National Human Genome Research Institute

Using Exome Data to Identify Malignant Hyperthermia Susceptibility Mutations

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Genetic Disease Research Branch

ABSTRACT

Background: Malignant hyperthermia susceptibility (MHS) is a life-threatening, inherited disorder of muscle calcium metabolism, triggered by anesthetics and depolarizing muscle relaxants. An unselected cohort was screened for MHS mutations using exome sequencing. The aim of this study was to pilot a strategy for the *RYR1* and *CACNA1S* genes.

Design: Multidisciplinary analysis of gene variants identified through exome sequencing.

Methods: Exome sequencing was performed on 870 volunteers not ascertained for MHS. Variants in *RYR1* and *CACNA1S* were annotated using an algorithm that filtered results based on mutation type, frequency, and information in mutation databases. Variants were scored on a six-point pathogenicity scale. Medical histories and pedigrees were reviewed for malignant hyperthermia and related disorders.

Results: The authors identified 70 RYR1 and 53 *CACNA1S* variants among 870 exomes. Sixty-three *RYR1* and 41 *CACNA1S* variants passed the quality and frequency metrics but the authors excluded synonymous variants. In *RYR1*, the authors identified 65 missense mutations, one nonsense, two that affected splicing, and one non-frameshift indel. In *CACNA1S,* 48 missense, one frameshift deletion, one splicing, and one non–frameshift indel were identified. *RYR1* variants predicted to be pathogenic for MHS were found in three participants without medical or family histories of MHS. Numerous variants, previously described as pathogenic in mutation databases, were reclassified by the authors as being of unknown pathogenicity.

Conclusions: Exome sequencing can identify asymptomatic patients at risk for MHS, although the interpretation of exome variants can be challenging. The use of exome sequencing in unselected cohorts is an important tool to understand the prevalence and penetrance of MHS, a critical challenge for the field.

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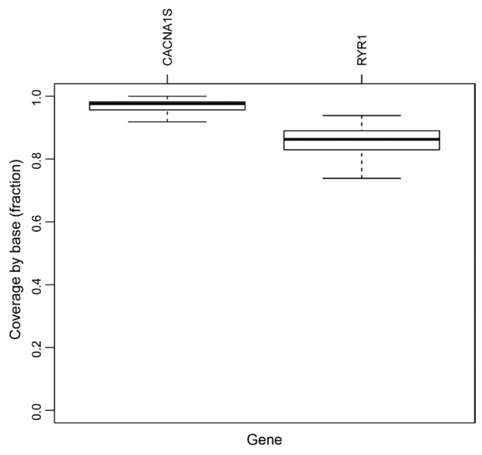
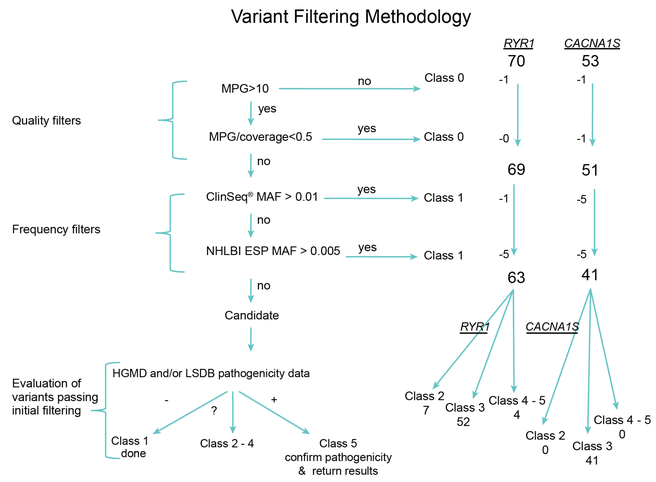
Display 1. Sequencing Coverage of the coding exons was 83% (*RYR1*) and 93% (*CACNA1S*)



Box and whisker plots showing base coverage for the *RYR1* and *CACNA1S* genes for a cohort of 870 probands.

