Sarcomere Protein Expression Studies Suggest Mutation-Specific Disease Mechanisms in Human Hypertrophic Cardiomyopathy (HCM)

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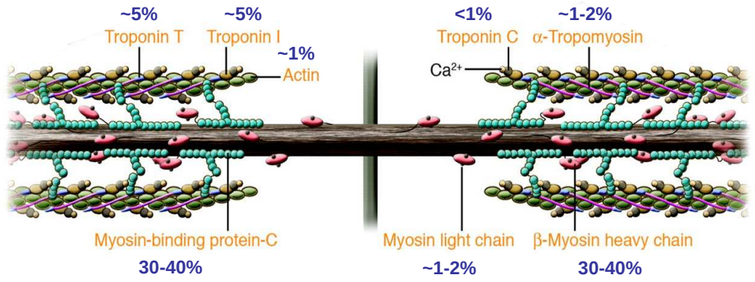
1. Introduction

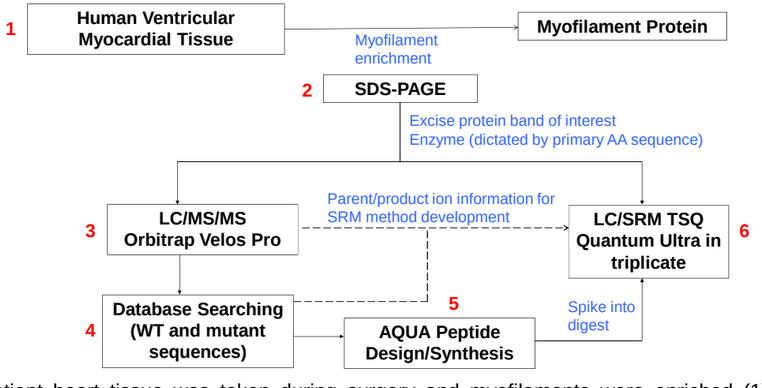
Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease. Heterozygous mutations in sarcomere genes account for the majority of cases, although mechanisms by which these mutant proteins exert their effects in human disease, and the functional relationship between co-expressed mutant and wild-type proteins, are largely unknown. Here, we present a study using targeted LC/SRM with AQUA peptides specific for wild type and mutant peptides in several proteins from myocardial tissue in HCM patients, in order to determine the ratio of WT to mutant expression.

The distribution of sarcomere gene mutations in HCM is summarized below:



Here, we measured 12 different missense mutations across MH7, TNNT2, TPM1, MYL2 and MYBPC3 from 18 patient samples.

2. Sample Preparation and Mass Spectrometry



Patient heart tissue was taken during surgery and myofilaments were enriched (1). Proteins were separated by SDS-PAGE and target proteins excised and digested using the most appropriate enzyme based on the primary sequence surrounding the mutation site (2). Data were acquired initially using data-dependent LC/MS/MS (3), and following database searching (4) peptides that contained the WT/mutant amino acid were synthesized with heavy labels (^{13}C,^{15}N) (5). The internal standards were spiked into the same digest and analyzed by LC/SRM (6). Peak areas for labeled and endogenous peptides were normalized, converted to mole values and summed for WT and mutant.

3. Allele-Specific Protein Expression Using AQUA

The results from AQUA analysis of 12 different mutations across 5 proteins and 18 samples are detailed below. In each case the WT/mutant amino acid is colored in red; the heavy labeled amino is indicated by an asterisk. Technical replicates are shown with 1 or 2 as a subscript.

