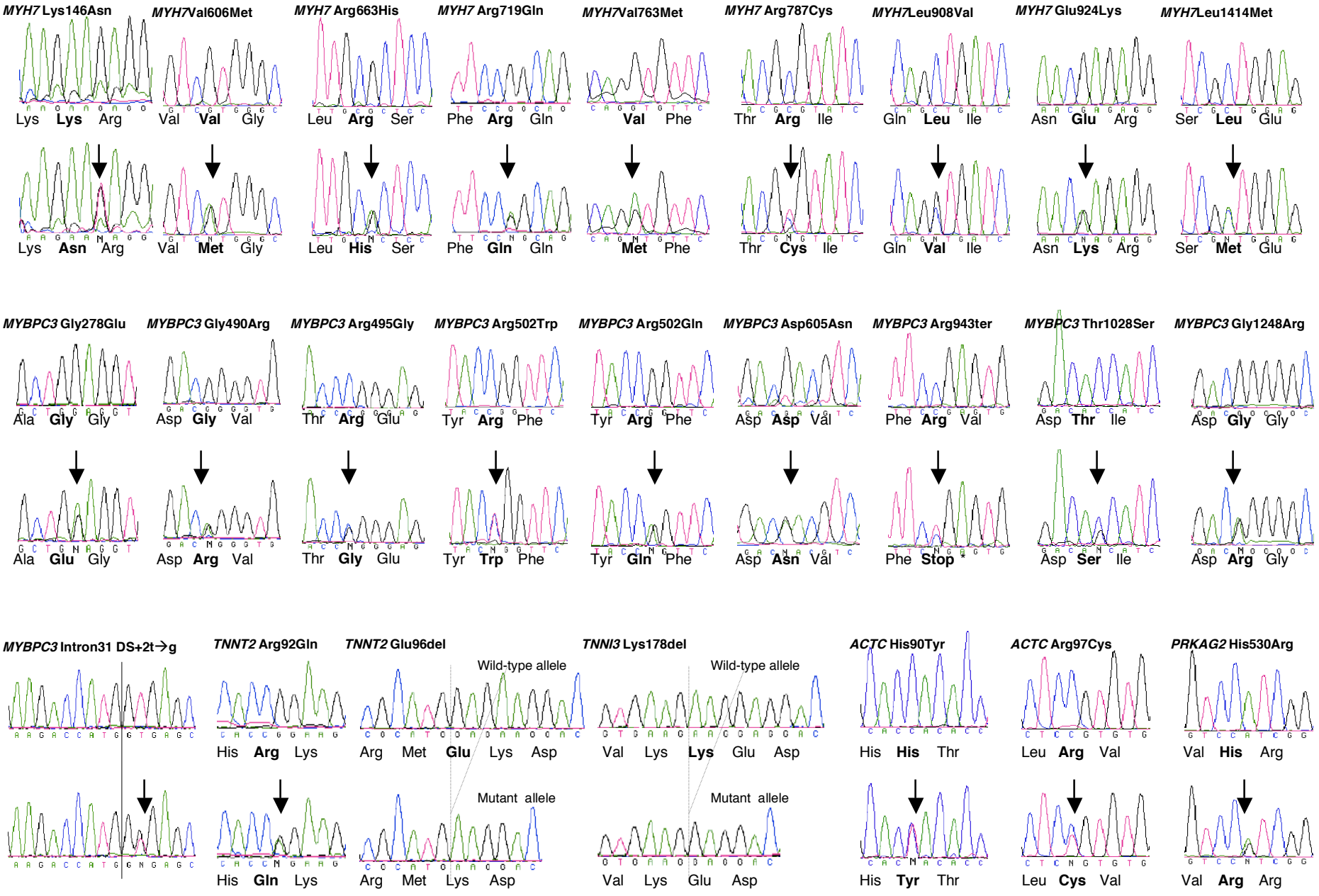
Lower letters indicate the mismatch bases; +, mutation creates the restriction site; -, mutation abolishes the restriction site.

