Pheno™ Analysis is a Highly Accurate and Robust Variant Classification Algorithm with High Tolerance for Reported Clinical History Errors

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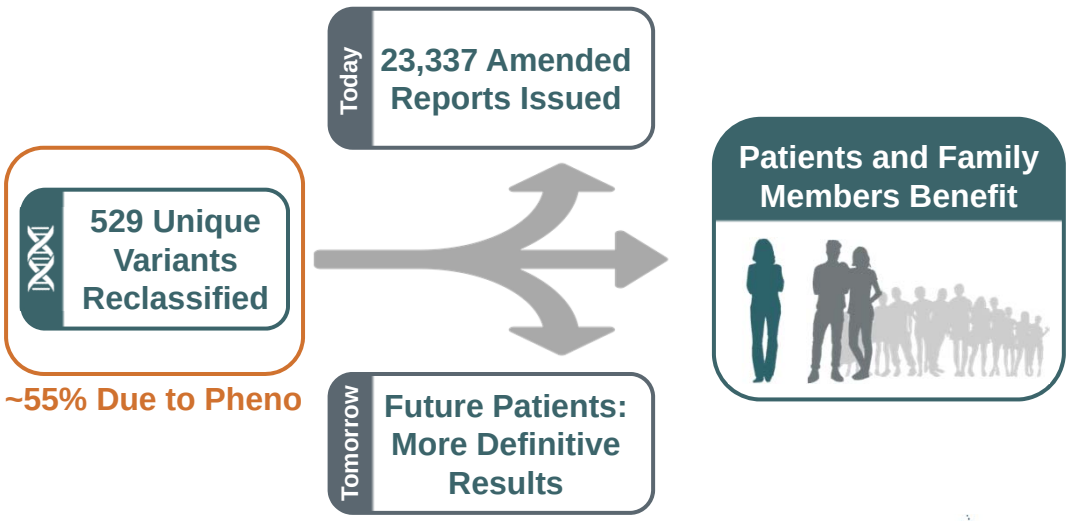
Session 1: 08:15 – 09:45

Disclosures

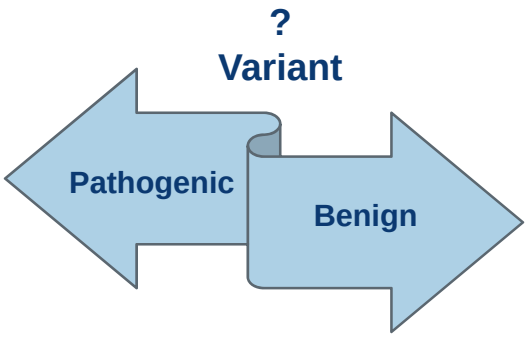
I am employed by Myriad Genetic Laboratories, Inc. and receive salary and stock as compensation.

Robust Variant Reclassification Tools Are in the Best Interest of Patient Care: Reclassification Impact 2016



Pheno statistically measures the severity of personal and family cancer history associated with a variant

If a variant is **pathogenic**, people who carry it typically have **strong personal and family histories of cancer.**



If a variant is **truly benign**, cancer histories may be **strong or weak**.

Methods

Clinical Testing: Informed Consent

- **Single syndrome**
- **Pan-cancer panel testing of up to 28 genes:** *BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, ATM, CHEK2, PALB2, MUTYH, APC, PTEN, TP53, STK11, SMAD4, CDH1, BARD1, BRIP1, CDKN2A, CDK4, BMPR1A, RAD51C, RAD51D, POLD1, POLE, GREM1*

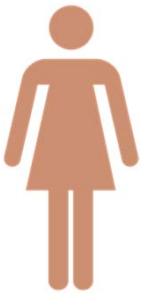
Clinical History: Test Requisition

- **Patient: Gender, ancestry, cancer(s) and age(s) of diagnosis, and current age**
- **Family members: Affected relatives with cancer type(s) and age(s) of diagnosis**

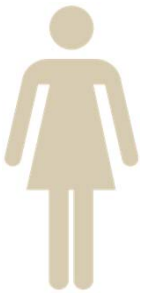
Cancers Assessed by Pheno

- **Assessed cancers are gene-specific**
- ***BRCA1/BRCA2:*** Female Breast, Breast + Ovarian, Breast *in situ,* DCIS, Ovarian, Multiple Breast, Male Breast, Female Pancreatic, Male Pancreatic, Prostate, Lobular

Pheno Starts By Scoring the Clinical History of Each Proband Carrying the Variant of Interest



