Precision Cardiovascular Medicine for **Multiethnic** Populations

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BULLS
2
© J. Warren

How can we make **rational** decisions with molecular data?

Amos Tversky Daniel Kahneman

probabilities + utilities

= **rational** decisions

… but we often stray

big data can help us
compute missing **probabilities**
and inform **rational** decisions
across **demographic** groups that are
underrepresented in past studies

The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL ARTICLE

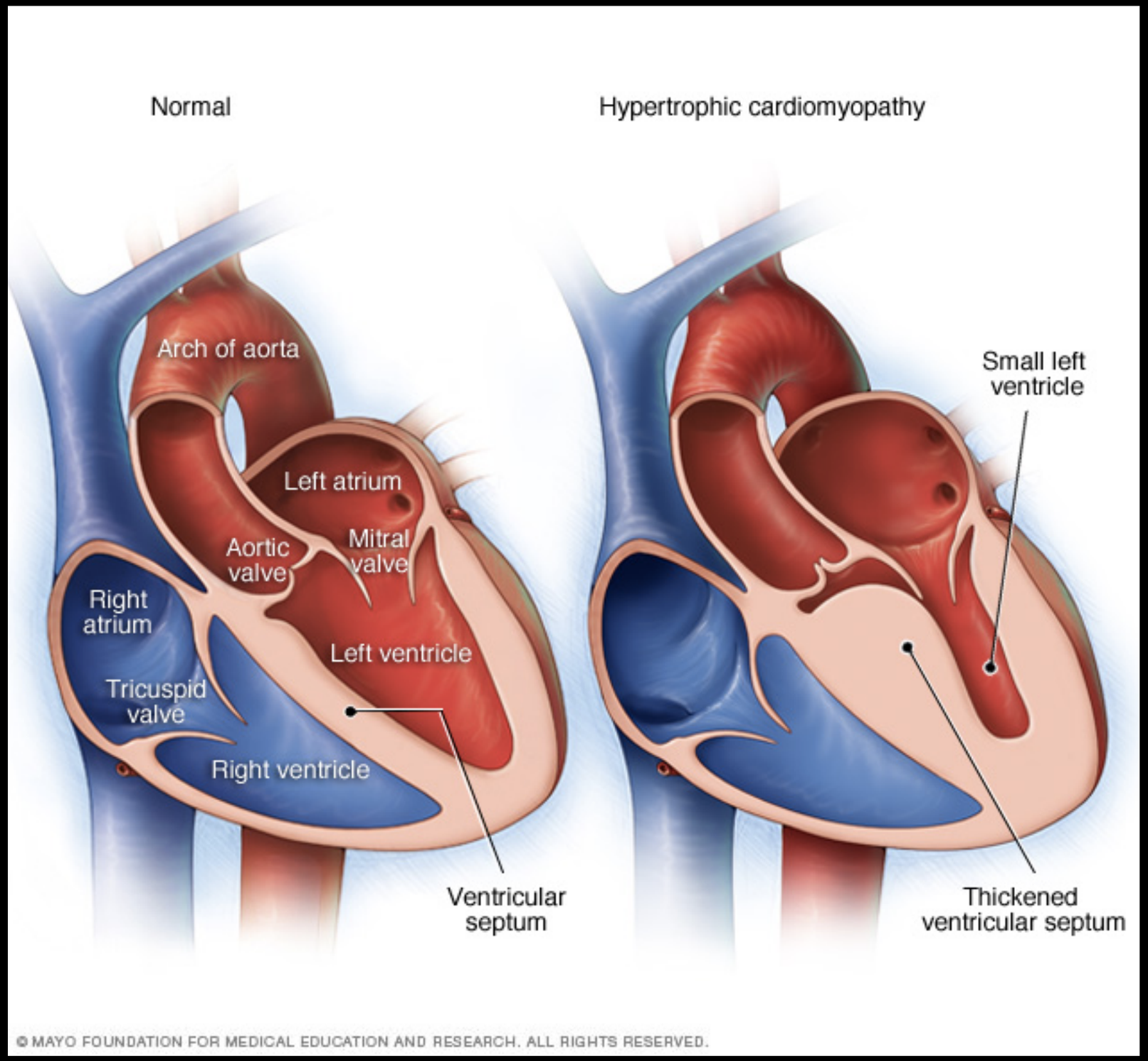
Genetic Misdiagnoses and the Potential for Health Disparities

Arjun K. Manrai, Ph.D., Birgit H. Funke, Ph.D., Heidi L. Rehm, Ph.D., Morten S. Olesen, Ph.D., Bradley A. Maron, M.D., Peter Szolovits, Ph.D., David M. Margulies, M.D., Joseph Loscalzo, M.D., Ph.D., and Isaac S. Kohane, M.D., Ph.D.

ABSTRACT

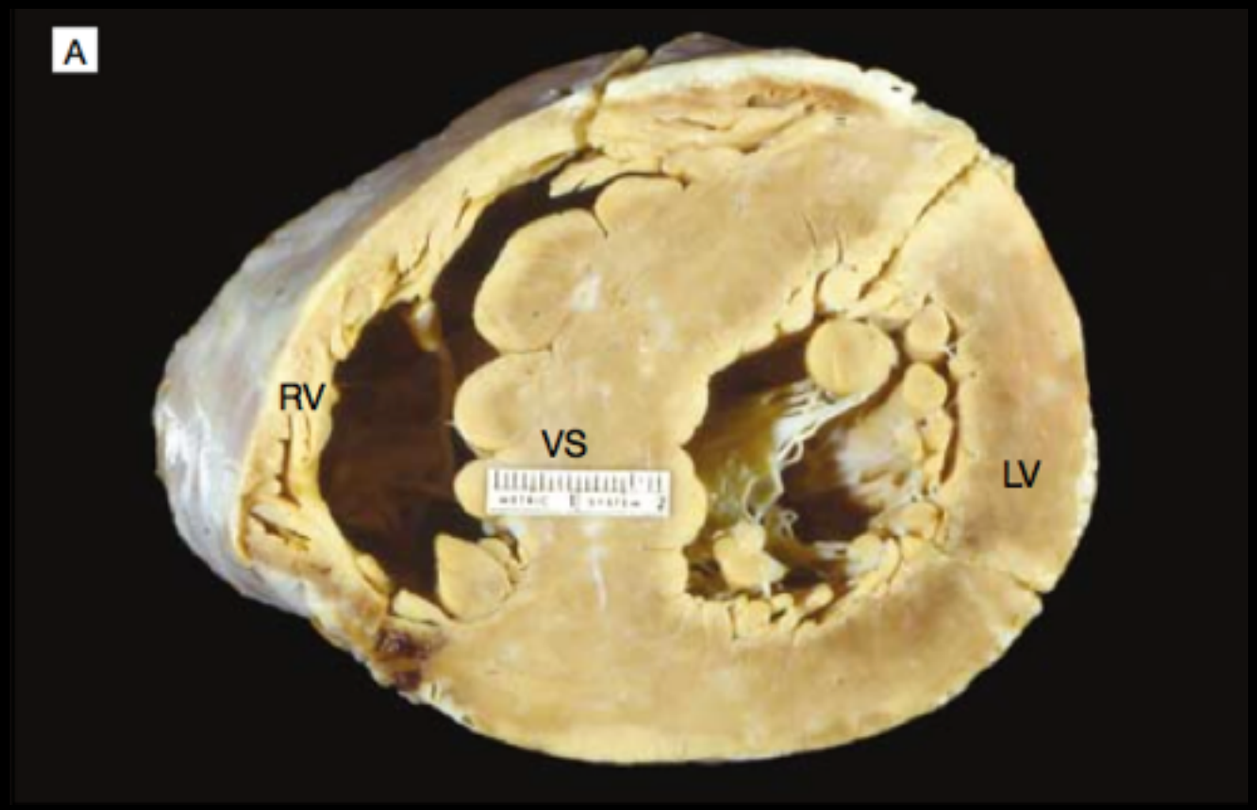
Manrai et al., *New England Journal of Medicine*, 2016

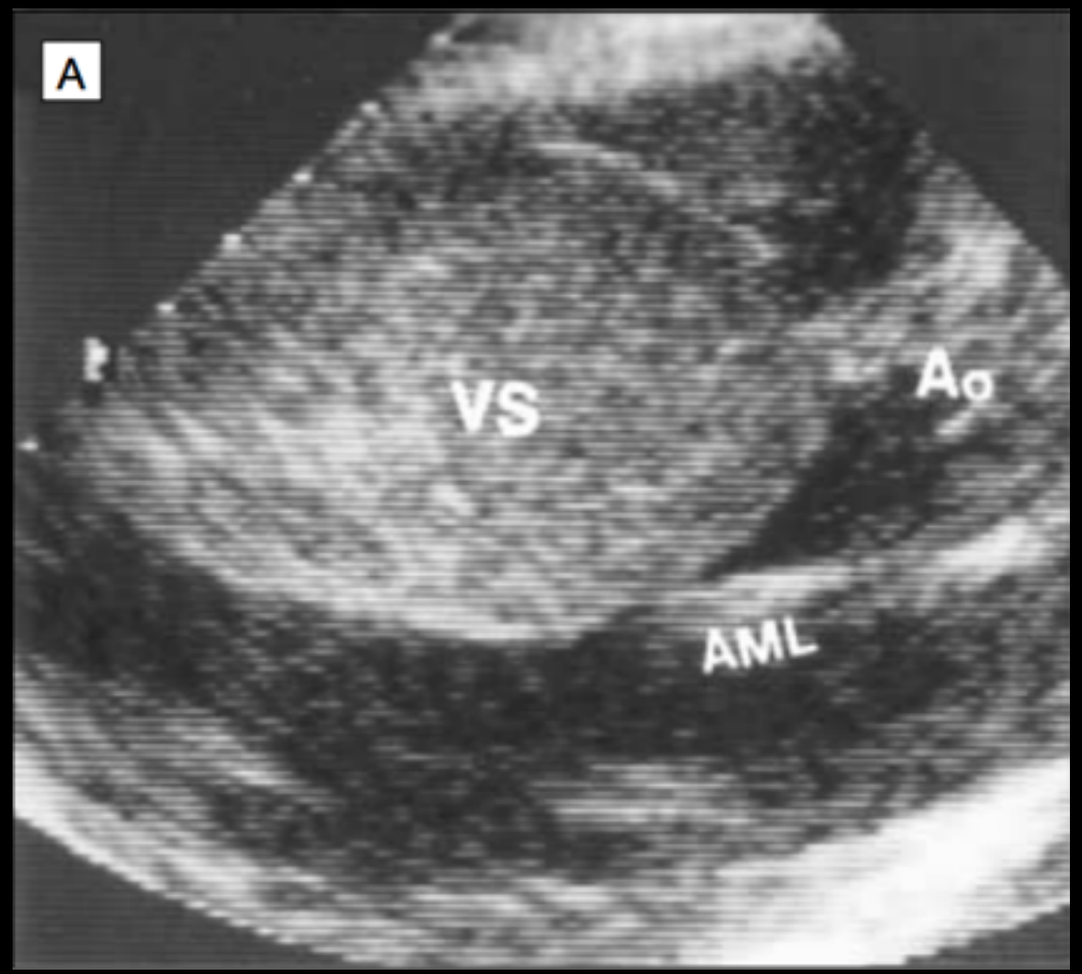
Hypertrophic Cardiomyopathy (HCM)