Display 2. Quality/ Frequency Filter Algorithm



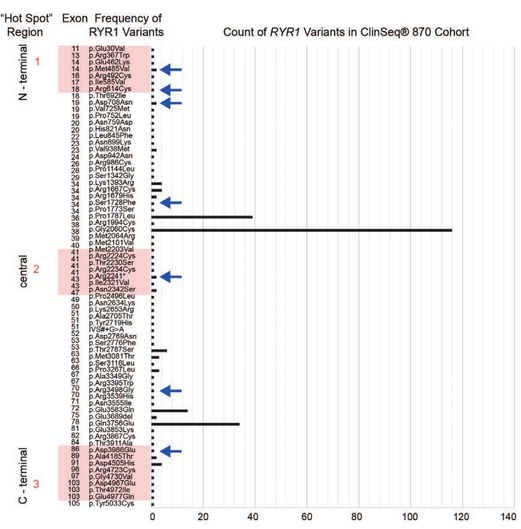
Variants filtered on genotype quality, coverage and allele frequencies (Class 0-1). Variants assessed for pathogenicity (Class 2-5) on data in the Human Gene Mutation Database (HGMD) and locus-specific databases (LSDBs). MPG =most probable genotype. MAF =minor allele frequency. NHLBI ESP =The National Heart, Lung, and Blood Institute, exome sequencing project.

Display 3. Variant Pathogenicity Classification System

Database Literature Designation	Novel (Not Published)		Published as pathogenic		Published as VUS	Published as benign
Mutation Type	**Missense In Frame Insertion/ Deletions**	**Nonsense Frameshift Splice**	**Missense**	**Nonsense Frameshift Splice**	**Any**	**Any**
Class 5 (pathogenic)		Similar to disease causing mutation and consistent family history	On EMHG list of 31 approved diagnostic (causative) mutations, and/or NAMH Group's mutation panel **OR** Two or more reports as pathogenic and no evidence against	On EMHG list of 31 approved diagnostic (causative) mutations, and/or **NAMH** Group's mutation panel **OR** Single report as pathogenic with supporting evidence		
Class 4 (Likely pathogenic)		Similar to disease causing mutation and inconsistent family history	Two or more reports as pathogenic with single evidence against **OR** Single report as pathogenic with supporting evidence	Two or more reports as pathogenic with single evidence against **OR** Single report as pathogenic without supporting evidence		
Class 3 (Uncertain)	All novel missense or in frame insertions/deletions without supporting publications	No similar disease causing mutation reported as pathogenic **OR** Inconsistent family history	Two or more reports as pathogenic with multiple evidence against **OR** Single report as pathogenic without supporting evidence	Two or more reports as pathogenic with multiple evidence against **OR** Single report as pathogenic with single evidence against	Reported as VUS (no convincing evidence they have a causative effect, no evidence to support polymorphism) **OR** single case reported as pathogenic	Single report as benign with insufficient supporting evidence
Class 2 (Likely not pathogenic)			Single report as pathogenic with multiple evidence against		Some evidence to support as polymorphism **OR** Multiple evidence against pathogenicity	On EMHG list of 156 nonpathogenic variants, **OR** Multiple cases reported as benign with insufficient evidence **OR** multiple report as benign with supporting evidence
Class 1 (Not pathogenic)	ClinSeq® / NHLBI ESP Minor Allele Frequency ≥ 1%/ 0.5%					

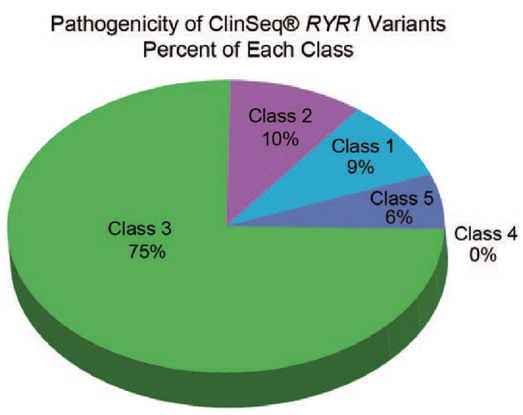
Criteria for assignment of pathogenicity class 1 to 5 for *RYR1* and *CACNA1S* variants based on data available in the HGMD, LSDBs, family history and the European Malignant Hyperthermia Group's (EMHG) list of diagnostic and non-pathogenic variants. VUS =variant of unknown significance.

