

Re-classification of variants: Implications from the cardiac perspective

Karen McGuire

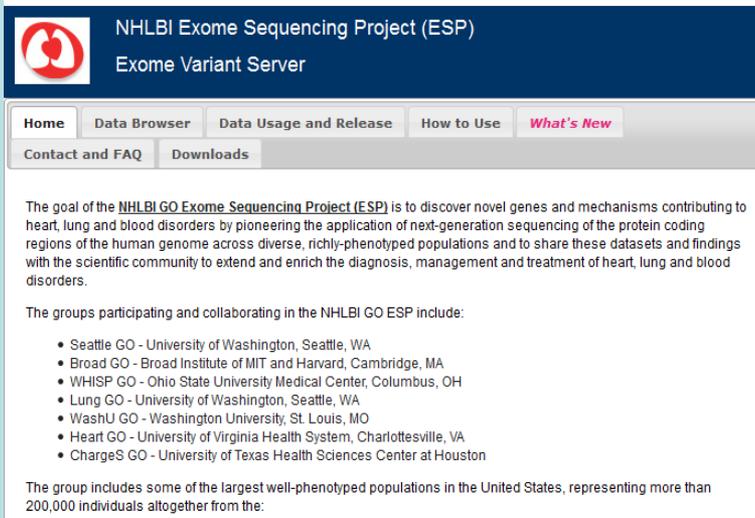
Oxford Medical Genetics Laboratories, Churchill Hospital, Old Road, Oxford, OX3 7LE.

Re-classification

New evidence that changes the original interpretation and classification of the variant

Enhance Pathogenicity	Question Pathogenicity
<i>De novo</i> occurrence	Detection in multiple phenotypes
Segregation with disease	Detected alongside another putative pathogenic variant
Additional functional studies e.g. RNA	Non segregation
	Frequency in population based cohorts
UPGRADE	DOWNGRADE

Population-based cohorts



The screenshot shows the NHLBI Exome Sequencing Project (ESP) Exome Variant Server website. The header includes the NHLBI logo and the text "NHLBI Exome Sequencing Project (ESP) Exome Variant Server". Below the header is a navigation menu with links for "Home", "Data Browser", "Data Usage and Release", "How to Use", "What's New", "Contact and FAQ", and "Downloads". The main content area contains a paragraph describing the goal of the NHLBI GO Exome Sequencing Project (ESP) and a list of participating groups, including Seattle GO, Broad GO, WHISP GO, Lung GO, WashU GO, Heart GO, and ChargeS GO. A note at the bottom states that the group includes some of the largest well-phenotyped populations in the United States, representing more than 200,000 individuals.

NHLBI Exome Sequencing Project (ESP)
Exome Variant Server

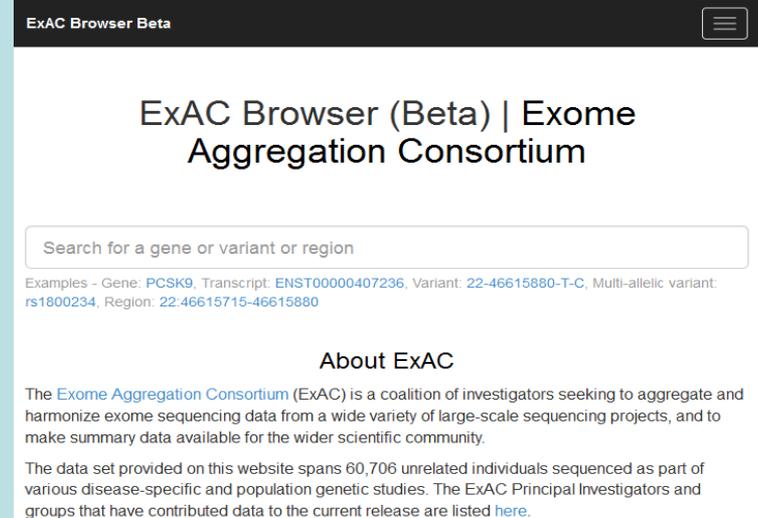
Home Data Browser Data Usage and Release How to Use **What's New**
Contact and FAQ Downloads

The goal of the [NHLBI GO Exome Sequencing Project \(ESP\)](#) is to discover novel genes and mechanisms contributing to heart, lung and blood disorders by pioneering the application of next-generation sequencing of the protein coding regions of the human genome across diverse, richly-phenotyped populations and to share these datasets and findings with the scientific community to extend and enrich the diagnosis, management and treatment of heart, lung and blood disorders.