Proband A
Self – Breast 38
Mother – Ovarian 55
Brother – Breast 55

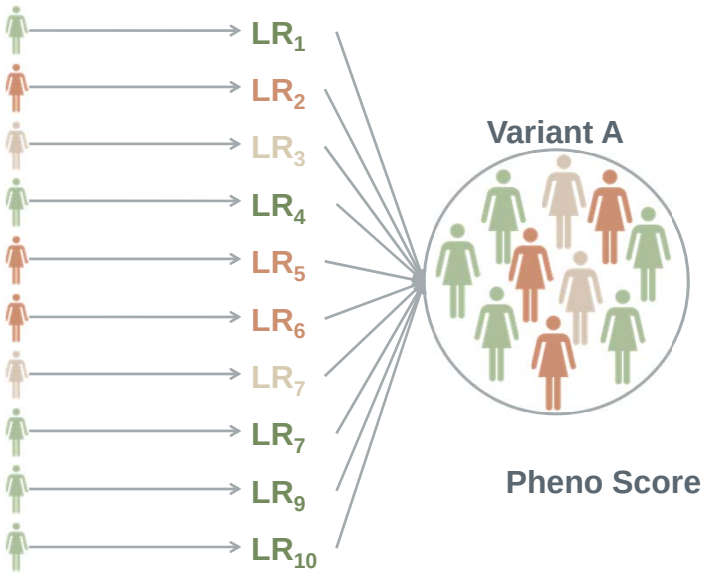


Proband B
Self – Breast 55
Mother – Breast 60
Mat. Aunt – Breast 80

Proband C
Self – Breast 78
Father – Prostate 82

More Severe ← **Severity of Cancer History** → **Less Severe**

Pheno Calculates an LR For Each Proband and Combines the LRs Into a Variant Pheno Score

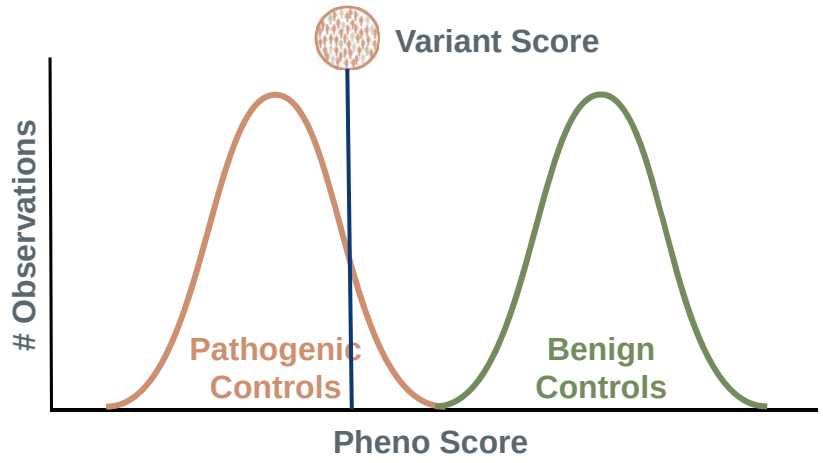


$$LR = \frac{(1 - P_{pro})P_{ave}}{P_{pro}(1 - P_{ave})}$$

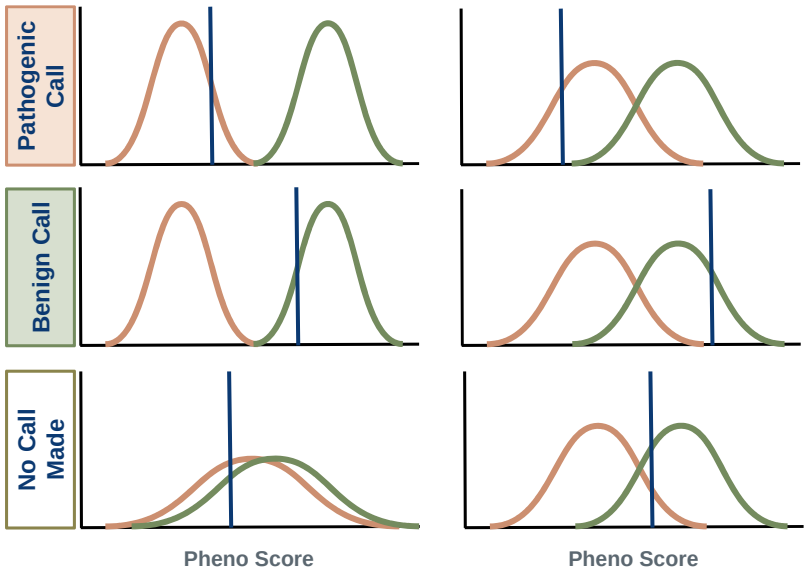
P_{pro} = Prob. proband carries a PV
P_{ave} = Average P_{pro} for all probands (with or without a mutation)