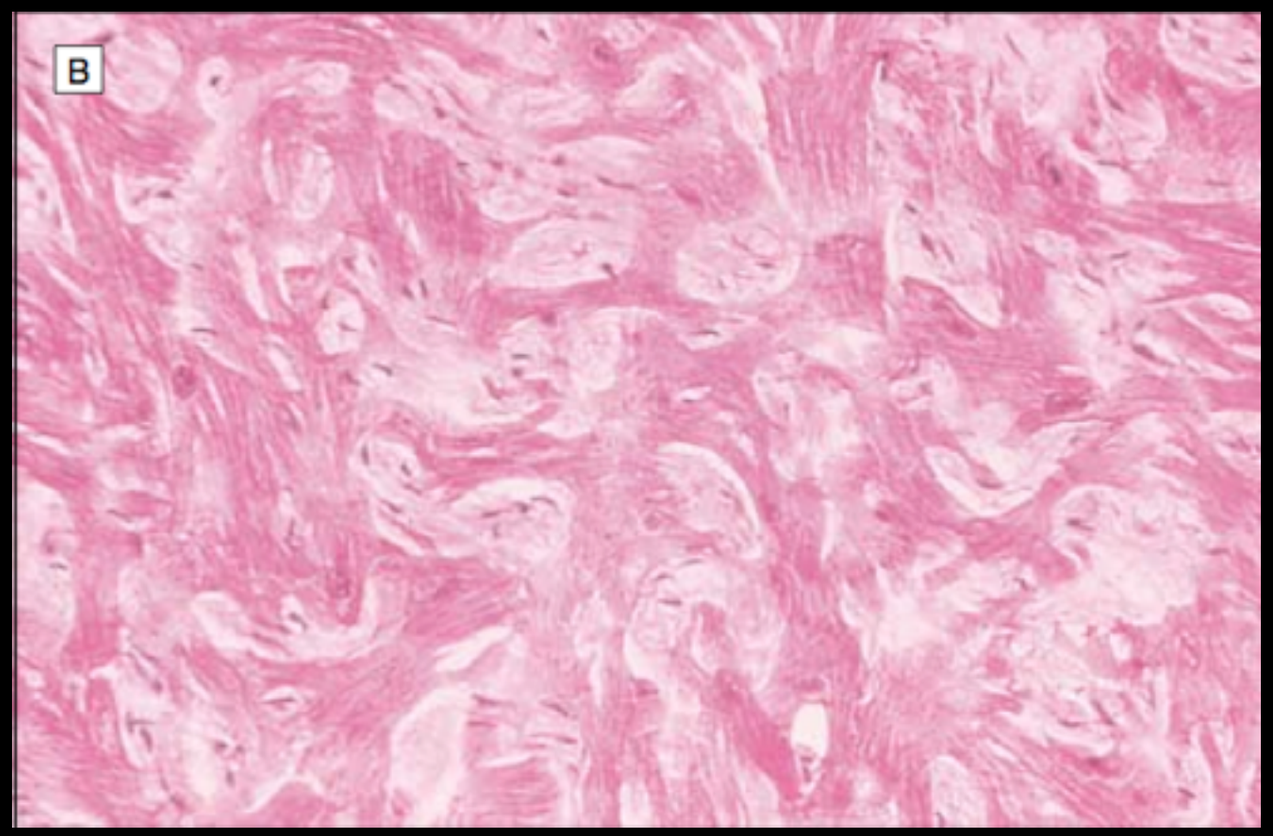


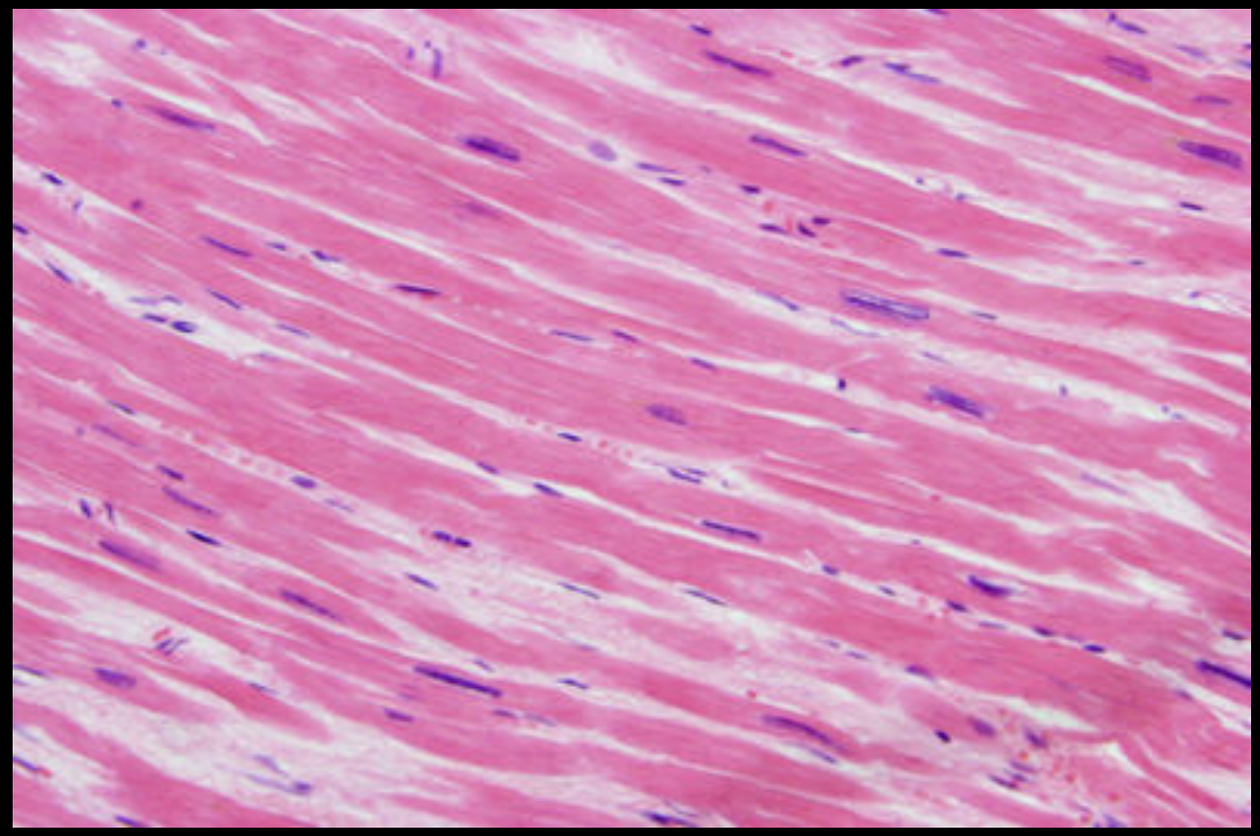
Heart failure
Arrhythmias
Obstructed blood flow
Infective endocarditis
Sudden cardiac death

Maron et al., *Circulation* 1995









Maron et al., *JAMA* 2002

Cell, Vol. 62, 999–1006, September 7, 1990, Copyright © 1990 by Cell Press

A Molecular Basis for Familial Hypertrophic Cardiomyopathy: A β Cardiac Myosin Heavy Chain Gene Missense Mutation

Anja A.T. Geisterfer-Lowrance★, Susan Kass†, Gary Tanigawa†, Hans-Peter Vosberg‡, William McKenna§, Christine E. Seidman★, J.G. Seidman†

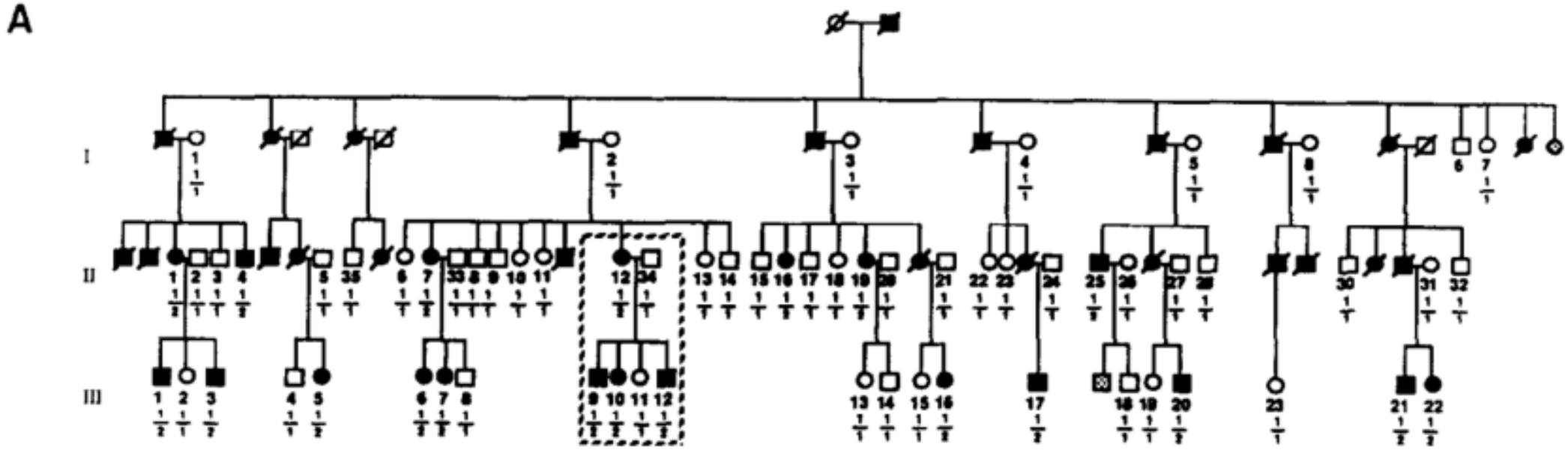
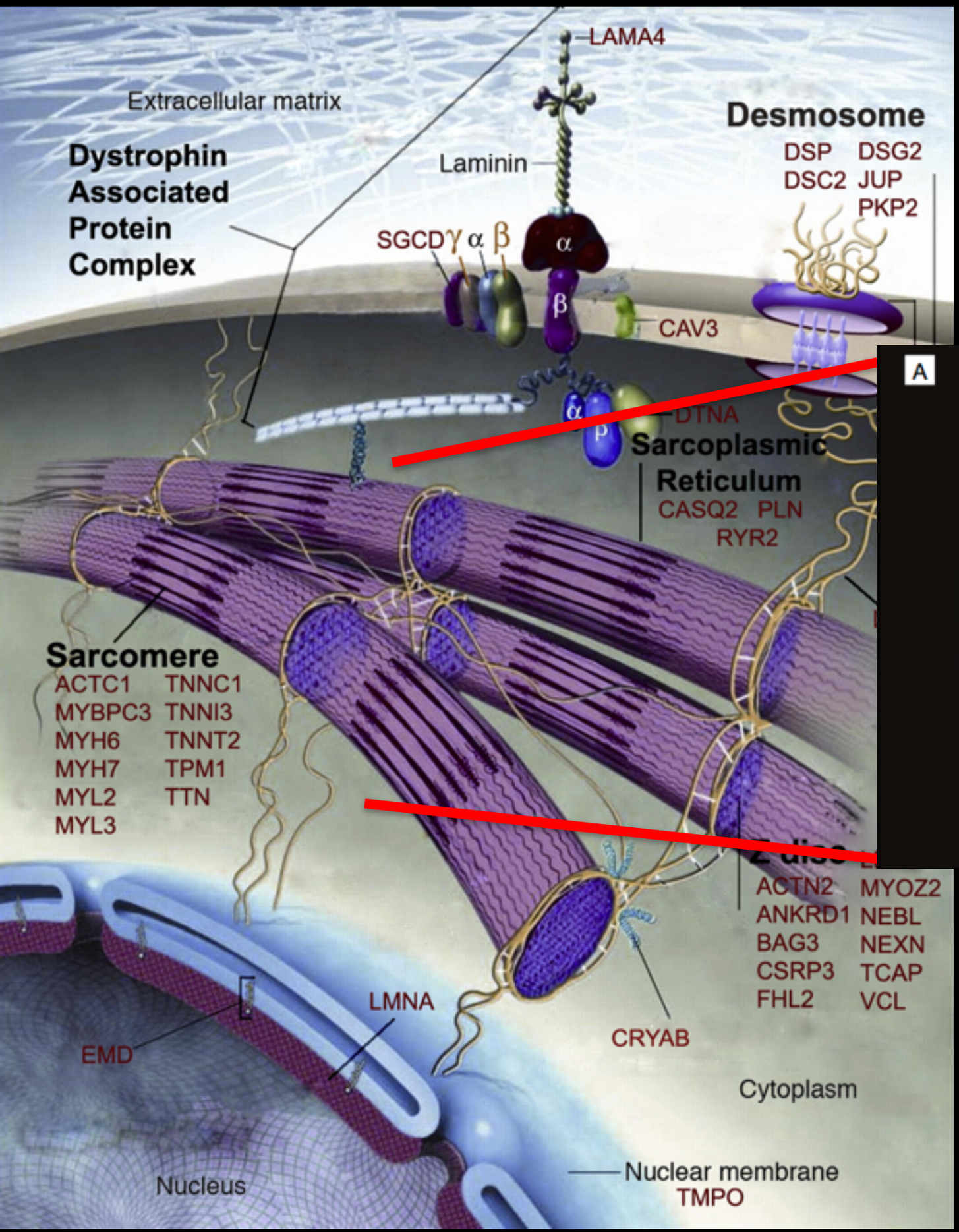
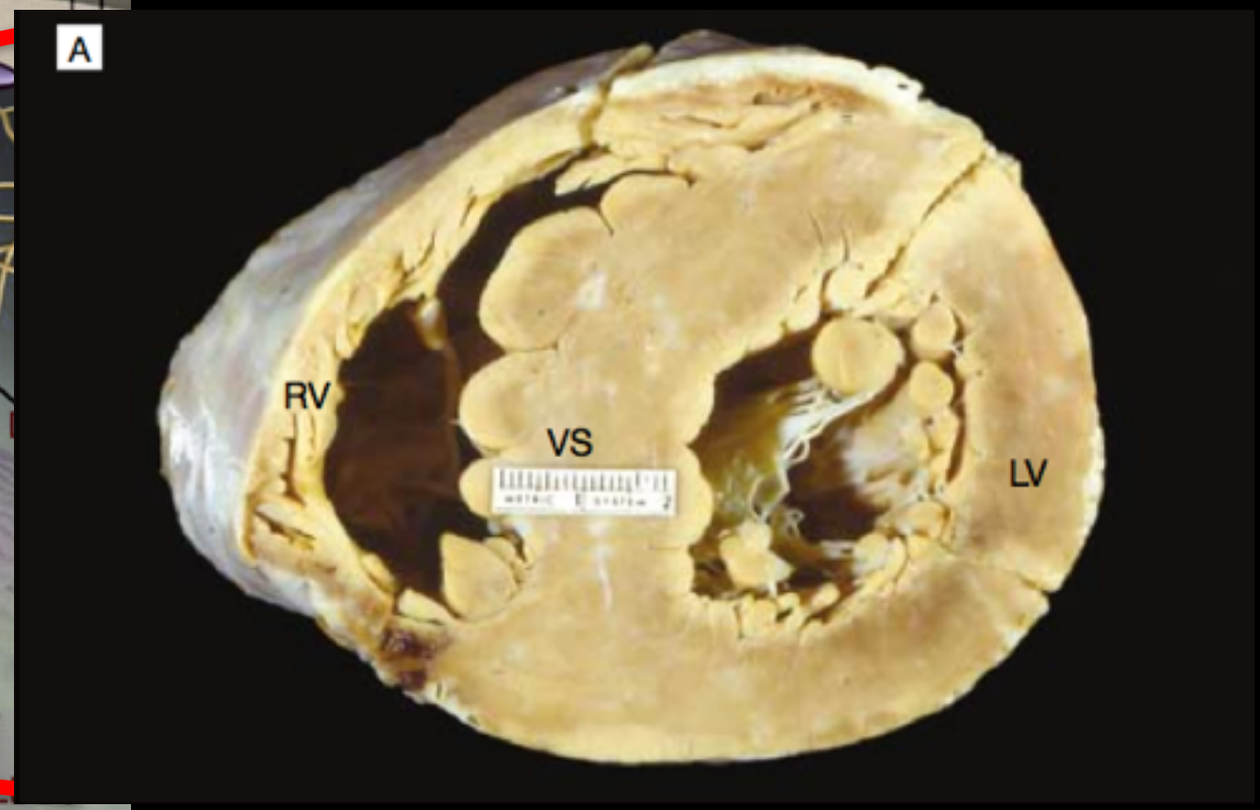


Figure 1. Inheritance of FHC and the βD-.425 Polymorphism in Family A

(A) The pedigree of family A is presented using standard nomenclature. Males (squares) and females (circles) are identified by generation and subject number. The disease status of each individual is indicated by shading: closed symbols, affected; open symbols, unaffected. Deceased individuals are represented by a slash. The genotype of each individual is shown. Allele 1 indicates a 425 bp fragment, and allele 2 represents a 385 bp fragment identified by βD-.425.

(B) Southern blot of DdeI-digested DNAs from members of a small nuclear family from family A (dashed box in [A]) hybridized to the βD-.425 probe (see Experimental Procedures).


LAMA4
Extracellular matrix
Desmosome
DSP DSG2
DSC2 JUP
PKP2
Dystrophin
Associated
Protein
Complex
Laminin
SGCD γ α β
α
β
CAV3
DTNA
Sarcoplasmic
Reticulum
CASQ2 PLN
RYR2
Sarcomere
ACTC1 TNNC1
MYBPC3 TNNI3
MYH6 TNNT2
MYH7 TPM1
MYL2 TTN
MYL3
ACTN2 MYOZ2
ANKRD1 NEBL
BAG3 NEXN
CSRP3 TCAP
FHL2 VCL
LMNA
CRYAB
EMD
Cytoplasm
Nucleus
Nuclear membrane
TMPO


A
RV
VS
LV

MOLECULAR DIAGNOSTICS REPORT

Specimen Type:	Blood, Peripheral	**Received Date:**	08/07/2008
Related Accession(s):		**Referring Facility:**	UNIV OF AMERICA
Referring Physician:	DR. SMITH	**Referring Fac. MRN:**	12345678
Copies To:	OTHER CONTACTS, MS, CGC SENDOUT UNIVERSITY OF AMERICA	**Lab Control Number:**	00-222-55555
		Family Number:	F000000

TEST DESCRIPTION – HCM Panel (18 Genes)
Sequence Confirmation Test
Copy Number Variation Analysis