The groups participating and collaborating in the NHLBI GO ESP include:

- Seattle GO - University of Washington, Seattle, WA
- Broad GO - Broad Institute of MIT and Harvard, Cambridge, MA
- WHISP GO - Ohio State University Medical Center, Columbus, OH
- Lung GO - University of Washington, Seattle, WA
- WashU GO - Washington University, St. Louis, MO
- Heart GO - University of Virginia Health System, Charlottesville, VA
- ChargeS GO - University of Texas Health Sciences Center at Houston

The group includes some of the largest well-phenotyped populations in the United States, representing more than 200,000 individuals altogether from the:



The screenshot shows the ExAC Browser Beta website. The header includes the text "ExAC Browser Beta" and a hamburger menu icon. The main content area features the title "ExAC Browser (Beta) | Exome Aggregation Consortium" and a search bar with the placeholder text "Search for a gene or variant or region". Below the search bar are examples of search results: "Gene: PCSK9, Transcript: ENST00000407236, Variant: 22-46615880-T-C, Multi-allelic variant: rs1800234, Region: 22:46615715-46615880". A section titled "About ExAC" describes the Exome Aggregation Consortium (ExAC) as a coalition of investigators seeking to aggregate and harmonize exome sequencing data from a wide variety of large-scale sequencing projects, and to make summary data available for the wider scientific community. It also mentions that the data set provided on this website spans 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies, and that the ExAC Principal Investigators and groups that have contributed data to the current release are listed [here](#).

ExAC Browser Beta

ExAC Browser (Beta) | Exome Aggregation Consortium

Search for a gene or variant or region

Examples - Gene: [PCSK9](#), Transcript: [ENST00000407236](#), Variant: [22-46615880-T-C](#), Multi-allelic variant: [rs1800234](#), Region: [22:46615715-46615880](#)

About ExAC

The [Exome Aggregation Consortium](#) (ExAC) is a coalition of investigators seeking to aggregate and harmonize exome sequencing data from a wide variety of large-scale sequencing projects, and to make summary data available for the wider scientific community.

The data set provided on this website spans 60,706 unrelated individuals sequenced as part of various disease-specific and population genetic studies. The ExAC Principal Investigators and groups that have contributed data to the current release are listed [here](#).

- Population ascertainment of samples
- Not pre-selected for having cardiomyopathy
- General prevalence of 1/500
- Allows investigation into general frequency of genetic variants in cardiac genes

Population-based cohorts

REPORT

Burden of Rare Sarcomere Gene Variants in the Framingham and Jackson Heart Study Cohorts

Alexander G. Bick,^{1,2} Jason Flannick,^{2,3} Kaoru Ito,¹ Susan Cheng,⁴ Ramachandran S. Vasan,^{5,6} Michael G. Parfenov,¹ Daniel S. Herman,¹ Steven R. DePalma,¹ Namrata Gupta,² Stacey B. G. Birgit H. Funke,^{7,15} Heidi L. Rehm,^{7,15} Emelia J. Benjamin,^{5,6,8} Jayashri Aragam,⁴ Herman A. Taylor, Jr.,^{9,10,16} Ervin R. Fox,⁹ Christopher Newton-Cheh,^{3,5,11} Sekar Kathiresan,^{3,12} Christopher J. O'Donnell,^{5,12} James G. Wilson,¹³ David M. Altshuler,^{2,3} Joel N. Hirschhorn,^{1,2} J.G. Seidman,¹ and Christine Seidman^{1,4,17,*}

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Population-Based Variation in Cardiomyopathy Genes Clinical Perspective
Jessica R. Golbus, Megan J. Puckelwartz, John P. Fahrenbach, Lisa M. Dellefave-Castillo, Don Wolfgeher and Elizabeth M. McNally
Circ Cardiovasc Genet 2012;5:391-399; originally published online July 4, 2012;
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EJHG Open

Evaluating Pathogenicity of Rare Variants From Dilated Cardiomyopathy Era

Nadine Norton, Peggy D. Robertson, Mark J. Rieder, Stephan Züchner, E Eden Martin, Duanxiang Li, Deborah A. Nickerson and Ray E. H

Circ Cardiovasc Genet. 2012;5:167-174; originally published online February 14, 2012
doi: 10.1161/CIRCGENETICS.111.961805

