MS Bioworks
Protein Mass Spectrometry Services

CS CUSTOM-WORKS

SRM Analysis of Sarcomere mutations

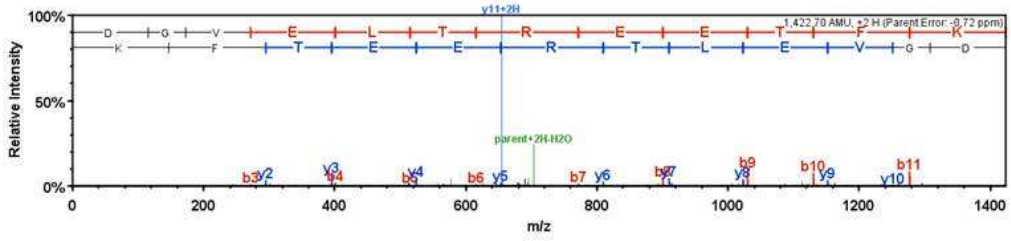
Hypertrophic cardiomyopathy (HCM) is the most common heritable cardiovascular disease. Heterozygous mutations in sarcomere genes account for the majority of cases, although mechanisms by which these mutant proteins exert their effects in human disease, and the functional relationship between co-expressed 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.

Methods

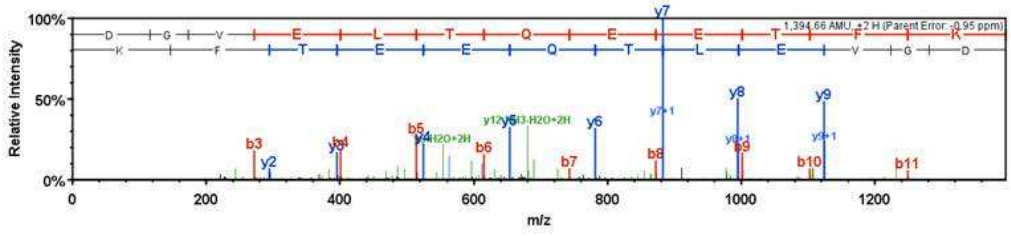
Patient heart tissue was taken during surgery and myofilaments were enriched. Proteins were separated by SDS-PAGE and target proteins excised and digested using the most appropriate enzyme based on the primary sequence surrounding the mutation site. Data were acquired initially using data-dependent LC/MS/MS, and following database searching peptides that contained the WT/mutant amino acid were synthesized with heavy labels (^{13}C,^{15}N). The internal standards were spiked into the same digest and analyzed by LC/SRM. Peak areas for labeled and endogenous peptides were converted to mole values and summed for WT and mutant. We measured 12 different missense mutations across MH7, TNNT2, TPM1, MYL2 and MYBPC3 from 18 patient samples.

Results

Example product ion spectra obtained during the initial data-dependent analysis are shown below for the MYBPC3 R495Q mutation. Both WT and mutant peptides were obtained following Lys-C digestion:



R495:
DGVELT**R**EETFK



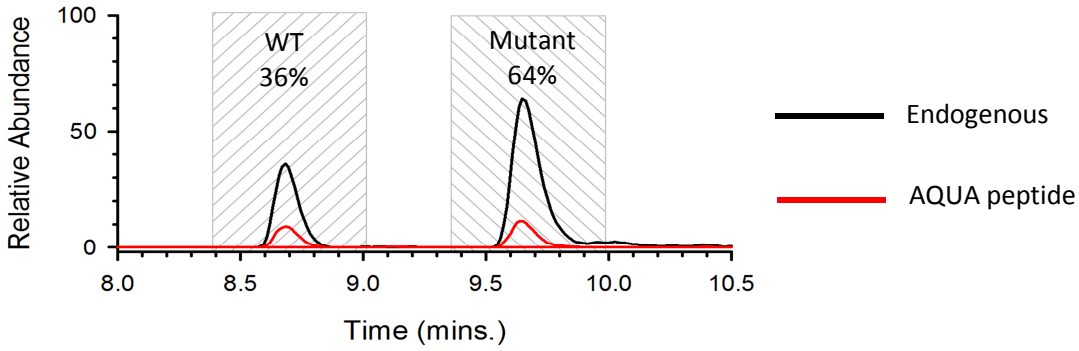
Q495:
DGVELT**Q**EETFK

MS Bioworks LLC, 3950 Varsity Drive, Ann Arbor, MI, 48108
Tel: 734-929-5083 Fax: 734-929-4637 Email: info@msbioworks.com
www.msbioworks.com