TEST PERFORMED – PCM-pnlB; SeqConfirm; CNV-a

INDICATION FOR TEST – Clinical features of HCM

RESULTS

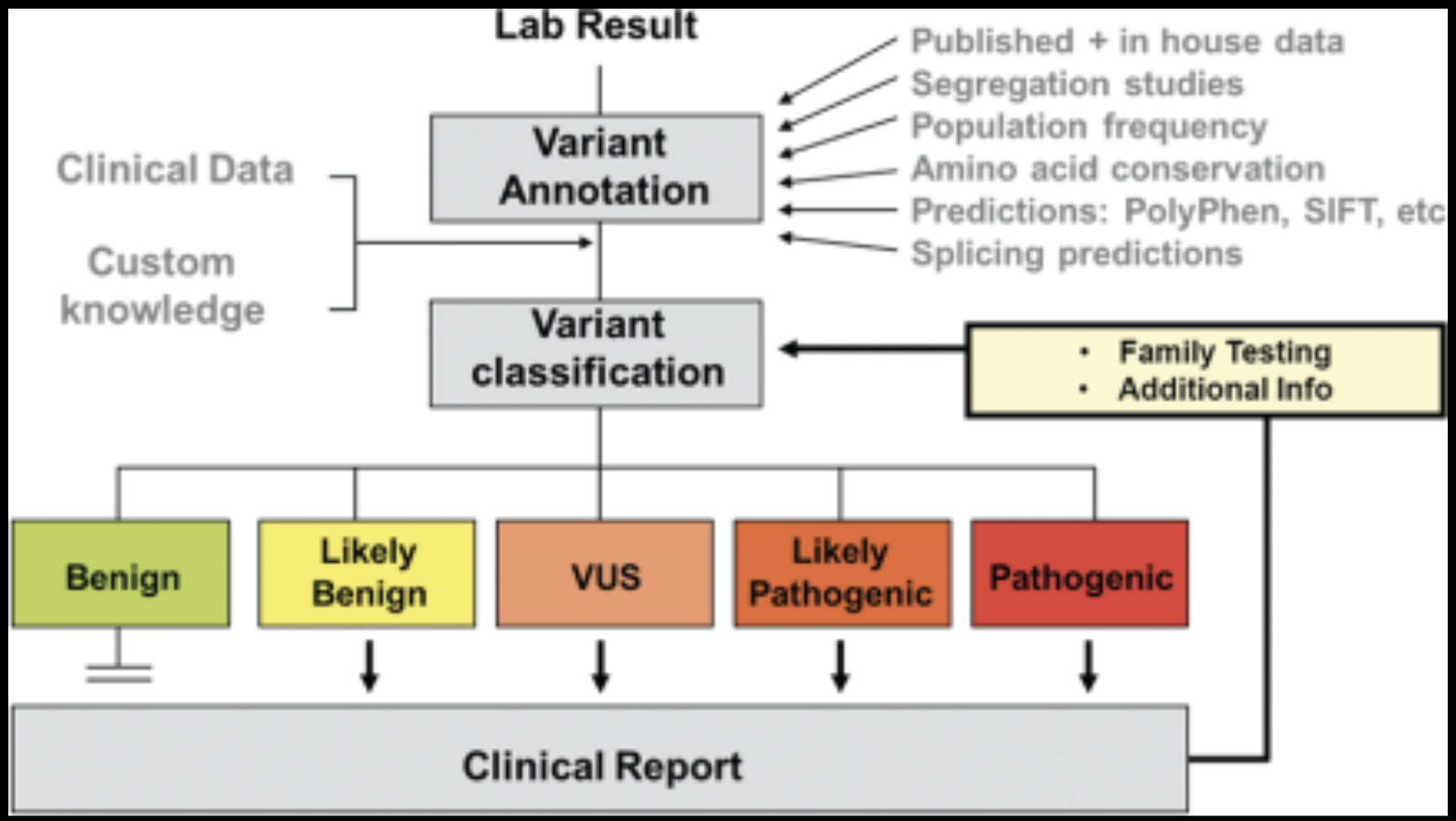
DNA VARIANTS:
Heterozygous c.1504C>T (p.Arg502Trp), Exon 17, MYBPC3, Pathogenic

INTERPRETATION:
Positive. DNA sequencing and copy number assessment of the coding regions and splice sites of ACTC1, ACTN2, CSRP3, GLA, LAMP2, MYBPC3, MYH7, MYL2, MYL3, MYOZ2, NEXN, PLN, PRKAG2, TNNC1, TNNI3, TNNT2, TPM1 and TTR identified the variant listed above.

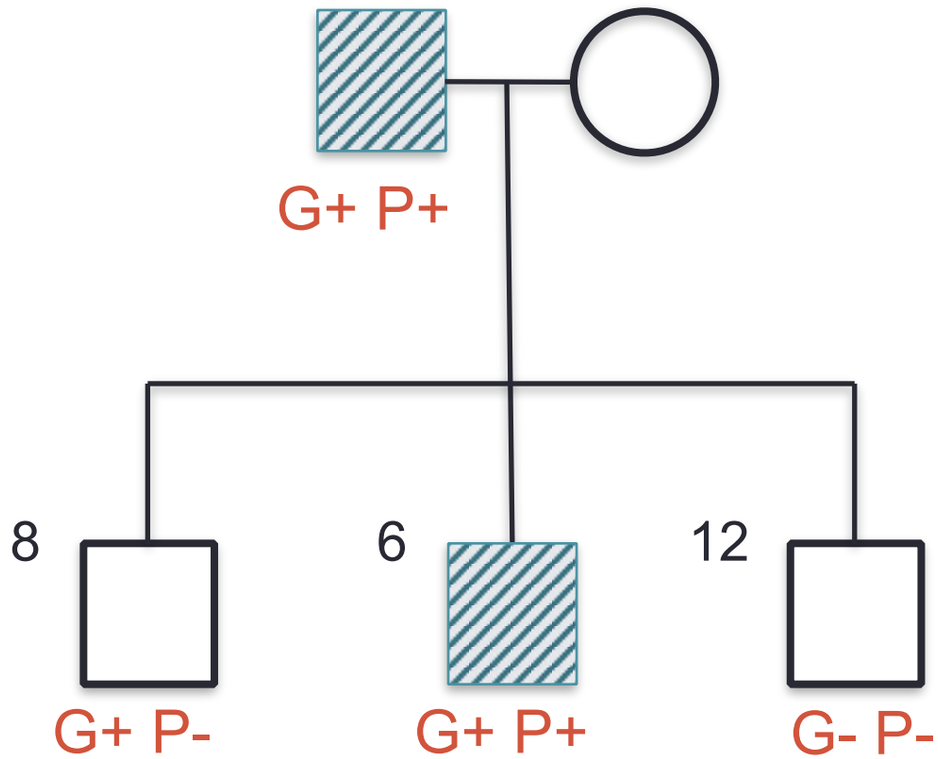
SUMMARY (see below for variant interpretations): This individual carries a pathogenic variant in MYBPC3, which is consistent with the clinical diagnosis of HCM.

Cardiomyopathy due to pathogenic variants in the MYBPC3 gene is typically inherited in an autosomal dominant pattern. Each first-degree relative has a 50% (or 1 in 2) chance of inheriting a variant and its risk for cardiomyopathy. Disease penetrance and severity can vary due to modifier genes and/or environmental factors. The significance of a variant should therefore be

Current scale for reporting variants



Duzkale et al. *Clinical Genetics* 2013.



Risk Stratification

KEY

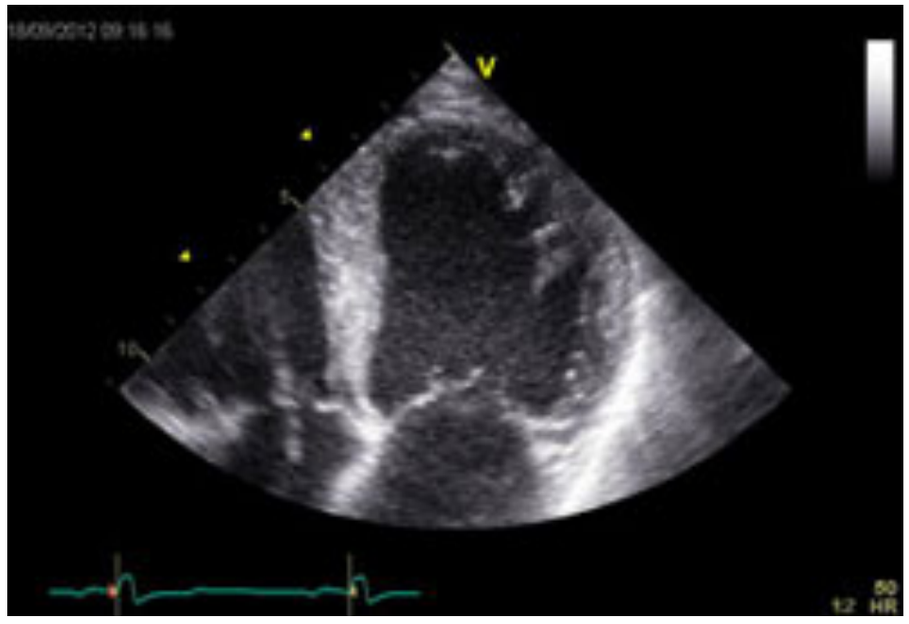
- Affected male
- Unaffected male
- Unaffected female
- Affected female

Cardiac follow-up, lifestyle modifications

NO Cardiac follow-up

P? resolve ambiguous clinical presentation

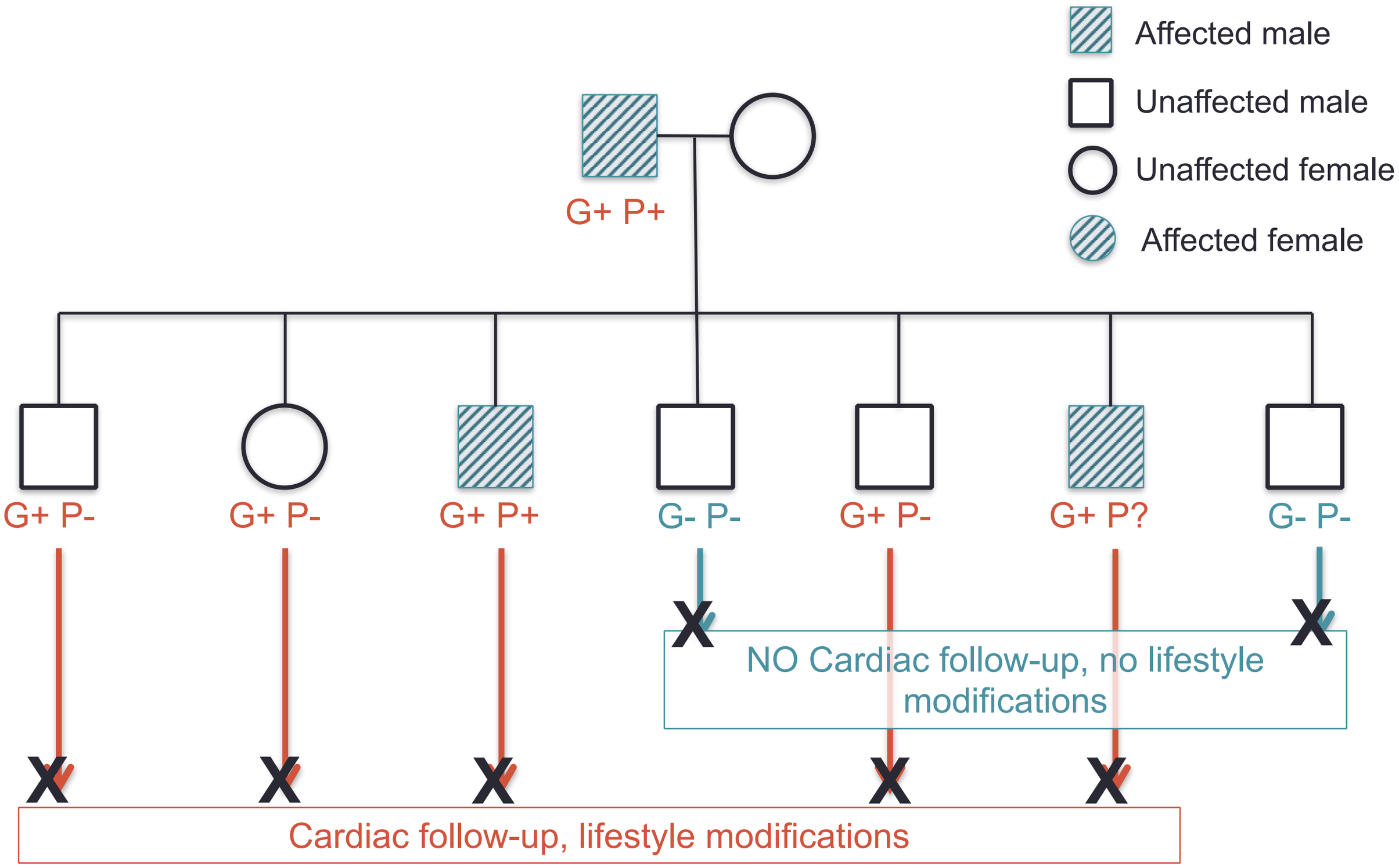
↑ICD / lifestyle modifications..etc.



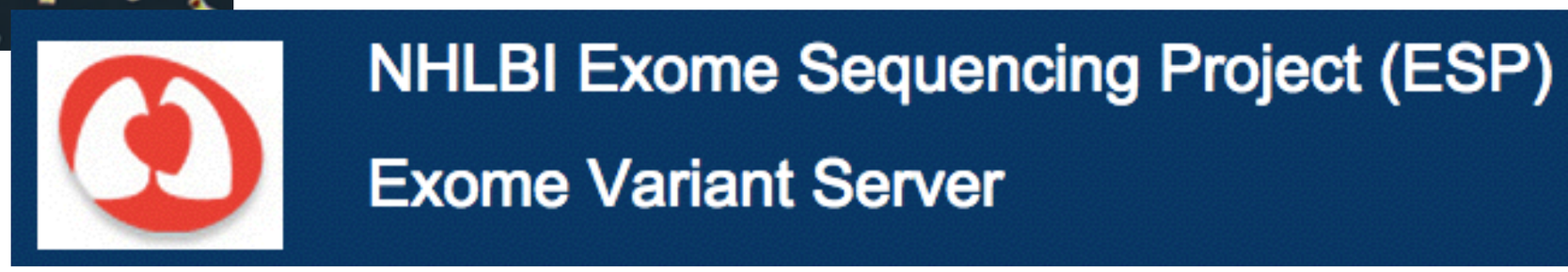
Challenge Question #1

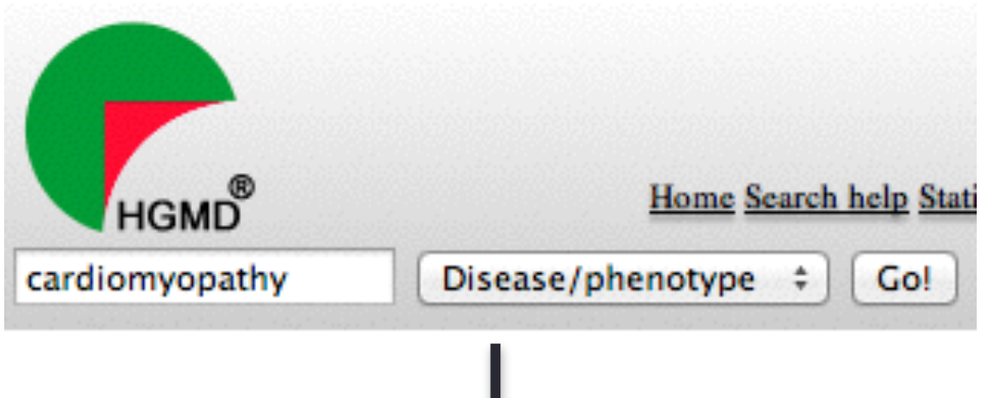
What are the chances that a son inherits his father's HCM pathogenic mutation in *MYBPC3* (Chr. 11)?

(a) 100%
(b) 50%
(c) 25%
(d) 0.2% (general population prevalence)



When do variant classifications change?