Display 4. Frequency Histogram of *RYR1* Variants



Frequency histogram of the 69 *RYR1* variants with predicted protein changes from the ClinSeq® 870 cohort.

Display 5.

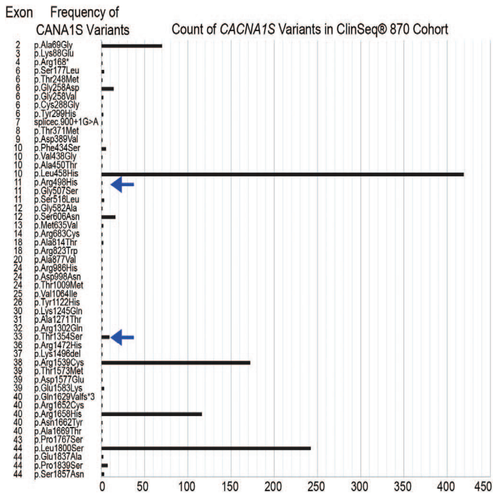


Display 6: Summary of Pathogenic *RYR1* Variants Identified in 870 ClinSeq® Exomes

Nucleotide Change	Predicted Protein Change	Associated Disease State Listed in HGMD	ClinSeq® Allele Count	NHLBI EVS Allele Count	ClinSeq® Pathogenicity Score
c.1840C>T	p.Arg614Cys	Malignant Hyperthermia	1/1,740	NF	5^
c.5183C>T	p.Ser1728Phe	Malignant hyperthermia	1/1,740	1/10,757	5
c.6721C>T	p.Arg2241X	Multi-minicore & Atypical Periodic Paralysis	2/1,740	NF	5
c.11958C>G	p.Asp3986Glu	Malignant hyperthermia	1/1,740	NF	5

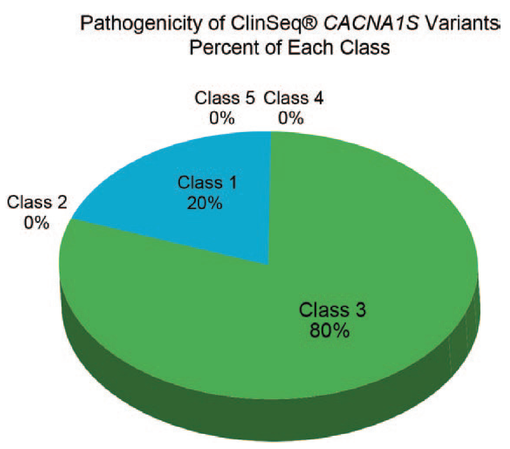
RYR1 Variants in Transcript NM_000540.2 Pathogenicity scores were determined as described in the Methods. NHLBI EVS =The National Heart, Lung, and Blood Institutes, Exome Sequencing Project, Exome Variant Server. NF =Not Found. **^ =**on European Malignant Hyperthermia Group's list of 31 approved diagnostic (causative) mutations.

Display 7. Frequency Histogram of *CACNA1S* Variants



Frequency histogram of 51 *CACNA1S* variants with predicted protein changes from the ClinSeq® 870 cohort.

Figure 8.



References: Accepted for publication July 18, 2013.
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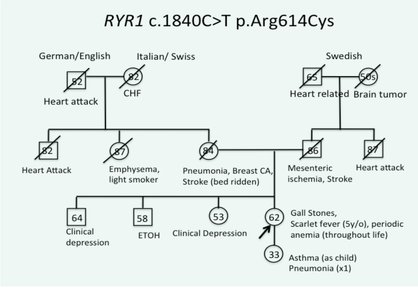
Figure 9: Illustration of Proband with MHS *RYR1* Variant

RYR1 p.Arg614Cys
Pathogenicity Classification: 5
Published causative mutation

Clinical Action:
a. Results disclosure
b. Provide MH management guidelines
c. Refer proband to Specialists for CHCT testing and NAMHA Registry



CONCLUSIONS:

- While the interpretation and assessment of pathogenicity can be challenging, causative mutations can be filtered from ES data
- Clinically relevant mutations can be identified as secondary (so-called incidental) findings in exomes sequenced for clinical care and clinical research
- The application of ES technology to large and diverse cohorts has the potential to accelerate the pace of MHS gene mutation discovery