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ARTICLE

New population-based exome data are questioning the pathogenicity of previously cardiomyopathy-associated genetic variants

Charlotte Andreassen^{1,2,5}, Jonas B Nielsen^{1,2,5}, Lena Refsgaard^{1,2}, Anders G Holst^{1,2}, Alex H Christensen^{1,2}, Louise Andreassen^{1,2}, Ahmad Sajadieh³, Stig Haunso^{1,2,4}, Jesper H Svendsen^{1,2,4} and Morten S Olesen^{*,1,2}

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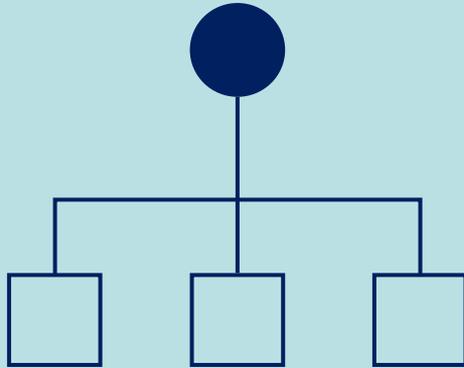


Reassessment of Mendelian gene pathogenicity using 7,855 cardiomyopathy cases and 60,706 reference samples

Roddy Walsh,  Kate Thomson,  James S Ware, Birgit H Funke, Jessica Woodley, Karen J McGuire, Francesco Mazarrotto, Edward Blair, Anneke Seller, Jenny C Taylor,  Eric V Minikel, Exome Aggregation Consortium, Daniel G MacArthur,  Martin Farrall,  Stuart A Cook,  Hugh C Watkins

Genetics In Medicine, in press.

TNNT2 p.Arg288Cys

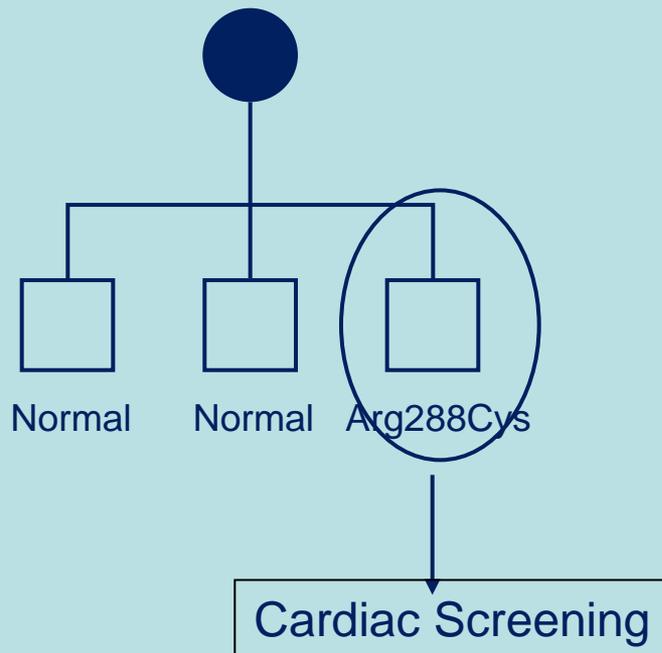


- Diagnosis of HCM aged 80
- No family history of HCM
- Targetted 4 gene screen
- *TNNT2* p.Arg288Cys detected

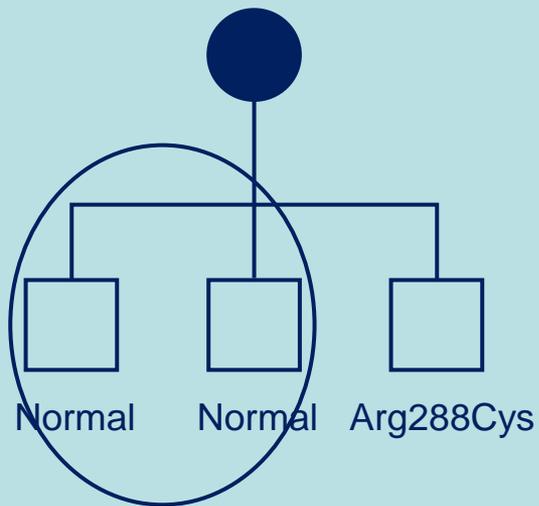
- Conserved across species
- Region of the protein where other pathogenic mutations were reported
- Detected in several individuals with HCM from multiple groups including UK^[1], Spanish^[2], Italian^[3], Australian^[4] and American^[5].
- Not detected in normal controls
- **Highly likely to be pathogenic (2007)**