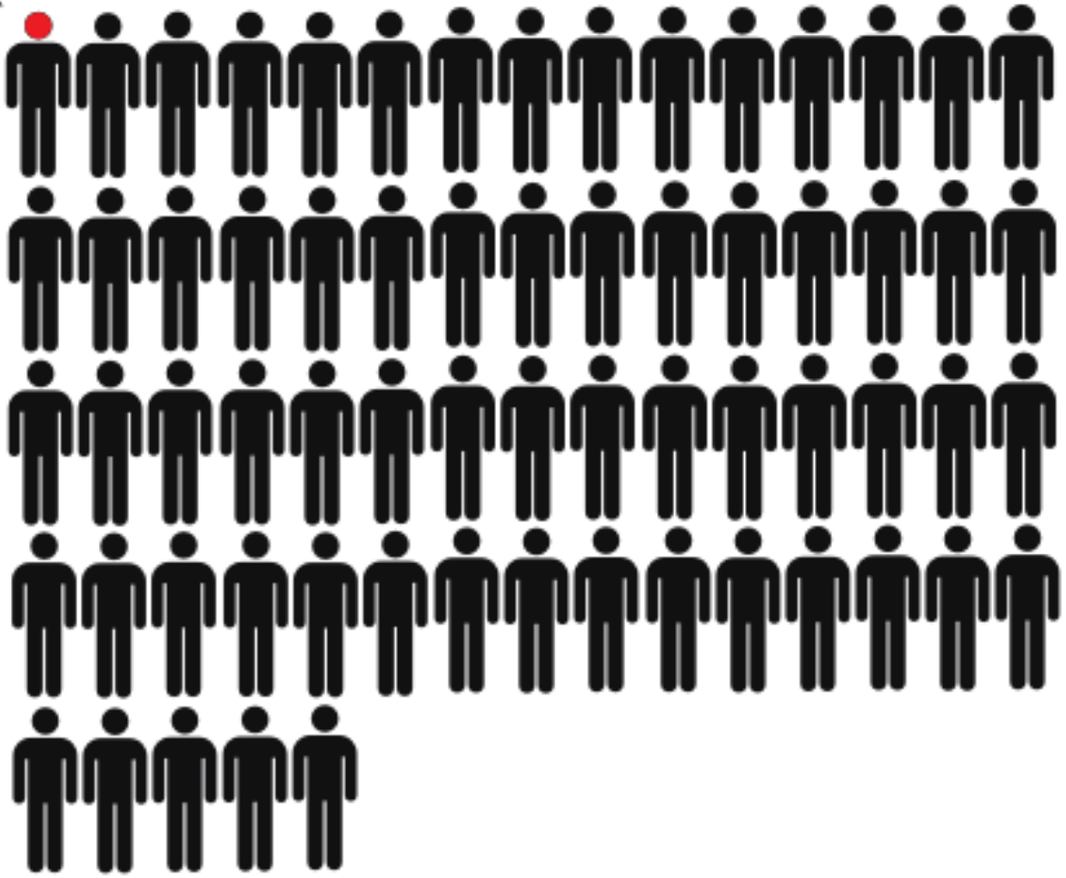


“pathogenic/disease causing” mutations in 84 cardiomyopathy genes

measure genotype frequency

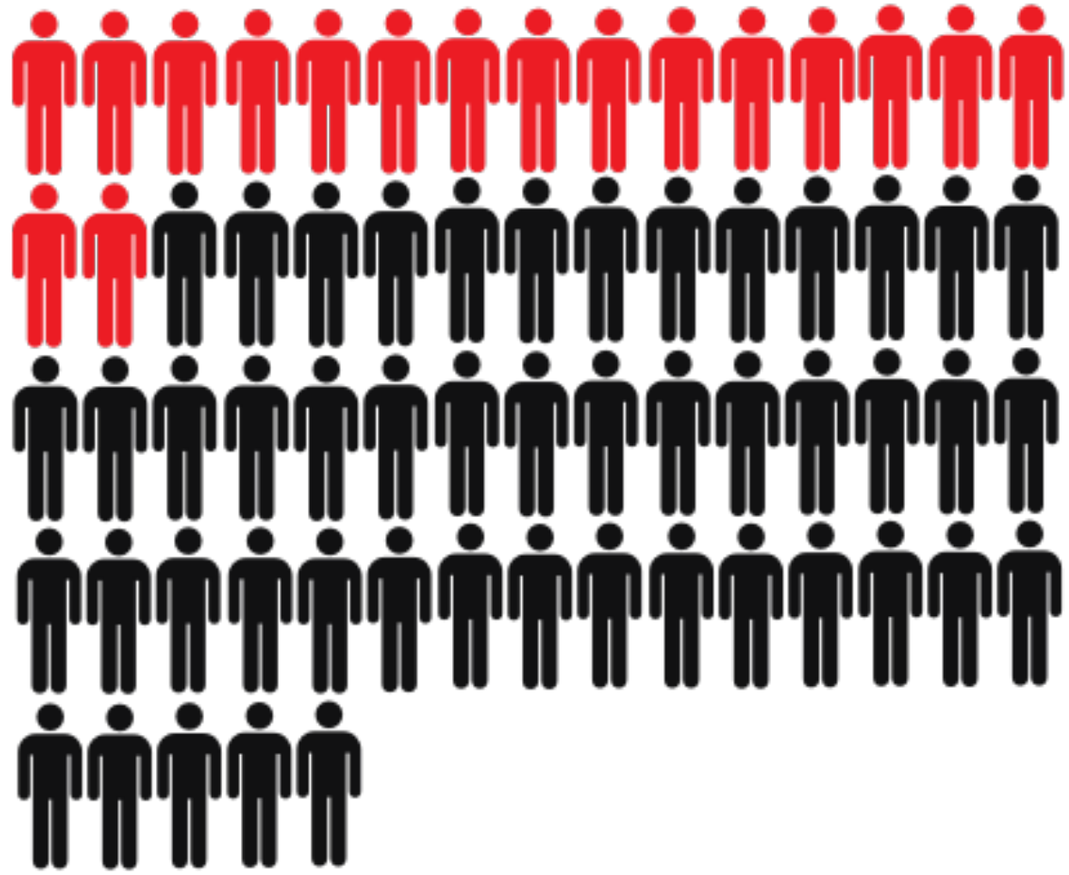
NHLBI ESP (4300 EAs, 2203 AAs)

NHLBI ESP



expected

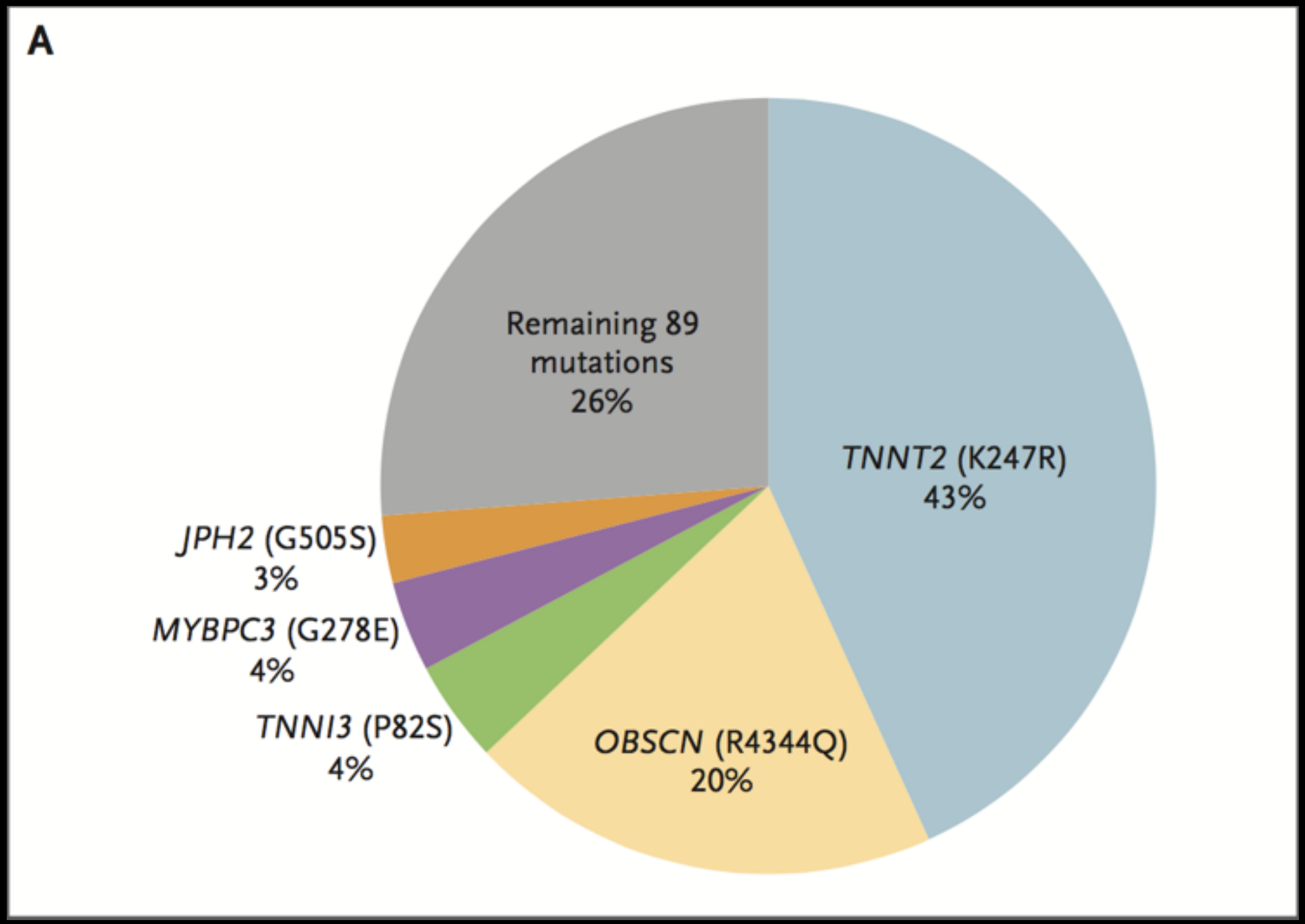
NHLBI ESP



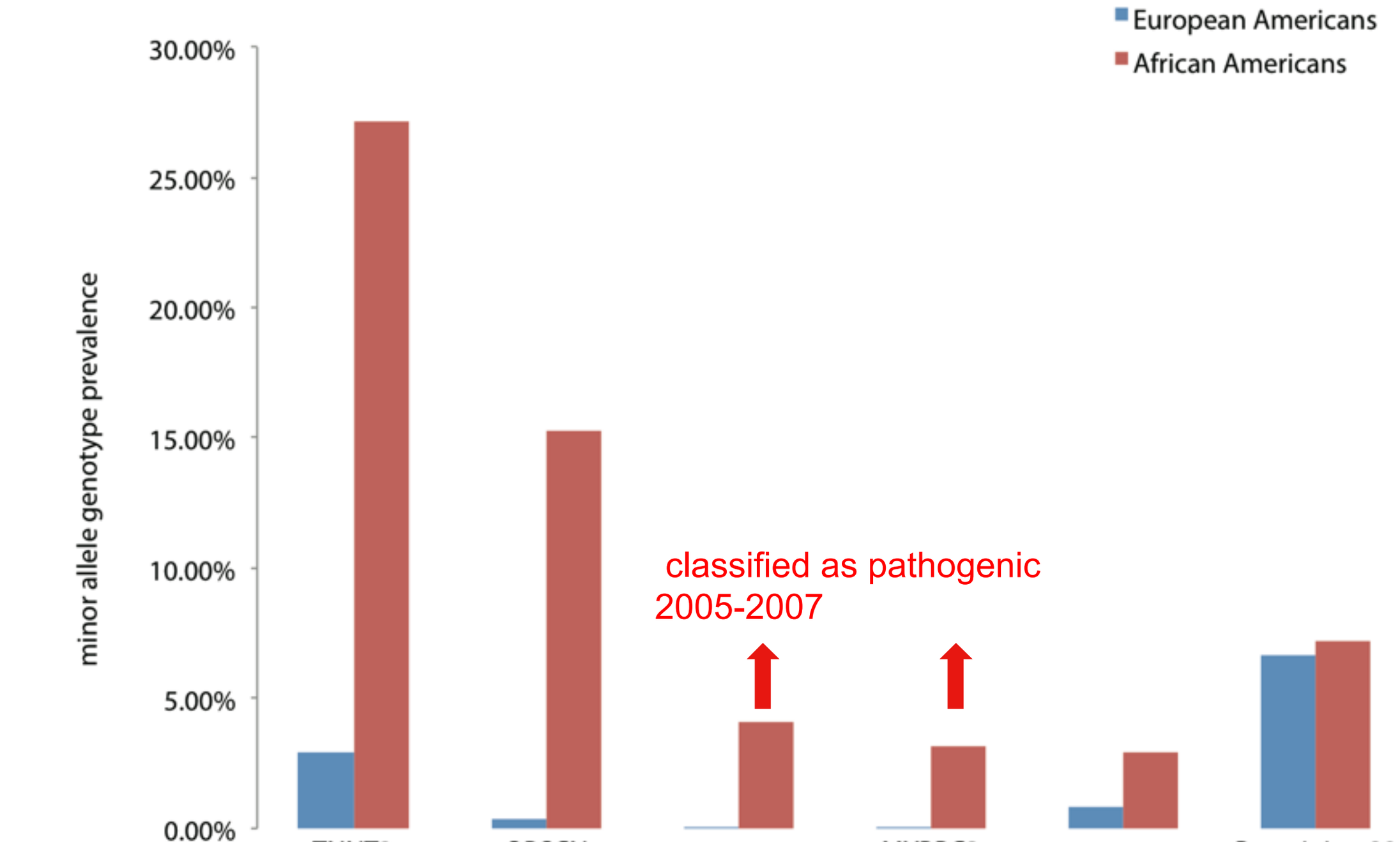
observed

100 individuals

HCM Prevalence = 1:500
HCM Inheritance = Autosomal Dominant



Manrai et al., *New England Journal of Medicine*, 2016



	TNNT2 (K247R)	OBSCN (R4344Q)	TNNI3 (P82S)	MYBPC3 (G278E)	JPH2 (G505S)	Remaining 89 mutations
European Americans	2.88%	0.33%	0.03%	0.02%	0.80%	6.67%
African Americans	27.14%	15.27%	4.07%	3.15%	2.92%	7.18%

All P/LP misclassifications in patients of African or unspecified ancestry

Table 1. Clinical Findings for High-Frequency Variants Associated with Hypertrophic Cardiomyopathy.

Originally Reported Status of Variant*	Patient's Age	Patient's Ethnic Background	Report Year	Report Result	Variant	Most Significant Pathogenic Variant†	Indication for Test
Pathogenic	46 yr	Unavailable	2005	Positive	*TNNI3* (P82S)	Yes	Clinical diagnosis of hypertrophic cardiomyopathy
Pathogenic	75 yr	Unavailable	2005	Positive	*TNNI3* (P82S)	Yes	Family history and clinical symptoms of hypertrophic cardiomyopathy
Presumed pathogenic	32 yr	African ancestry	2005	Positive	*TNNI3* (P82S)	No	Clinical diagnosis of hypertrophic cardiomyopathy
Pathogenicity debated	34 yr	African ancestry	2005	Positive	*TNNI3* (P82S)	No	Clinical diagnosis and family history of hypertrophic cardiomyopathy
Unknown significance	12 yr	African ancestry	2006	Inconclusive	*TNNI3* (P82S)	Yes	Family history of hypertrophic cardiomyopathy
Unknown significance	40 yr	African ancestry	2007	Inconclusive	*TNNI3* (P82S)	Yes	Clinical diagnosis of hypertrophic cardiomyopathy
Unknown significance	45 yr	African ancestry	2007	Inconclusive	*TNNI3* (P82S)	Yes	Clinical features of hypertrophic cardiomyopathy
Unknown significance	16 yr	Asian ancestry	2008	Positive	*TNNI3* (P82S)	No	Clinical diagnosis and family history of hypertrophic cardiomyopathy
Presumed pathogenic	59 yr	African ancestry	2006	Positive	*MYBPC3* (G278E)	Yes	Clinical features of hypertrophic cardiomyopathy
Presumed pathogenic	15 yr	African ancestry	2007	Positive	*MYBPC3* (G278E)	Yes	Clinical diagnosis of hypertrophic cardiomyopathy
Presumed pathogenic	16 yr	African ancestry	2007	Positive	*MYBPC3* (G278E)	Yes	Clinical diagnosis of hypertrophic cardiomyopathy
Presumed pathogenic	22 yr	African ancestry	2007	Positive	*MYBPC3* (G278E)	No	Clinical diagnosis and family history of hypertrophic cardiomyopathy
Unknown significance	48 yr	African ancestry	2008	Positive	*MYBPC3* (G278E)	No	Clinical diagnosis of hypertrophic cardiomyopathy

* All variants subsequently have been reclassified as benign.
† Information in this column indicates whether the variant was unequivocally the most pathogenic variant in the original report that was provided to the patient.