Gene	Mutation	Enzyme	Wild-Type Peptide(s)	WT fmol (%)	Mutant Peptide(s)	Mutant fmol (%)
MYH7	A797T	Trypsin	GVL**A**R*	107.9_1 (51.2%) 310.5_2 (48.2%)	GVL**T**R*	102.7_1 (48.8%) 333.0_2 (51.8%)
MYH7	R1606H	Trypsin	VVDSLQTSLDAET**R***	77.4_1 (63.6%) 1031.6_2 (52.1%)	VVDSLQTSLDAET**H**SR*	44.4_1 (36.4%) 949.1_2 (47.9%)
MYH7	R1606C	Trypsin	VVDSLQTSLDAET**R***	387.9 (62.9%)	VVDSLQTSLDAET**C**SR*	228.6 (37.1%)
MYH7	D1096Y	Trypsin	IE**D**EQALGSQLQK* IE**D**EQALGSQLQKK* **Total**	87.5 248.9 336.4 (53.1%)	IE**Y**EQALGSQLQK* IE**Y**EQALGSQLQKK* **Total**	192.7 104.4 297.1 (46.9%)
MYH7	T1377M (LV septum)	Trypsin	YE**T**DAIQR* TKYE**T**DAIQR* TKYE**T**DAIQRTEELEEAK* **Total**	1.6 50.4 6.3 58.3 (50.6%)	YE**M**DAIQR* TKYE**M**DAIQR* TKYE**M**(ox)DAIQR* **Total**	1.0 47.1 8.6 56.8 (49.4%)
MYH7	T1377M (LV Lateral)	Trypsin	YE**T**DAIQR* TKYE**T**DAIQR* TKYE**T**DAIQRTEELEEAK* **Total**	3.3 68.5 7.6 79.3 (45.9%)	YE**M**DAIQR* YE**M**(ox)DAIQR* TKYE**M**DAIQR* TKYE**M**(ox)DAIQR* TKYE**M**DAIQRTEELEEAK* **Total**	2.5 0.5 62.7 20.3 7.6 93.6 (54.1%)
MYH7	T1377M (RV)	Trypsin	YE**T**DAIQR* TKYE**T**DAIQR* TKYE**T**DAIQRTEELEEAK* **Total**	2.8 108.5 17.4 128.7 (49.5%)	YE**M**DAIQR* TKYE**M**DAIQR* TKYE**M**(ox)DAIQR* TKYE**M**DAIQRTEELEEAK* TKYE**M**(ox)DAIQRTEELEEAK* **Total**	1.4 86.7 20 14.4 8.6 131.1 (50.5%)
MYH7	I323N	Asp-N	DYAFISQGETTV*AS**I** DYAFISQGETTV*AS**I**D **Total**	100.6 37.9 138.5 (31%)	DYAFISQGETTV*AS**N** DYAFISQGETTV*AS**N**D **Total**	301.73 0 301.73 (69%)
MYH7	G708A (LV septum)	Trypsin	K**G**FPNR*	250.1 (91%)	K**A**FPNR*	24.1 (9%)
MYH7	G708A (LV lateral)	Trypsin	K**G**FPNR*	677.1 (90%)	K**A**FPNR*	74.8 (10%)
MYH7	G708A (RV)	Trypsin	K**G**FPNR*	277.5 (89%)	K**A**FPNR*	33.3 (11%)
TNNT2	D86A	Trypsin	V**D**FDDIHR*	4.6_1 (19.2%) 9.72_2 (12.3%)	V**A**FDDIHR*	19.4_1 (80.8%) 69.25_2 (87.7%)
TPM1	I284V	Trypsin	AISEEL*DHALNDMTS**I** AISEEL*DHALNDM(ox)TS**I** **Total**	1.4_1 536.2_2 57_1 603.8_2 58.4_1 (59%) 1140.0_2 (52.1%)	AISEEL*DHALNDMTS**V** AISEEL*DHALNDM(ox)TS**V** **Total**	4.2_1 449.9_2 36.3_1 596.3_2 40.5_1 (41%) 1046.2_2 (47.9%)
MYL2	H161R	Chymo	VHIIT**H**GEEK*D KNLV**H**IITHGEEK*D **Total**	133.7 66.1 199.2 (69.2%)	VHIIT**R**GEEK*D KNLVHIIT**R**GEEK*D **Total**	67 21.8 88.8 (30.8%)
MYBPC3	R495Q (a)	Lys-C	DGVELT**R**EETFK*	52.9_1 (25.6%) 23.1_2 (36.6%)	DGVELT**Q**EETFK*	153.4_1 (74.4%) 40.0_2 (63.4%)
MYBPC3	R495Q (b)	Lys-C	DGVELT**R**EETFK*	36.9_1 (36.3%) 35.3_2 (39.7%)	DGVELT**Q**EETFK*	64.8_1 (63.7%) 53.7_2 (60.3%)
MYBPC3	E542Q (a)	Chymo	IVQ**E**KK*LEVY	2.10 (95.2%)	IVQ**Q**KK*LEVY	0.11 (4.8%)
MYBPC3	E542Q (b)	Chymo	IVQ**E**KK*LEVY	16.3 (98.4%)	IVQ**Q**KK*LEVY	0.26 (1.6%)