References: [1] Watkins *et al* (1995) *NEJM* **332** 1058 [2] Garcia-Castro *et al.* (2003) *Clin Chem* 49(8):1279-1285 [3] Torricelli *et al.* (2003) *Am J Cardiol* 92(11):1358-1362 [4] Ingles *et al.* (2005) *J Med Genet* 42(10)e59 [5] Van Driest *et al* (2003) *Circ.* 108(4):445-451

TNNT2 p.Arg288Cys

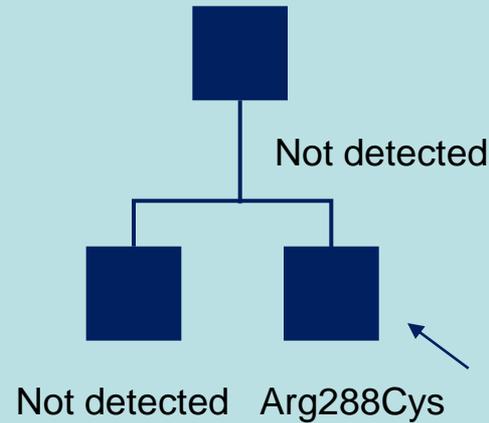


TNNT2 p.Arg288Cys



No clinical screening

***TNNT2* p.Arg288Cys**



Is this variant not a pathogenic mutation?

or

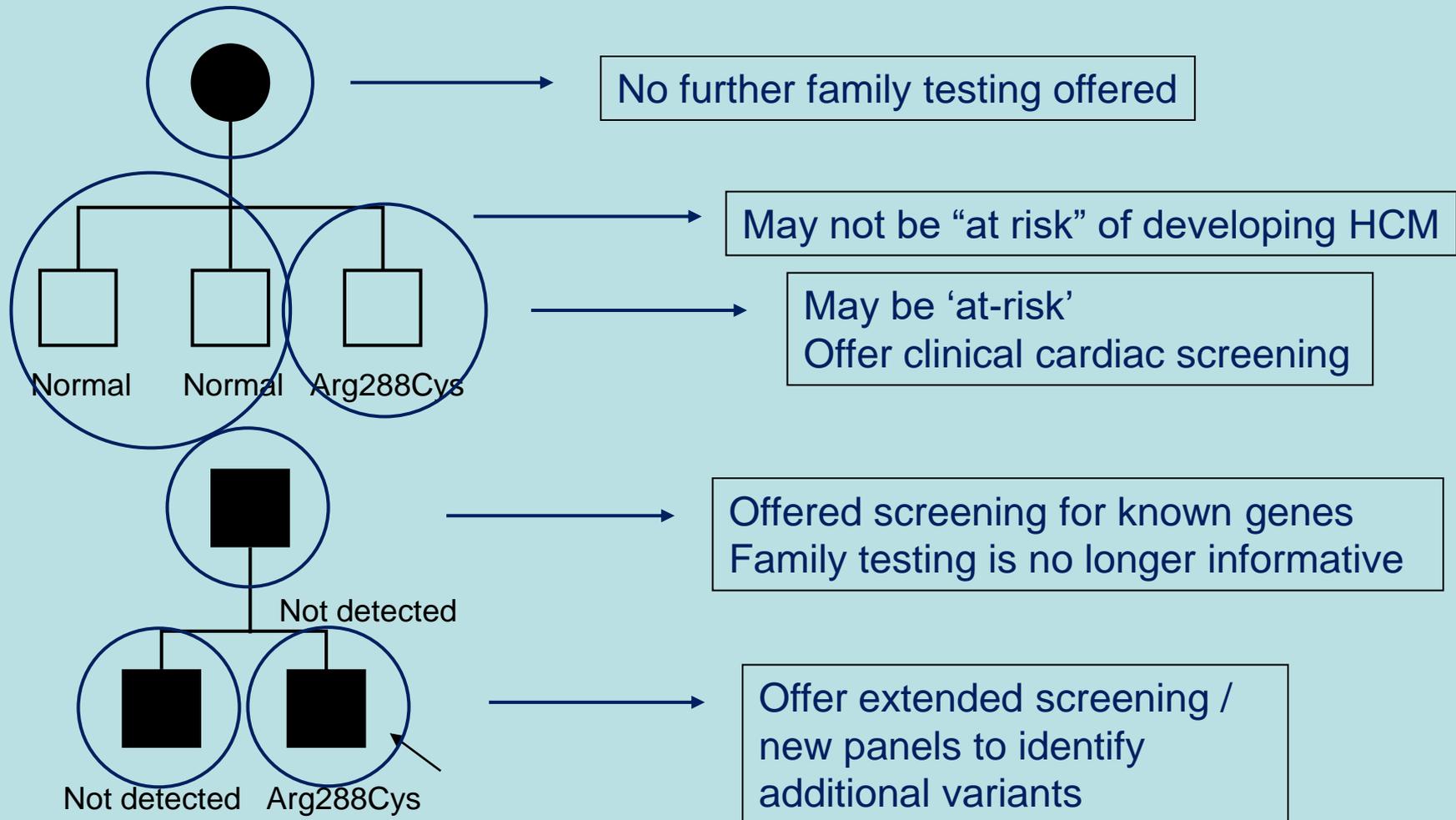
Is there a second pathogenic variant in this family?