Manrai et al., *New England Journal of Medicine*, 2016

Table 2. Studies That Initially Implicated High-Frequency Variants Associated with Hypertrophic Cardiomyopathy.

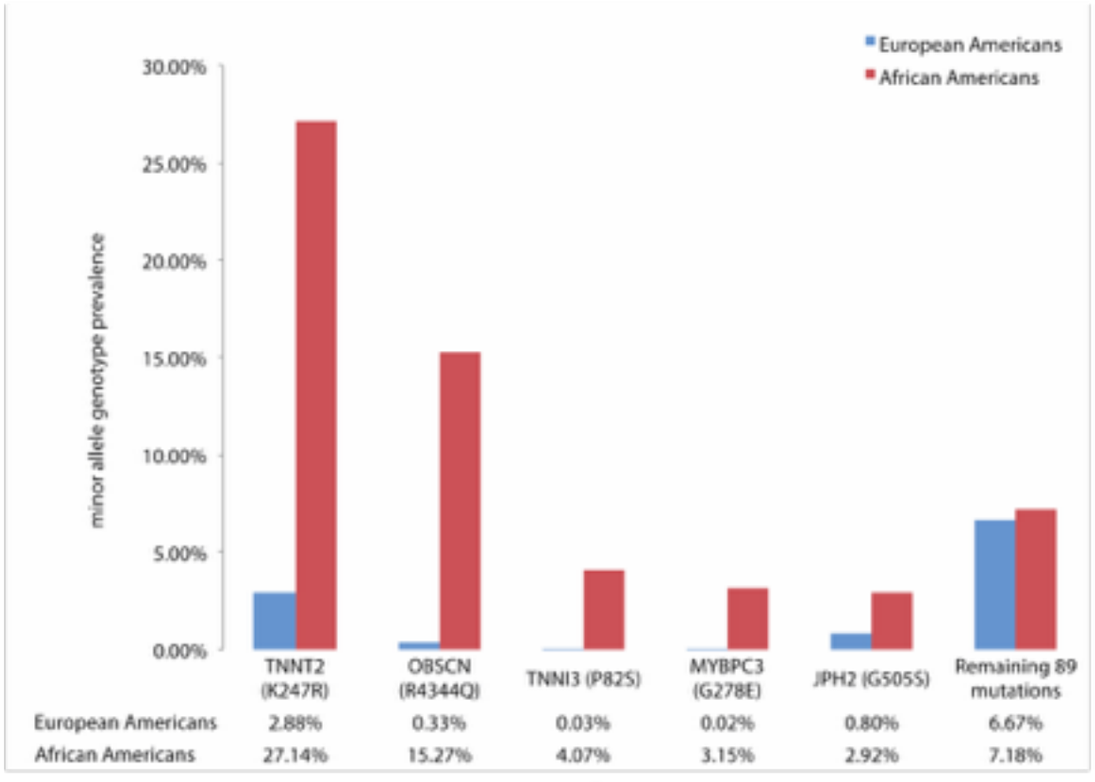
Gene (Variant)	Reference	Discovery Phase	No. of Cases	No. of Controls	Variant Assessment		Country	Included in LMM Clinical Panel
					In Vitro	In Vivo		
TNNT2 (K247R)	García-Castro et al.[21]	Targeted gene sequencing of unrelated cases and controls from Asturias	30	200	No	No	Spain	Yes
*OBSCN** (R4344Q)	Arimura et al.[22]	Targeted gene sequencing of unrelated Japanese cases and controls	144	288	Yes	No	Japan	No
TNNI3 (P82S)	Niimura et al.[23]	Targeted gene sequencing of unrelated cases and controls†	31	85	No	No	United States	Yes
MYBPC3 (G278E)	Richard et al.[24]	Targeted gene sequencing of unrelated cases and controls‡	197	100	No	No	France	Yes
*JPH2** (G505S)	Matsushita et al.[25]	Targeted gene sequencing of Japanese cases and controls	195	236	Yes	No	Japan	No

* *OBSCN* and *JPH2* have never been included in cardiomyopathy testing at the Laboratory for Molecular Medicine (LMM).
† No specific ethnic background was provided, but "informed consent was obtained in accordance with human subject committee guidelines at Brigham and Women's Hospital, St. George's Hospital Medical School [U.K.], and Minneapolis Heart Institute Foundation."[23]
‡ "Patients were recruited in France, and most of them were of European origin."[24] The sample of patients included persons of African ancestry (Richard P: personal communication).

Manrai et al., *New England Journal of Medicine*, 2016

Studies took place around the world but not in Africa

TNNI3, P82S

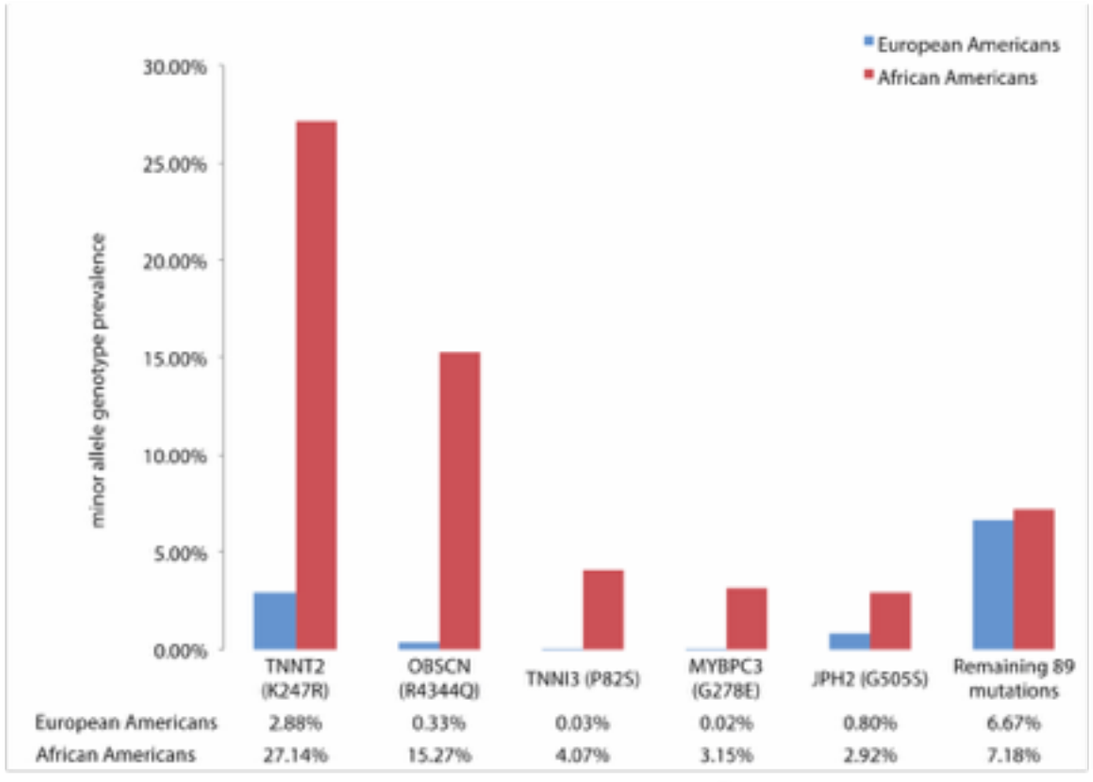


No ethnicity information provided, but three separate populations

85 controls

Niimura et al. 2002

MYBPC3, G278E

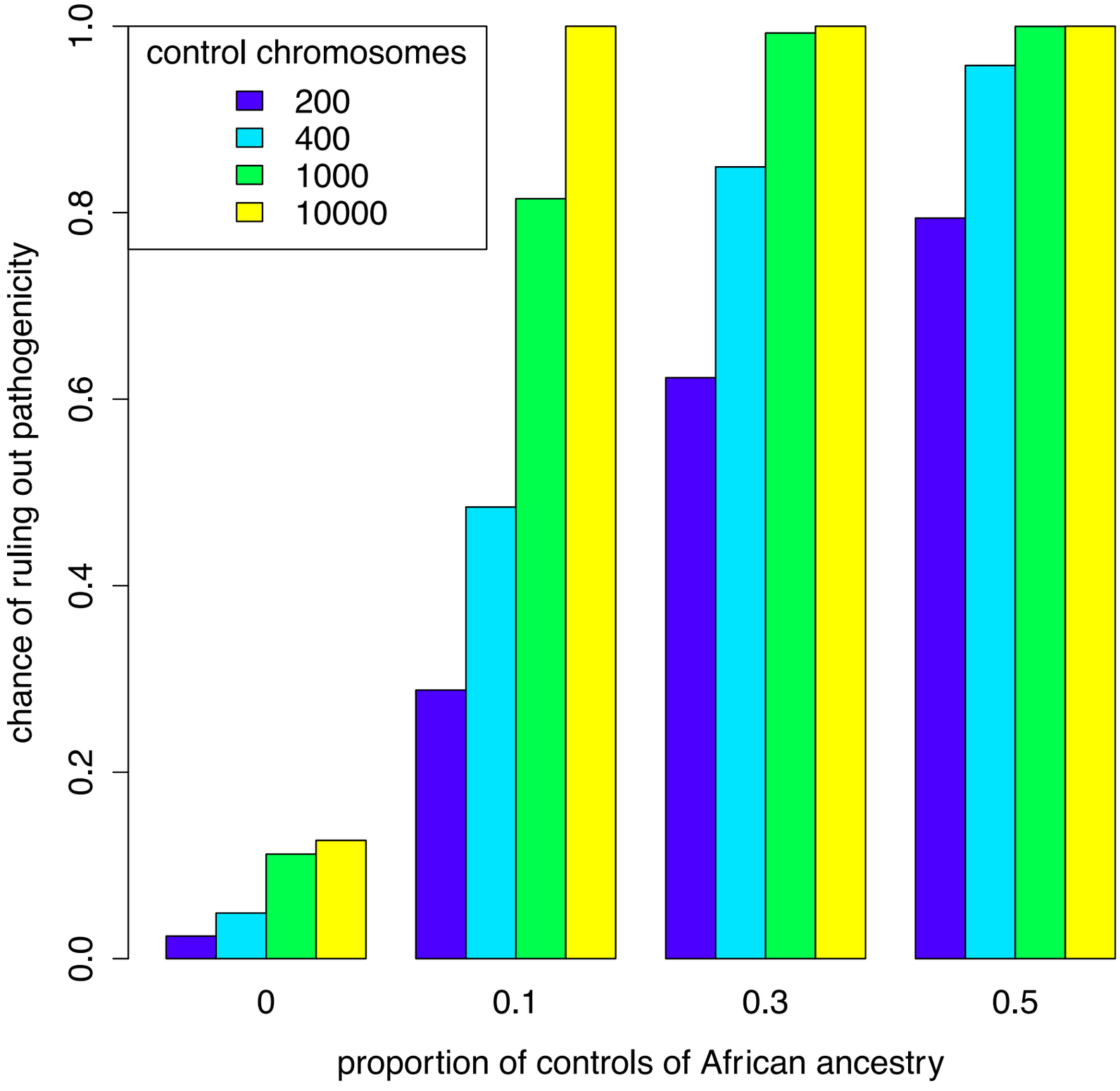


"Patients were recruited in France, and most of them were of European origin."