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

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

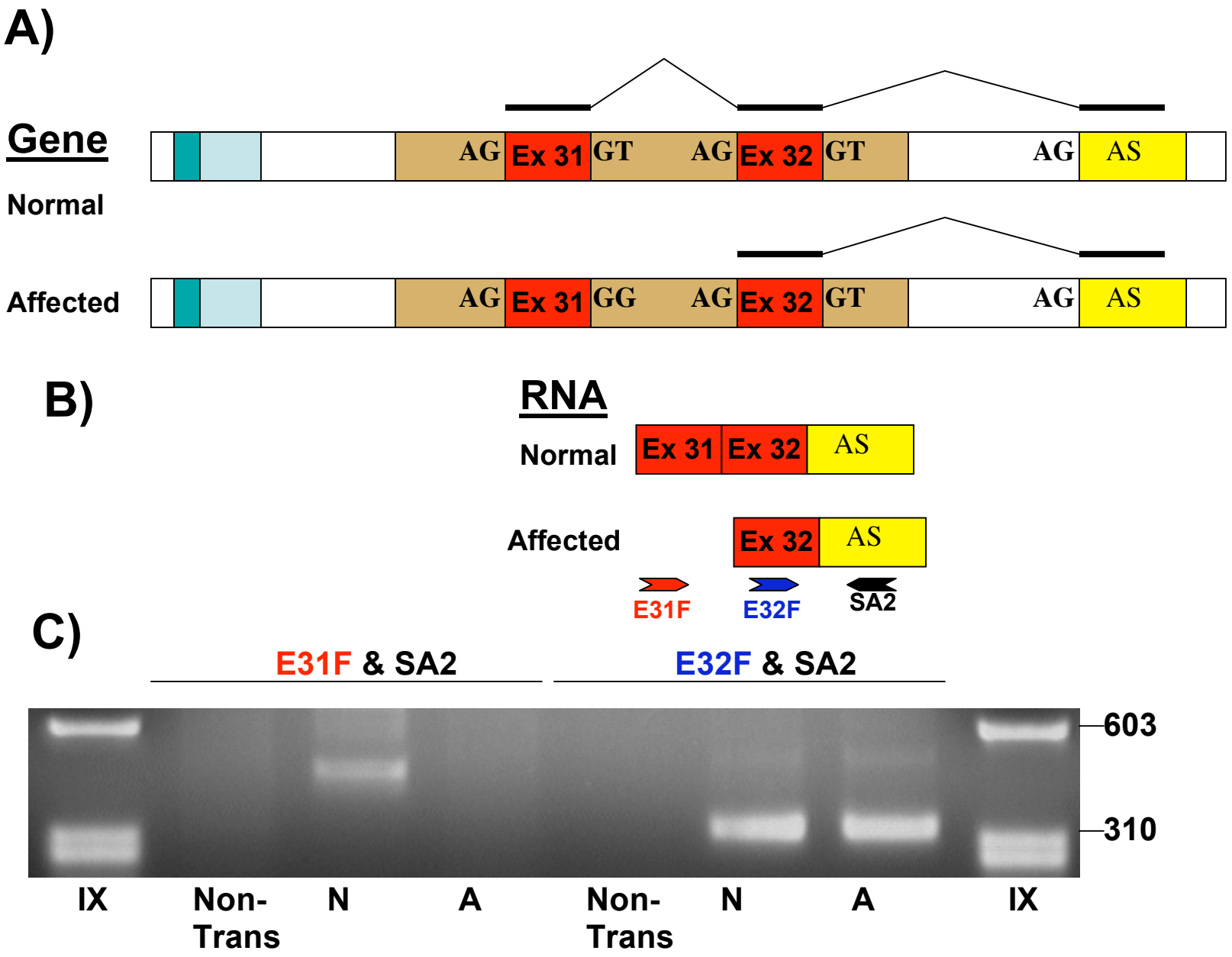
Designation, mutations are denoted by normally encoded amino acid, residue number, substituted amino acid or termination signal (ter) or altered splice signal (IVS); Consequence, mutational effects on protein; Charge, altered charge by mutant amino acid change; Reported. HCM mutations previously described-yes (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