TNNT2 p.Arg288Cys

Detected in 5 of >4,000 individuals (0.1%) of European ancestry and 1 of >2,000 individuals (0.04%) of African ancestry in NHLBI-EVS cohort.	Is this a rare polymorphism or population specific polymorphism?
Detected in 12 of 3,000 individuals in our own cohort (0.4%); however, 4 of these individuals also had a second putative pathogenic variant. This information is reflected in cases reported in the literature.	Does this variant work in concert with other mutations to cause cardiomyopathy? Is it a mild variant? Is it sufficient to cause disease alone?
Functional studies <i>in vitro</i> indicated that the variant does not have a significant effect on protein function.	Is this variant sufficient to cause disease as a sole variant?

Re-Classified as Unknown Pathogenicity

Clinical impact of re-classification



Re-classifications 2013 - present

Mutation details	Class change	Families	Individuals
<i>TNNT2</i> c.862C>T p.(Arg288Cys)	Highly likely to UV	15	31
<i>MYBPC3</i> c.2429G>A p.(Arg810His)	Highly likely to UV	7	13
<i>MYBPC3</i> c.13G>A p.(Gly5Arg)	Likely to unlikely	13	45
<i>MYBPC3</i> c.2003G>A p.(Arg668His)	Likely to unlikely	4	6
<i>MYH7</i> c.2945T>C p.(Met982Thr)	Likely to unlikely	10	21
<i>MYBPC3</i> c.2441_2443delAGA p.(Lys814del)	Likely to UV	4	14
<i>MYBPC3</i> c.3392T>C p.(Ile1131Thr)	Likely to UV	1	1
<i>MYH7</i> c.5135G>A p.(Arg1712Gln)	Likely to UV	2	2
<i>MYBPC3</i> c.530G>A p.(Arg177His)	UV to unlikely	2	2
<i>MYBPC3</i> c.649A>G p.(Ser217Gly)	UV to unlikely	3	7
<i>MYBPC3</i> c.961G>A p.(Val321Met)	UV to unlikely	3	6
<i>MYBPC3</i> c.1519G>A p.(Gly507Arg)	UV to unlikely	3	5
<i>MYBPC3</i> c.2870C>G p.(Thr957Ser)	UV to unlikely	2	2
<i>MYBPC3</i> c.2914C>T p.(Arg972Trp)	UV to unlikely	1	1
<i>PRKAG2</i> c.250C>T p.(Arg84Trp)	UV to unlikely	1	1
	Total	70	156

Variant Review

This data highlights a need for all clinical laboratories to review variants in light of new information.

- Review active variants with a frequency of 12 months
 - Variant detected in a new proband
 - Family test requested
 - Requested by the referring Clinician
 - Large new cohort of data is released
- Always report on the current classification
 - Sometimes discrepancy between different laboratories classifications
 - Contact with clinical team - OK to proceed?
 - Additional explanatory information on the report
- Review variants prior to clinic date for family testing

Future improvements

- **Guidelines on:**
 - Who is responsible for flagging variants for review?
 - How often should variants be reviewed?
 - What is the mechanism for review?
 - Which re-classifications require communication?
- **Bioinformatic solutions:**
 - Automatically extrapolate new information for review
 - Automatic variant reviews
 - Administration of re-classification letters
- Consistent approach to variant review and re-classification amongst ACGS laboratories.

Acknowledgements

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Department of Clinical Genetics

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Geneticist

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