100 controls

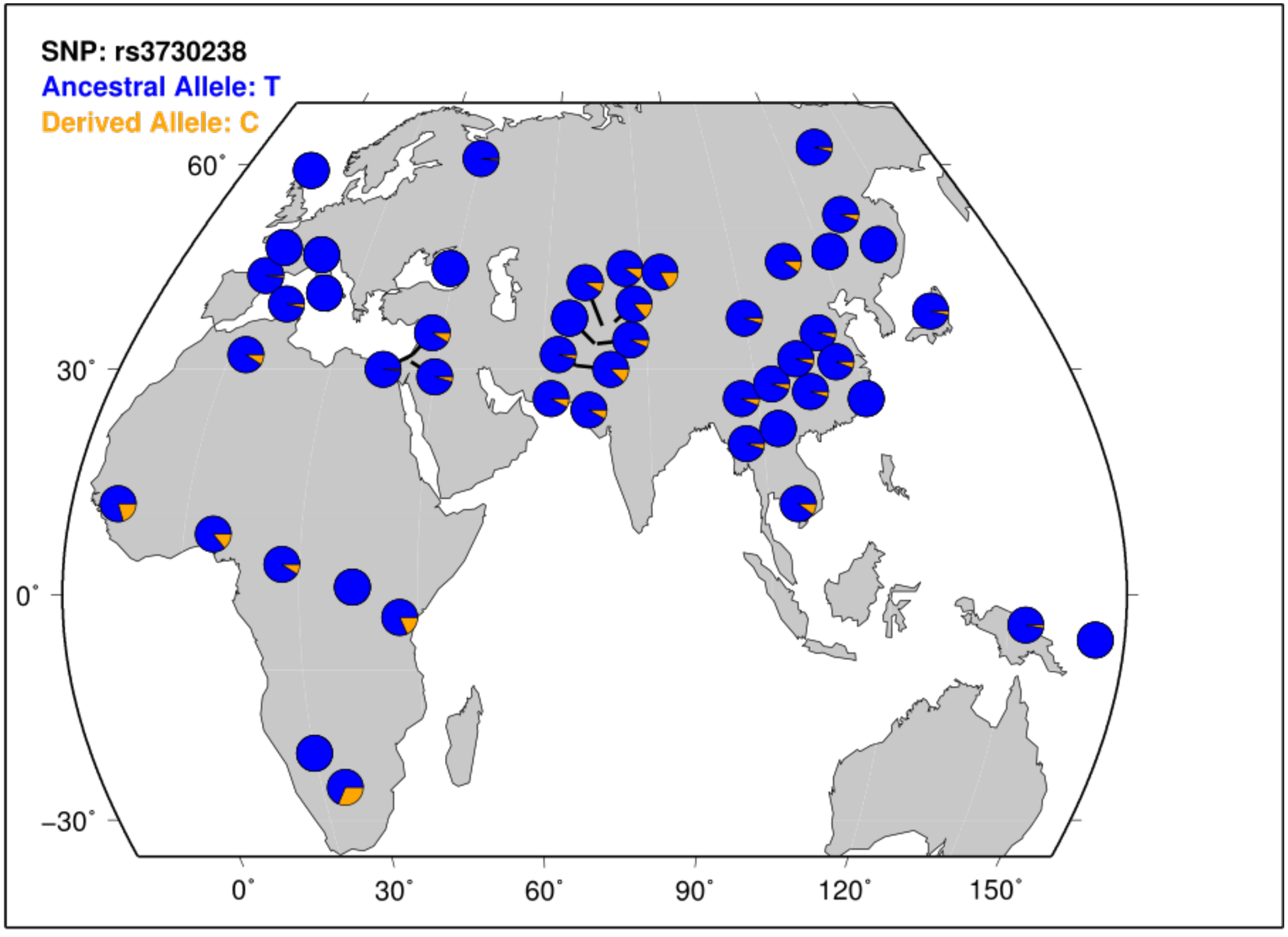
Richard et al. 2003

Including African American controls would have ruled out pathogenicity

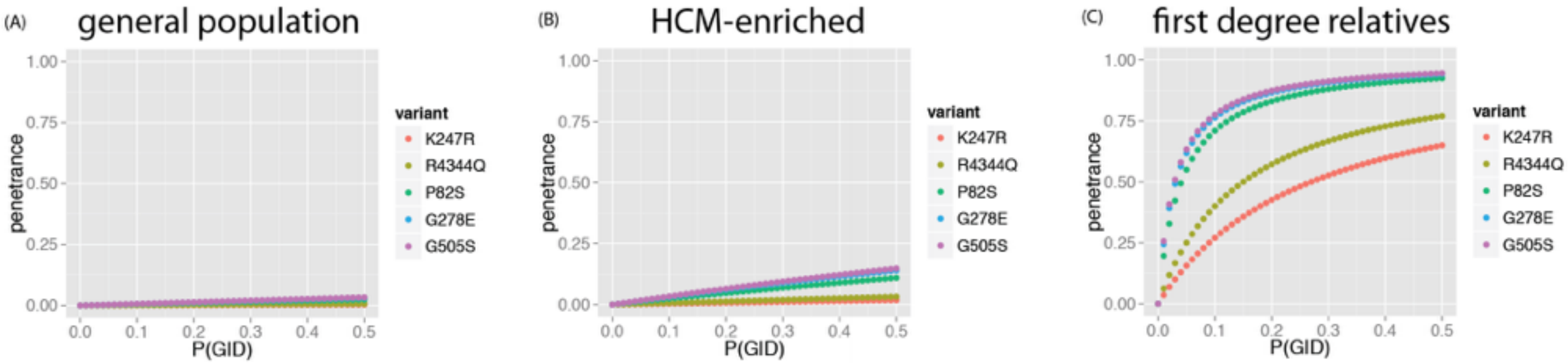


Manrai et al., *New England Journal of Medicine*, 2016

TNNT2 K247R

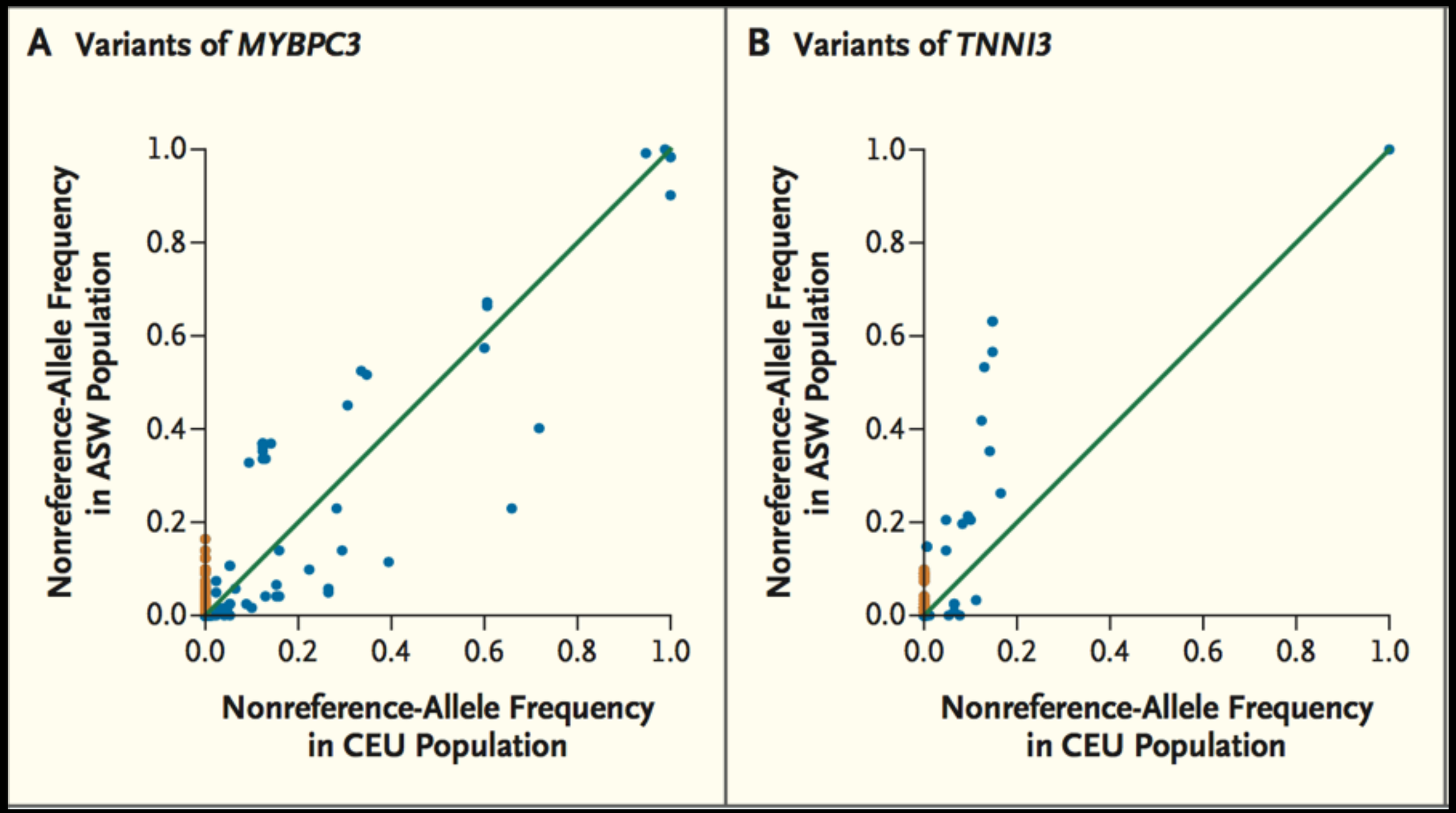


Manrai et al., *New England Journal of Medicine*, 2016



$$P(D|G) = \frac{P(G|D)P(D)}{P(G|D)P(D) + P(G|\overline{D})P(\overline{D})}$$

Manrai et al., *New England Journal of Medicine*, 2016



Manrai et al., *New England Journal of Medicine*, 2016

Table 3 Current Criteria Used to Determine Probability for Pathogenicity of an HCM Mutation*

Pathogenicity Criterion	Description	Potential Limitations for Interpretation
Cosegregation	Determine whether mutation is present in relatives with LVH and absent in those without LVH	Often impracticalFamily size may be small/relatives unavailableFamily compliance unpredictableRequires resources for imaging/DNA studies in ≥3 relatives (other than proband) including ≥1 with HCM phenotype†
Prior evidence of pathogenicity	Documentation that mutation is HCM disease–causing in ≥1 patient in published literature, or in the individual experience of a testing laboratory	Absence of established comprehensive, curated, and cooperative database tabulating mutations‡High rate of novel (de novo; "private") mutations in 65% of probandsInterpretation of pathogenicity can be inconsistent among testing laboratories
Control population	Confidence for pathogenicity increased when mutation absent from large, ethnicity-matched ostensibly healthy population	Often insufficient size§Control subjects should be unrelated, ethnicity-specific and free of the disease in questionPotentially pathogenic variants can occur in subjects judged clinically normalMany rare benign (missense) variants in normals, termed "background noise"
Major disruption protein structure, and function	Mutant proteins are judged to have substantially altered physical propertiesı	Inferred from evidence obtained from in nonhuman sources¶

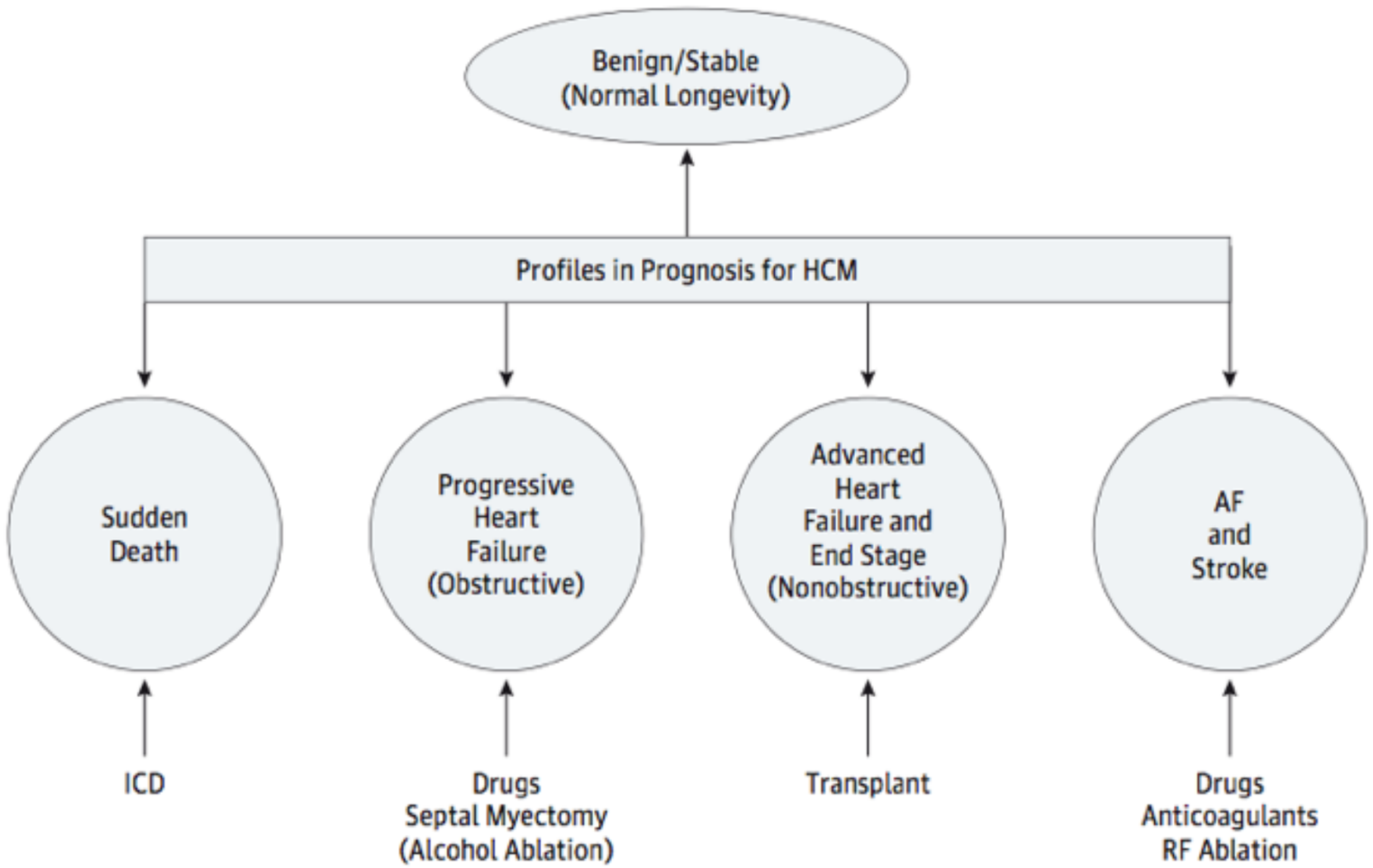
Clinical Review & Education

Review

How Hypertrophic Cardiomyopathy Became a Contemporary Treatable Genetic Disease With Low Mortality
Shaped by 50 Years of Clinical Research and Practice

Barry J. Maron, MD; Ethan J. Rowin, MD; Susan A. Casey, RN; Martin S. Maron, MD

Figure 5. Prognostic Pathways and Primary Treatment Strategies Within the Broad Clinical Spectrum of Hypertrophic Cardiomyopathy (HCM)



Most patients have an uncomplicated and benign course without major complications. However, individual patients can experience adverse disease progression along 1 or more of the complication pathways, each nevertheless associated with a potentially effective treatment strategy. AF indicates atrial fibrillation; ICD, implantable cardioverter-defibrillator; and RF, radiofrequency.

Maron et al. *JAMA Cardiology*. 2016.