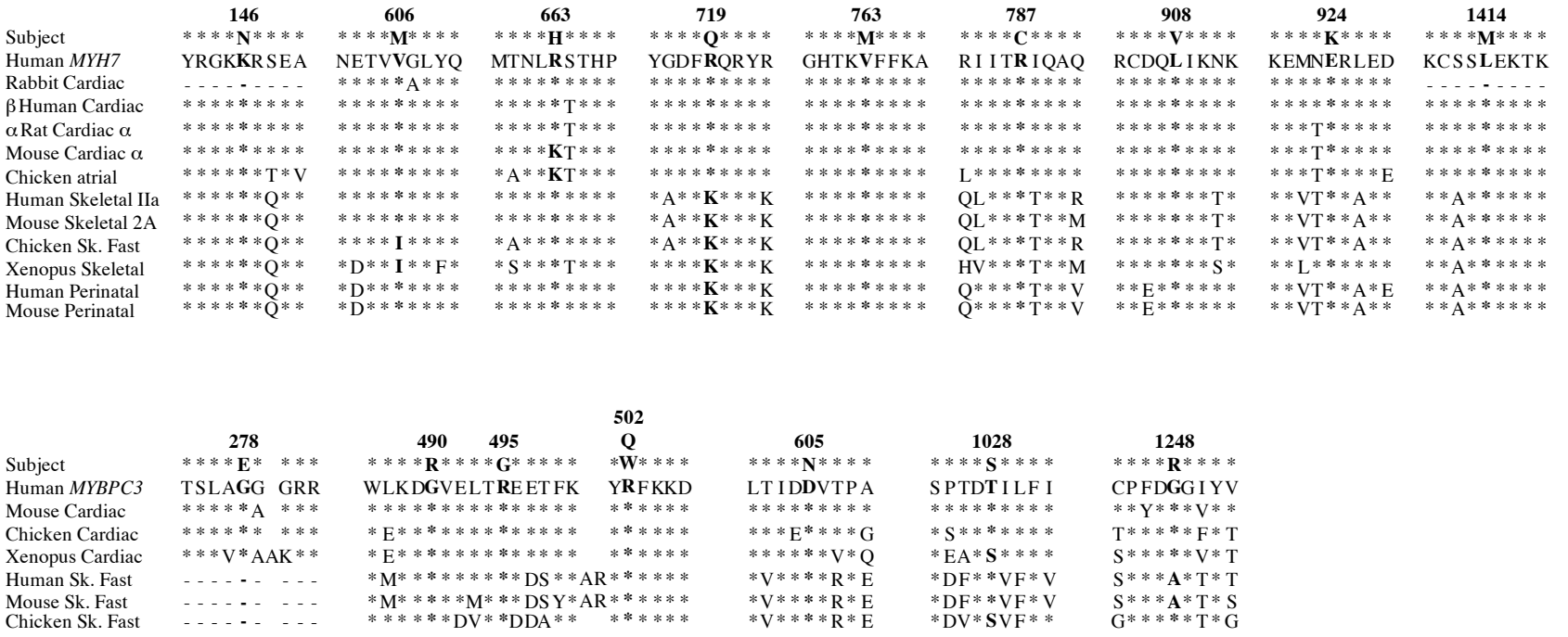
Supplemental Figure 1 Legend. Sequence analyses of genomic DNA from 25 variants; β-cardiac myosin heavy chain (*MYH7*) Lys146Asn, Val606Met, Arg663His, Arg719Gln, Val763Met, Arg787Cys, Leu908Val, Glu924Lys, Leu1414Met; cardiac myosin binding protein-C (*MYBPC3*) Gly278Glu, Gly490Arg, Arg495Gly, Arg502Trp/Gln, Asp605Asn, Arg943Stop, Thr1028Ser, Gly1248Arg, IVS31+2t→g; cardiac troponin T (*TNNT2*) Arg92Gln, Glu96del; cardiac troponin I (*TNNI3*) Lys178del; cardiac actin (*ACTC*) His90Tyr, Arg97Cys and γ2 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