Technical duplicates had a CV of 4-14%. Analytical replicates per sample (n=3) had CVs of less than 10%.

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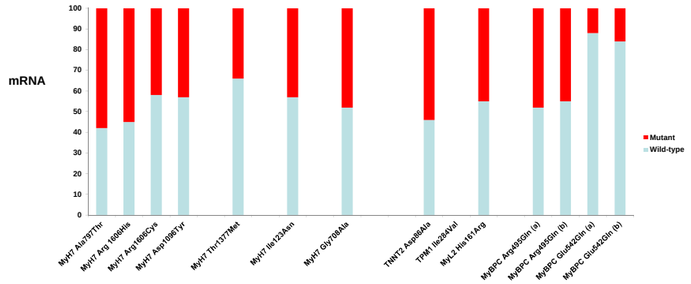
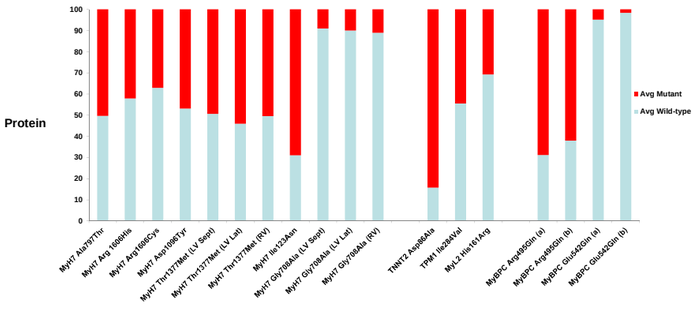
4. Correlation With mRNA Measurement

Quantification of wild-type and mutant sarcomere missense gene transcripts by custom-designed single base extension reactions was performed:



Protein level measurements are summarized below:



Five out of seven MYH7 samples showed the wild-type to mutant protein ratio was at or near the expected 1:1, as with mRNA. MYH7 I323N showed the mutant peptide was more abundant (70% of total) and MYH7 G708A showed the mutant peptide comprised only 10%. Both were different to the mRNA ~1:1 ratio. There was no regional variation across septum, right ventricle and lateral wall in two explanted hearts (T1377M and G708A). The TPM1 mutant protein was present in ~1:1 ratio with wild-type. MYL2 and TNNT2 showed marked heterogeneity. MYBPC E542Q was predominantly WT, matching the mRNA missense data. Note MYBPC E54Q exists as both a missense and nonsense mutation.

5. Summary

Here, we used a quantitative mass spectrometry (LC/SRM) approach with AQUA internal standard peptides to determine the stoichiometric ratio of wild-type to mutant proteins in the human heart sarcomere for twelve missense mutations in MYH7, MYBPC3, TNNT2, MYL2 and TPM1. Data reveal at the protein level that missense mutations comprise on average 40% and between 10-84%. Six of the samples were acquired in technical replicate several months apart and showed CVs of 4-14%. These data demonstrate allele-specific expression patterns for HCM mutations and that the basis for allelic variation would appear to be at the post-transcriptional level in several cases, when compared to the mRNA levels.