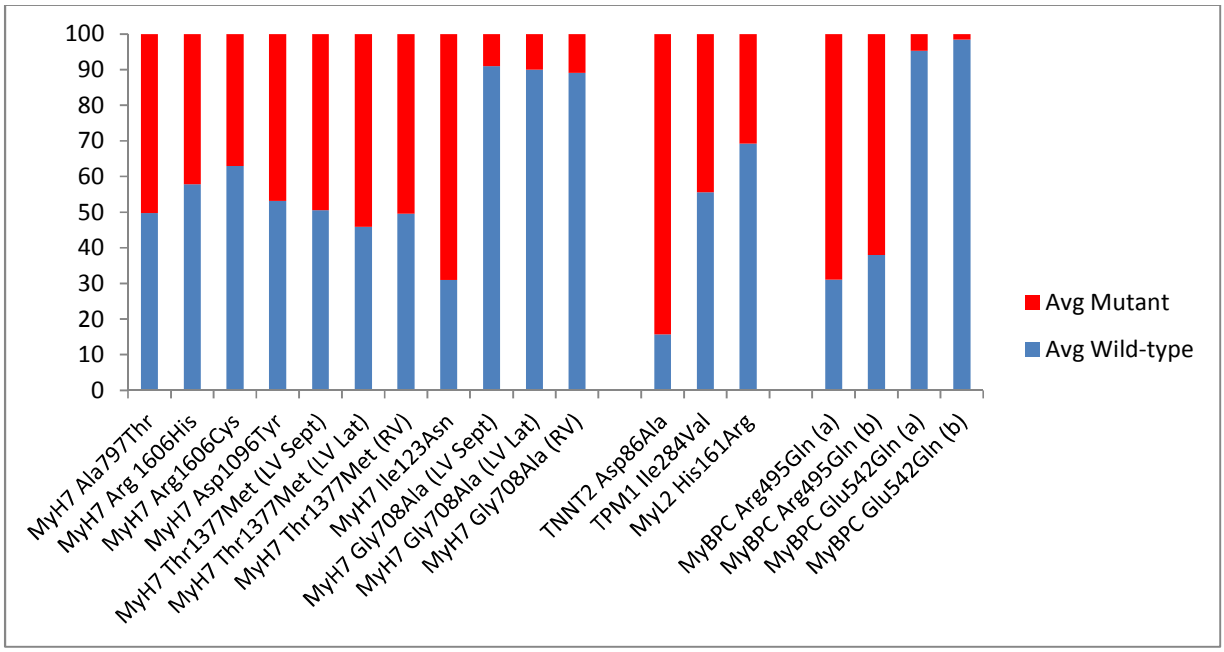
MS Bioworks
Protein Mass Spectrometry Services

CS CUSTOM-WORKS

Following synthesis of the corresponding AQUA peptides, the LC/SRM analysis for MYBPC3 R495Q gave the following chromatograms:



The results from the 12 different mutations across 5 proteins and 18 samples are detailed below. Data reveal at the protein level that missense mutations comprise on average 40% and between 10-84% and allele-specific expression patterns exist for HCM mutations; the basis for allelic variation would appear to be at the post-transcriptional level in several cases, when compared to the mRNA levels.



MS Bioworks LLC, 3950 Varsity Drive, Ann Arbor, MI, 48108
Tel: 734-929-5083 Fax: 734-929-4637 Email: info@msbioworks.com
www.msbioworks.com