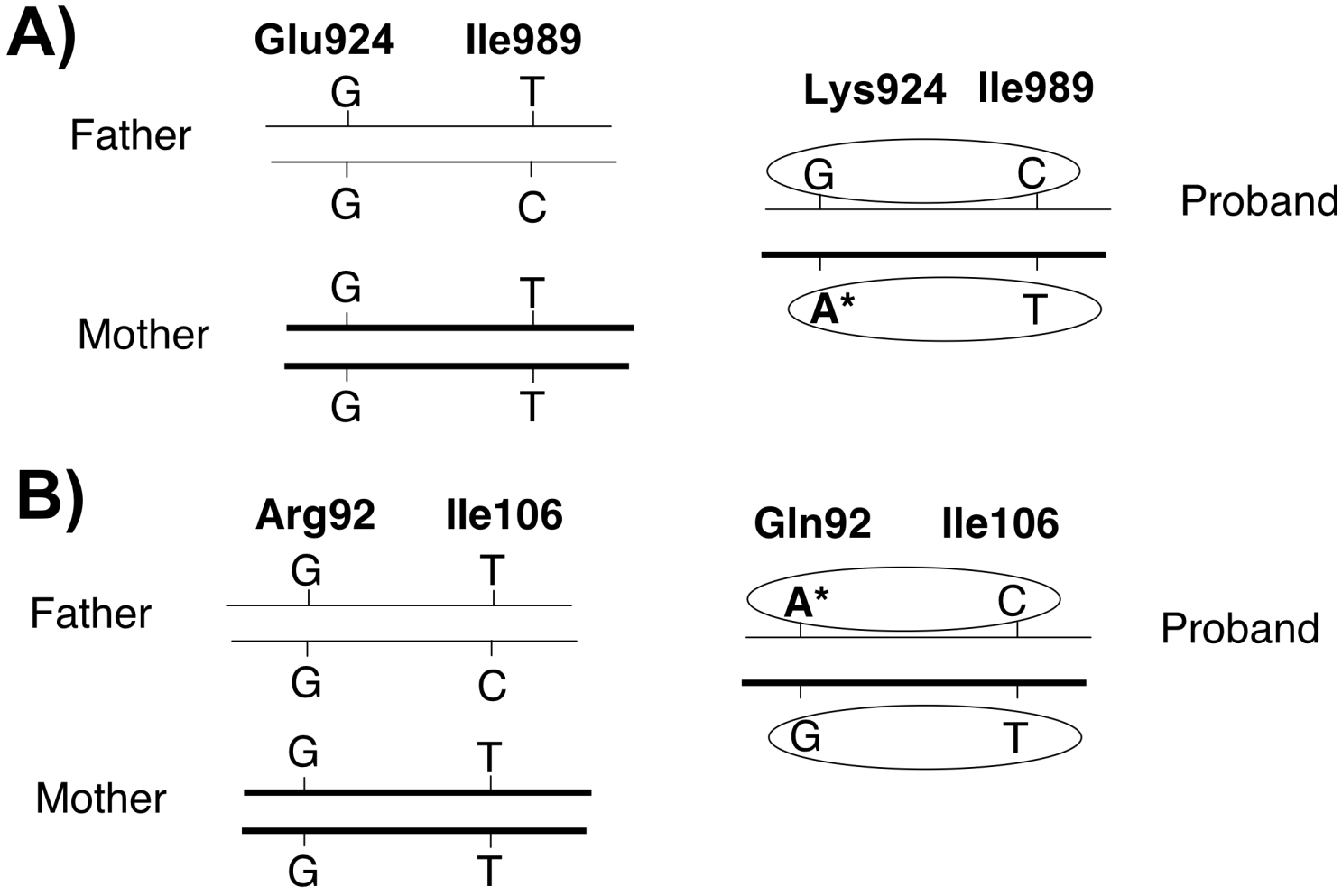
Supplemental Figure 2 Legend. Assessment of splicing site mutations. Using the exon trapping vector pSPL3, the effect *MYBPC3* IVS31+2t>g variant on splicing was tested. Vectors containing the wildtype or mutant genomic fragment (A) were transfected in COS-7 cells. Splicing was assessed using forward 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 prosessed 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



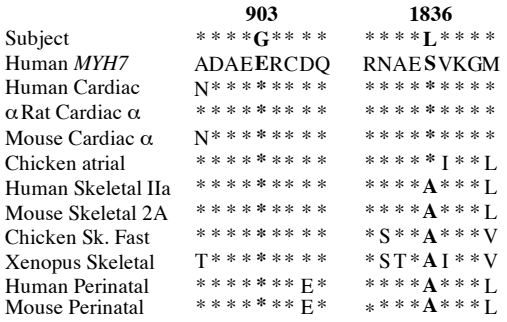
Supplemental Figure 3 Legend. Conservation of amino acid residues altered by missense or deletion mutations in presumed sporadic cases of childhood-onset LVH. β-cardiac myosin heavy chain (*MYH7*) Lys146Asn, Val606Met, Arg663His, Arg719Gln, Val763Met, Arg787Cys, Leu908Val, Glu924Lys, Leu1414Met; cardiac 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 γ2 regulatory subunit of AMP-activated protein kinase (*PRKAG2*) His530Arg. The altered residue and eight flanking amino acid residues are displayed in single letter code.

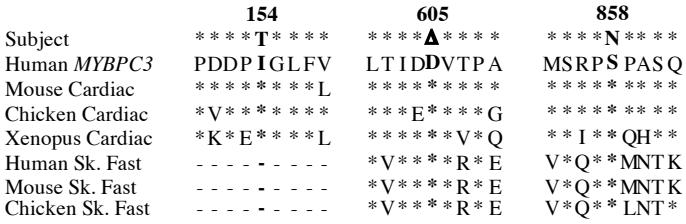
Supplemental Figure 4

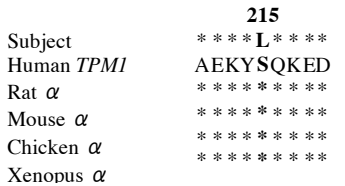


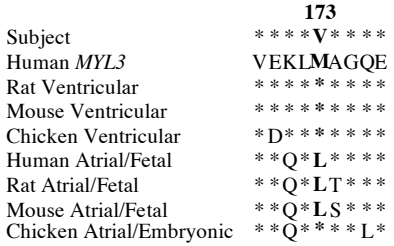
Supplemental Figure 4 Legend. Parental origin of the chromosomes containing *de novo* mutations. (A) The region flanking nucleotides corresponding to Glu924 and Ile989 (rs 7157716) in *MYH7* was amplified followed by subcloning using TOPO TA cloning kit (Invitrogen). The *de novo* mutation *MYH7* Glu924Lys (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









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