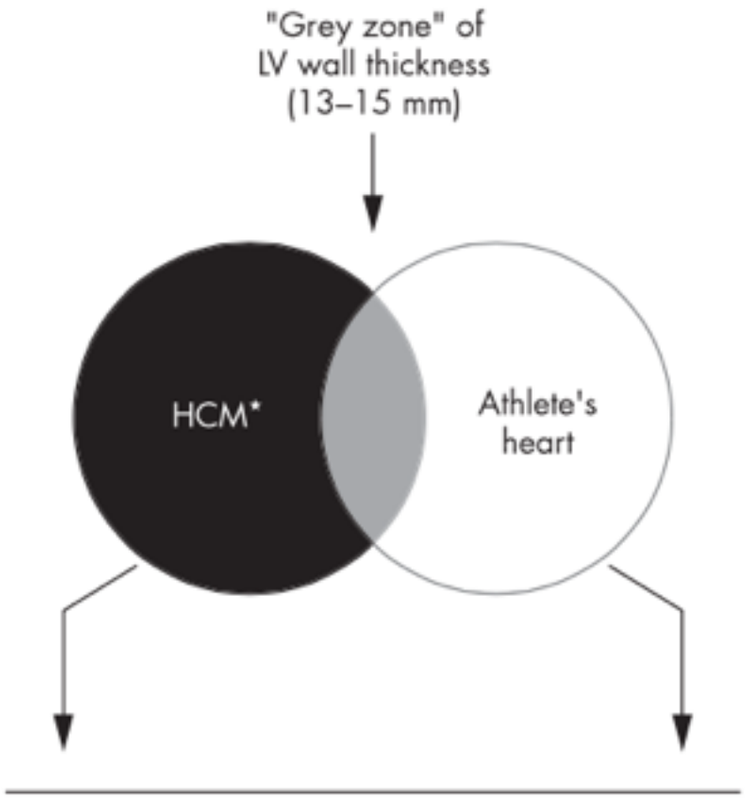


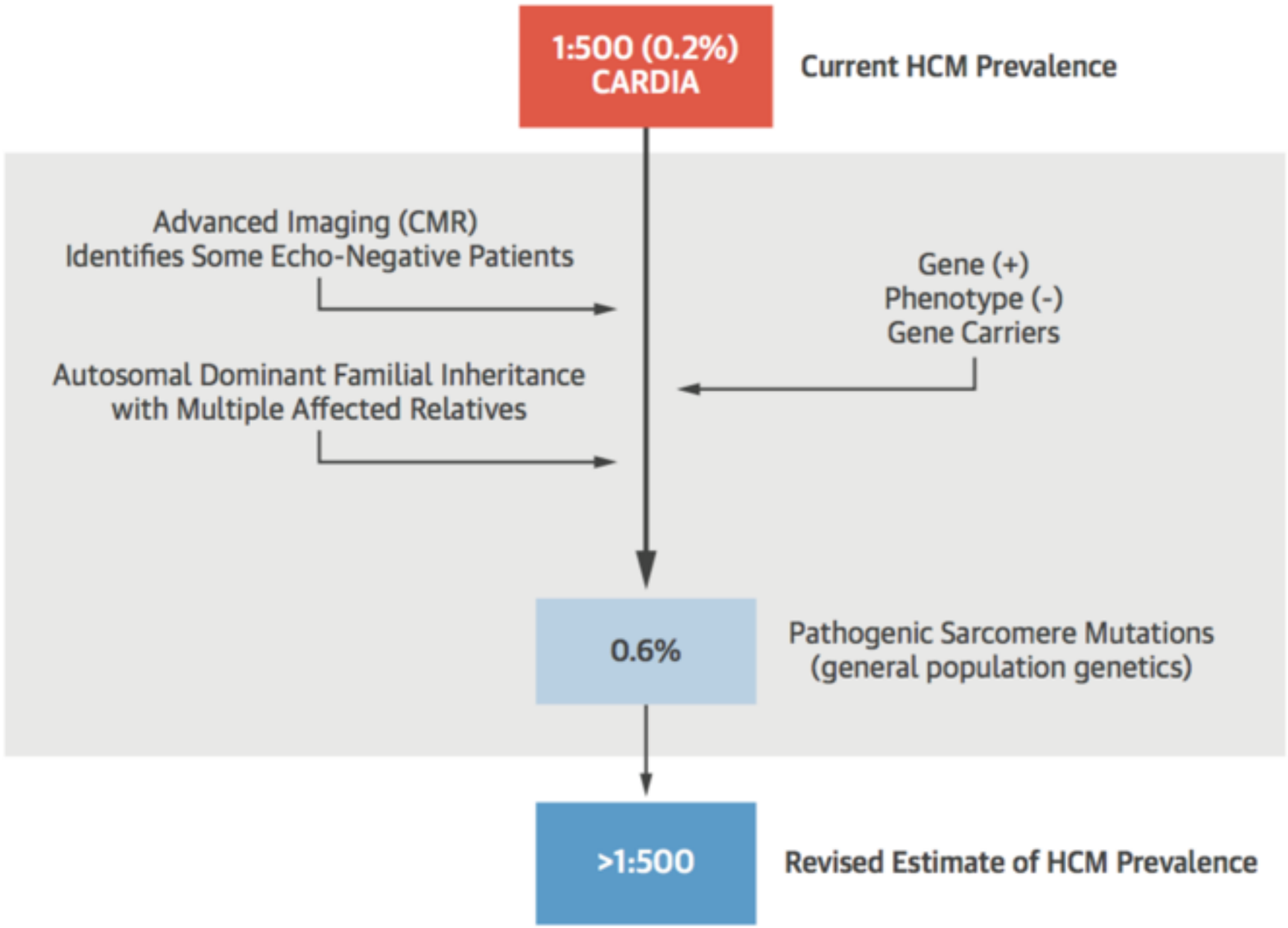
Figure 1 Criteria used to distinguish hypertrophic cardiomyopathy (HCM) from athlete's heart when the left ventricular (LV) wall thickness is within the shaded "grey zone" of overlap, consistent with both diagnoses. ↓ indicates decreased; LA, left atrial; LVH, left ventricular hypertrophy. Reproduced from Maron *et al*,[11] with permission of American Heart Association.

Genetic testing

The most definitive resolution of this important differential diagnosis can come from genetic testing. Indeed, a rapid genetic test is now available,[12] analysing by direct DNA sequencing mutations in the eight most common HCM causing genes. While a positive test result in an athlete can resolve the diagnostic ambiguity between athlete's heart and HCM, there is however significant potential for false negative test results in which a HCM diagnosis cannot be excluded.

Maron. *Heart*. 2005.

CENTRAL ILLUSTRATION **Factors Contributing to the Revised Estimate for the Prevalence of HCM**



Semsarian, C. et al. J Am Coll Cardiol. 2015; 65(12):1249-54.

The initial estimate of the prevalence of hypertrophic cardiomyopathy (HCM) came largely from the CARDIA (Coronary Artery Risk Development in Young Adults) study, which relied on echocardiographic identification of probands. Among the factors contributing to the revised estimate of more common than 1 in 500 were the identification of gene carriers who are negative for the HCM phenotype; enhanced clinical identification of the HCM phenotype with advanced imaging; recognition that because of the autosomal-dominant inheritance pattern, multiple relatives of probands (and carriers) would be affected by HCM; and recognition that up to 0.6% of the population may carry HCM-causing sarcomere mutations. CMR = cardiac magnetic resonance.

Semsarian. *JACC*. 2015.

Valsartan for Attenuating Disease Evolution In Early Sarcomeric HCM (VANISH)

This study is currently recruiting participants. (see Contacts and Locations)

Verified April 2016 by New England Research Institutes

Sponsor:
New England Research Institutes

Collaborator:
National Heart, Lung, and Blood Institute (NHLBI)

Information provided by (Responsible Party):
New England Research Institutes

ClinicalTrials.gov Identifier:
NCT01912534

First received: June 5, 2013
Last updated: May 16, 2016
Last verified: April 2016
History of Changes

Eligibility Criteria ICMJE	Inclusion Criteria: 1. All subjects must have a Pathogenic or Likely Pathogenic HCM Sarcomere Mutation a. The following categories of mutations are considered acceptable for subjects who have previously undergone clinical genetic testing. If results are ambiguous, they will be reviewed by the Clinical Coordinating Center to determine eligibility. • Laboratory for Molecular Medicine (Pathogenic, Likely Pathogenic) • Transgenomics/ PGXHealth (Class I) • GeneDx (Disease causing; Variant; likely disease-causing; Published, disease-causing mutation; Novel, likely disease-causing, mutation) • Correlagen (Associated; Probably Associated)

P defines meaningful G

G defines meaningful P???

Table 2. Comparison of LVH in African American and White Athletes

	Athlete Group		
	African American (n = 406)	White (n = 107)	*P* Value
Maximum mean LVWT, mm			
Unadjusted (95% CI)	11.2 (11.1-11.3)	10.5 (10.3-10.7)	<.001
Adjusted (95% CI)[a]	11.2 (11.1-11.4)	10.4 (10.2-10.6)	
Mean LVMI, g/m^2			
Unadjusted (95% CI)	106.3 (104.6-108.0)	102.2 (99.0-105.4)	.03
Adjusted (95% CI)[a]	106.5 (104.8-108.2)	101.7 (98.4-105.0)	
Mean RWT			
Unadjusted (95% CI)	0.39 (0.38-0.40)	0.35 (0.34-0.36)	<.001
Adjusted (95% CI)[a]	0.39 (0.38-0.40)	0.35 (0.34-0.36)	
LVH, No. (%)[b]			
Concentric nondilated	60 (53.1)	4 (19.0)	.004
Eccentric nondilated	7 (6.2)	0	.60
Concentric dilated	19 (16.8)	7 (33.3)	.13
Eccentric dilated	27 (23.9)	10 (47.6)	.03

Abbreviations: LVH, left ventricular (LV) hypertrophy; LVMI, LV mass index; LVWT, LV wall thickness; RWT, relative wall thickness.

[a] Linear regression was used to calculate adjusted means after adjustment for age, body surface area, and systolic and diastolic blood pressure.

[b] Pattern of hypertrophy is shown as percentages of African American and white athletes with subtypes of hypertrophy.

The ‘**N**ormal **B**ig **A**thlete’

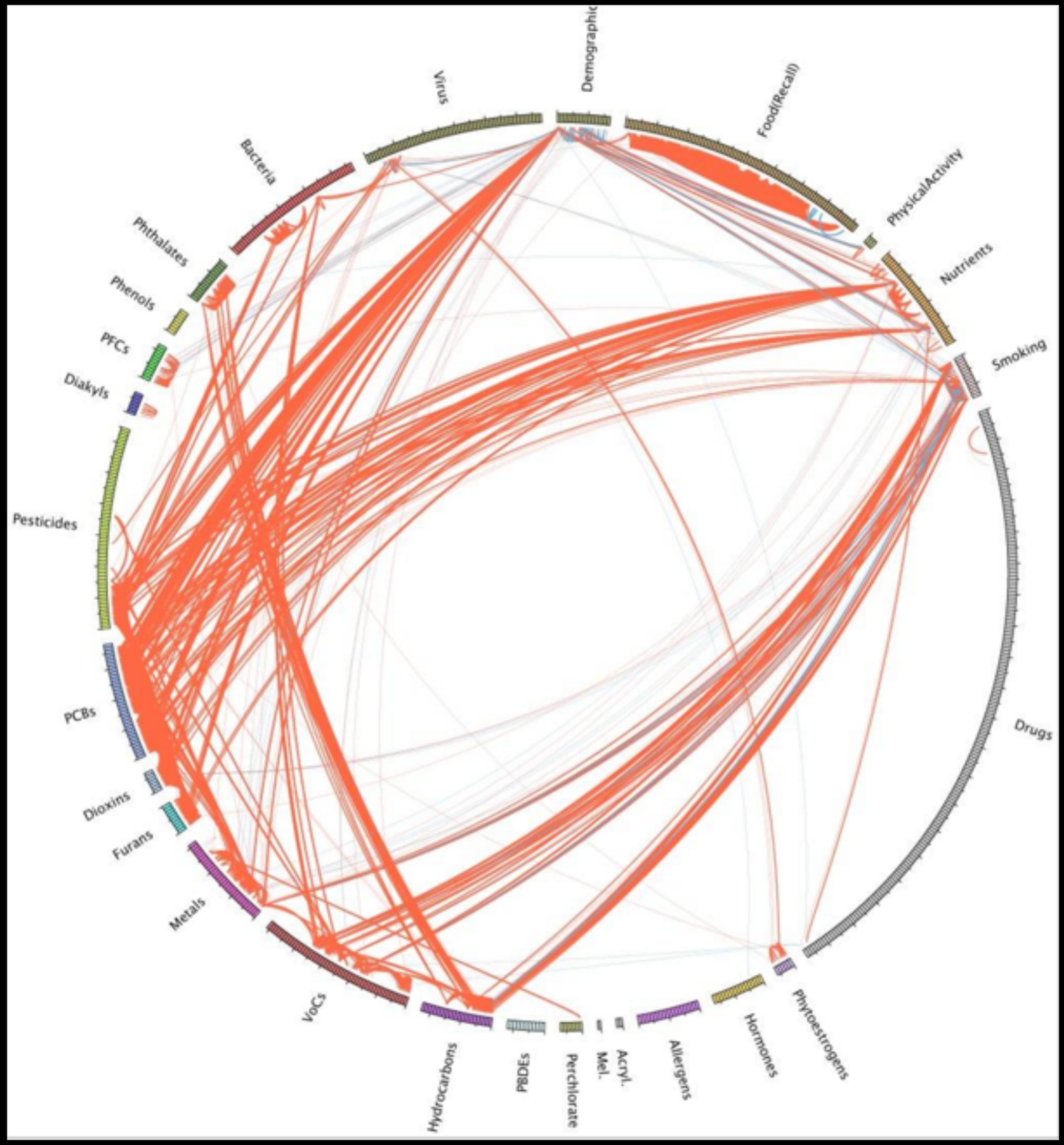
Engel et al.. *JAMA Cardiology*. 2016.

Challenge Question #2

Causes of left ventricular hypertrophy other than hypertrophic cardiomyopathy (HCM) include:

(a) Systemic hypertension
(b) Athletic conditioning
(c) Aortic valve stenosis
(d) a and b
(e) a, b, and c

$$P = G + E$$



Patel and Manrai. *PSB*. 2015.

HCM is one test of many

GTR: GENETIC TESTING REGISTRY

All GTR | **Tests** | Conditions/Phenotypes | Genes | Labs | GeneReviews | Advanced search for tests

Search Tests

Find tests by searching test names, disease names, phenotypes, gene symbols and names, protein names, laboratory names, directors and locations.

YouTube GTR Tutorials

IMPORTANT NOTE: NIH does not independently verify information submitted to the GTR; it relies on submitters to provide information that is accurate and not misleading. NIH makes no endorsements of tests or laboratories listed in the GTR. GTR is not a substitute for medical advice. ***Patients and consumers*** with specific questions about a genetic test should contact a health care provider or a genetics professional.

NIH thanks labs for registering over 26,000 tests for 5,400 conditions and 3,700 genes!

YouTube basic search video

(4/5) ◂◂ II ▸▸

Quick Links

- Panels with 5 or more genes including *BRCA1* and *BRCA2*
- All Comparative Genomic Hybridization tests
- All pharmacogenetic responses and links to those tests
- Labs that offer genomic testing services
- All single-gene tests (NOT panels)
- All GTR content

Tell us what other quick links you need!

Screenshot taken April 22, 2015

Challenge Question #3

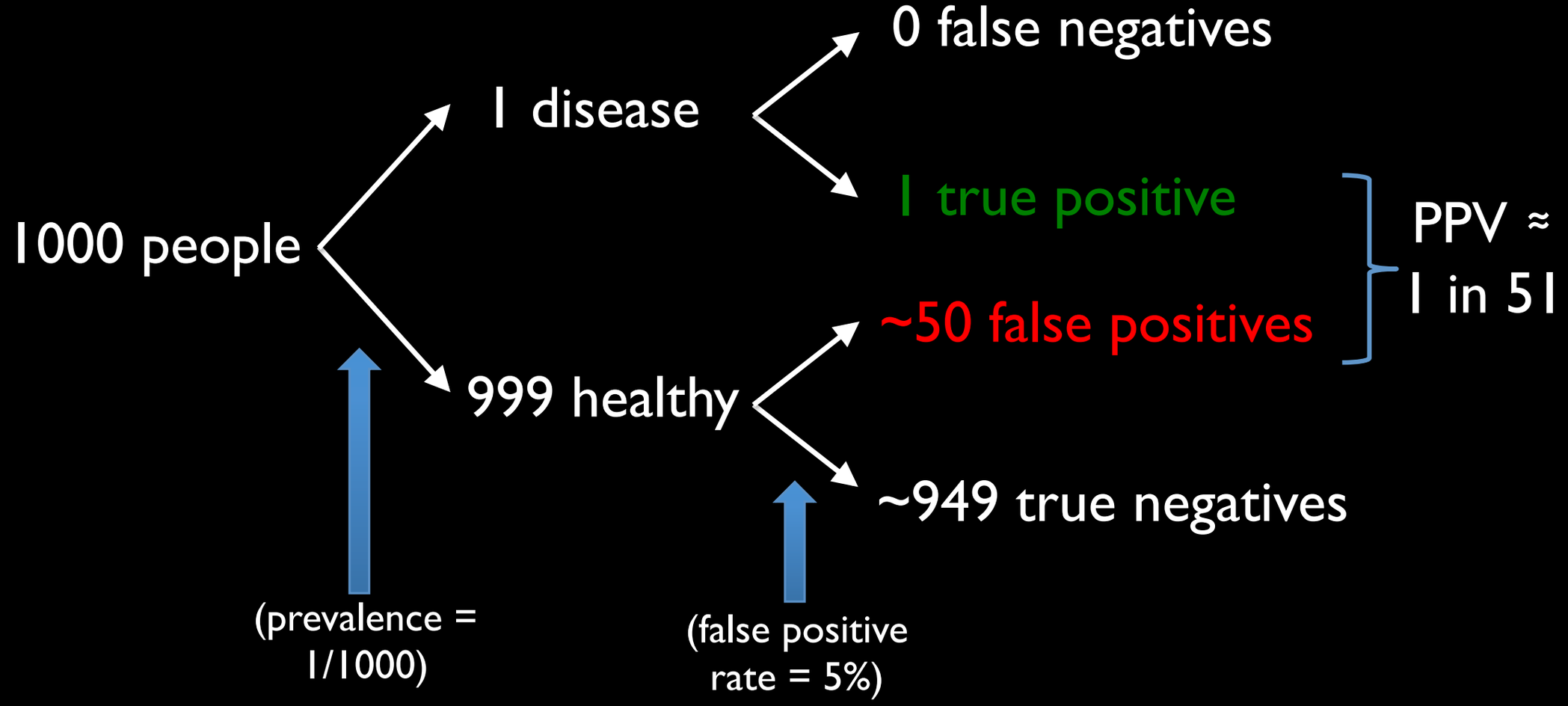
If a test to detect a disease whose prevalence is 1/1000 has a false positive rate of 5 percent, what is the chance that a person found to have a positive result actually has the disease, assuming you know nothing about the person's symptoms or signs?

(a) 100%
(b) 95%
(c) 50%
(d) 25%
(e) 2%

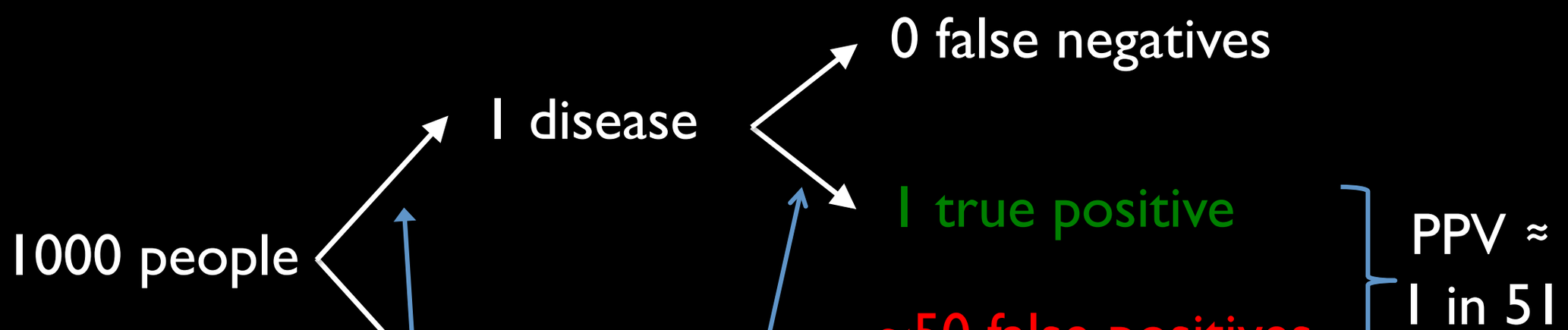
Formal approach: Bayes' Rule

$$P(D^+ \mid T^+) = \frac{P(D^+)P(T^+ \mid D^+)}{P(D^+)P(T^+|D^+) + P(D^-)P(T^+|D^-)}$$

Intuitive approach

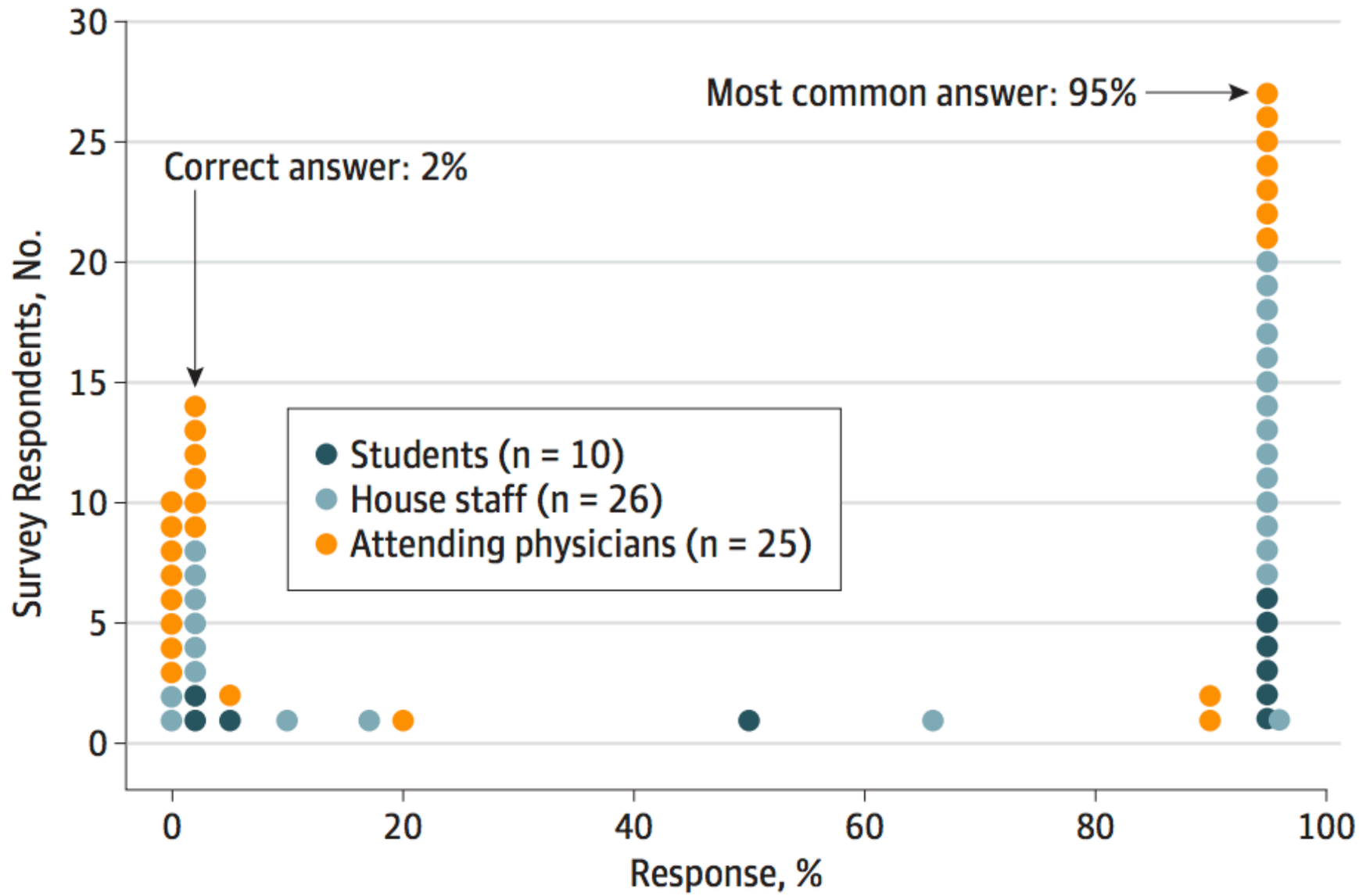


Reconciling with Bayes



$$\frac{P(D^+)P(T^+ \mid D^+)}{P(D^+)P(T^+|D^+) + P(D^-)P(T^+|D^-)} = P(D^+ \mid T^+)$$

Figure. Distribution of Responses to Survey Question Provided in the Article Text



Manrai et al. *JAMA Internal Medicine.* 2014

Common Mistakes

- "true positive rate" = 1 – "false positive rate"
  - Specificity = 1 – "false positive rate"
- 95% specificity is "very good"
  - Prevalence influences the quality of a test
- Positive test makes the disease *less* likely (8 respondents)
  - Even a completely random positive test result will not decrease PPV below prevalence

The NEW ENGLAND JOURNAL of MEDICINE

INTERPRETATION BY PHYSICIANS OF CLINICAL LABORATORY RESULTS

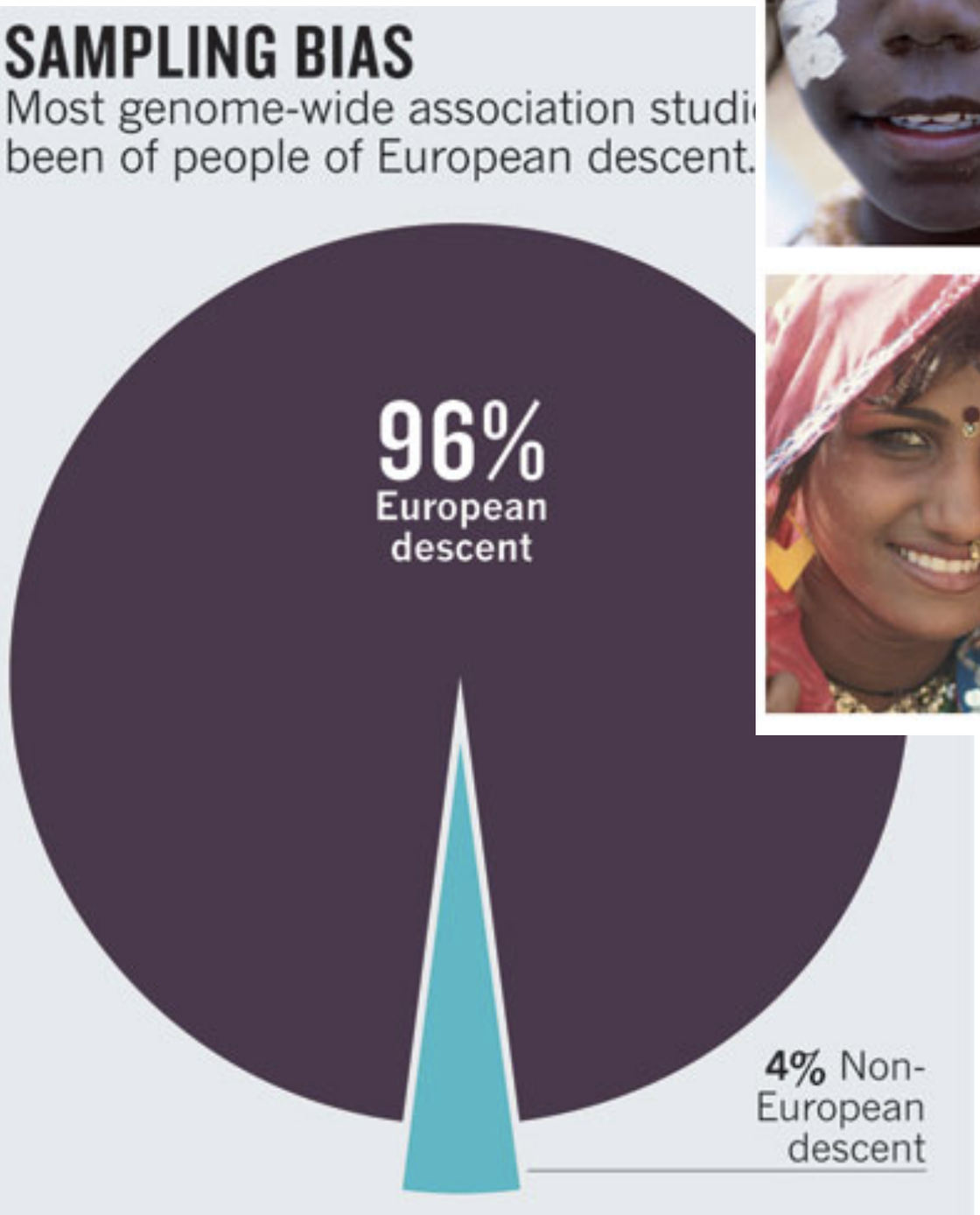
WARD CASSCELLS, B.S., ARNO SCHOENBERGER, M.D., AND THOMAS B. GRABOYS, M.D.

AS both the number and cost of clinical laboratory tests continue to increase at an accelerating rate,[1] physicians are faced with the task of comprehending and acting on a rising flood tide of information. We conducted a small survey to obtain some idea of how physicians do, in fact, interpret a laboratory result.

Genomics for the world

Carlos D. Bustamante, Francisco M. De La Vega & Esteban G. Burchard

Affiliations | **Corresponding author**



Bustamante, De La Vega, Burchard. *Nature*. 2011.

Genomics is failing on diversity

Alice B. Popejoy & **Stephanie M. Fullerton**

12 October 2016

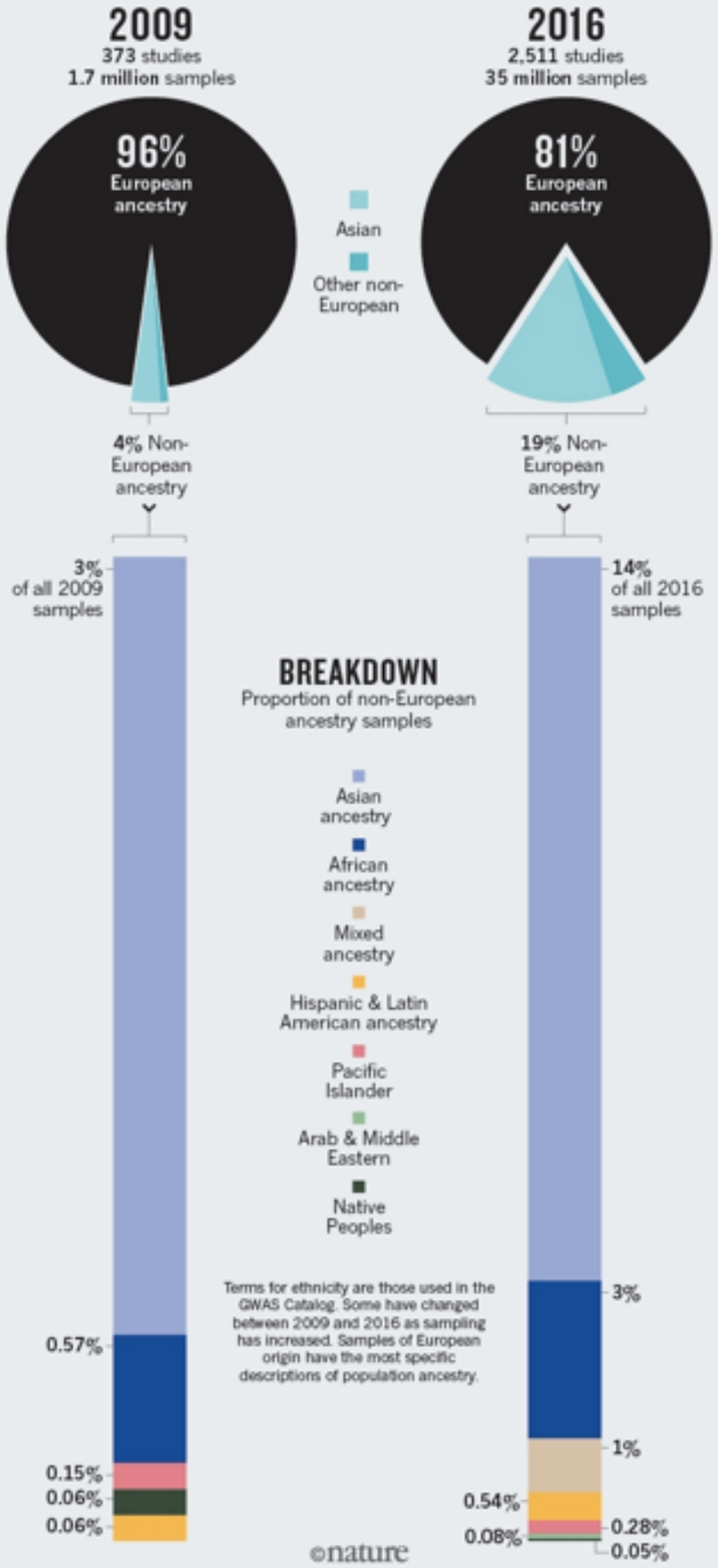
An analysis by Alice B. Popejoy and Stephanie M. Fullerton indicates that some populations are still being left behind on the road to precision medicine.

Subject terms: Diseases · Genetics · Genomics · Health care

Popejoy & Fullerton. *Nature*. 2016.

PERSISTENT BIAS

Over the past seven years, the proportion of participants in genome-wide association studies (GWAS) that are of Asian ancestry has increased. Groups of other ancestries continue to be very poorly represented.



Popejoy & Fullerton. *Nature*. 2016.

Summary

- We identified common (benign) genetic variants **misclassified** (as pathogenic) exclusively in African Americans
- This creates the potential for **healthcare disparities** due to genomic misdiagnosis
- Variants vetted in **diverse** control populations can help prevent false positives
- **Statistics** over calculus for **rational** decision making

http://probasketballtalk.nbcsports.com/

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Thank you