Pheno Score = $(LR_1)(LR_2),\ldots,(LR_n)$

Variant-Specific Pheno Scores are Compared to Scores from 10,000 Composite Pathogenic or Benign Variants



Pheno Makes Call When a Variant-Specific Score is Well Within One Control Curve and Not Significantly Within the Other Curve



Pheno is Optimized for Each Gene Individually:
PPV and NPV Greater than 99.5%

	Gene	Classification	# Variants Tests	NPV (%)	PPV (%)
High Risk	*BRCA1*	Pathogenic Benign	27,000 52,000	99.90	99.72
	BRCA2	Pathogenic Benign	27,000 52,000	99.90	99.71
	MLH1	Pathogenic Benign	27,000 52,000	99.92	99.79
	MSH2	Pathogenic Benign	27,000 52,000	99.93	99.72
	MSH6	Pathogenic Benign	27,000 52,000	99.90	99.86

Pheno is Optimized and Re-Validated At Least Every Two Years to Verify that PPV and NPV Remain Greater than 99.5%

	Gene	Classification	# Variants Tests	NPV (%)	PPV (%)
Moderate Risk	*ATM*	Pathogenic Benign	26,000 51,000	99.94	99.81
	CHEK2	Pathogenic Benign	26,000 51,000	99.87	99.87
	PALB2	Pathogenic Benign	26,000 6,000	99.97	N/A*
	BARD1	Pathogenic Benign	26,000 6,000	99.85	N/A*

* Pheno is not currently used for *BARD1* or *PALB2* upgrades to pathogenic or likely pathogenic

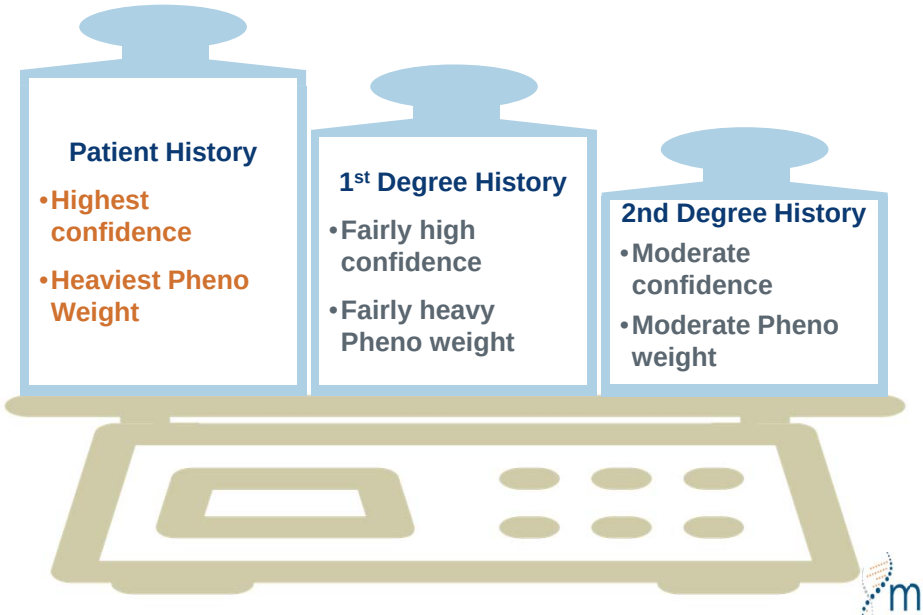
Will Pheno Work if Personal and Family History Data Contains Errors?

Fact: Patient cancer histories are obtained from the test requisition form, and patients may not report family histories correctly.

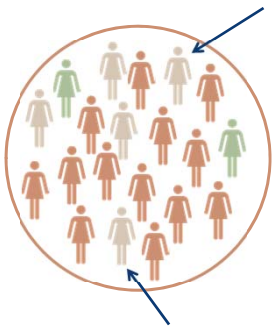
Concern: If the data used to generate a Pheno score isn't perfect, how can we trust the Pheno classification?

Response: Pheno has multiple design features which minimize the effect of clinical history errors on accuracy.

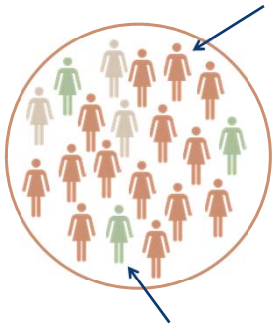
Pheno Places the Highest Weight on the Most Accurate Clinical Information



Pheno Scores are Based on Multiple Patients, Reducing the Impact of Limited History Errors



Pheno Score Based on Reported Cancer Histories

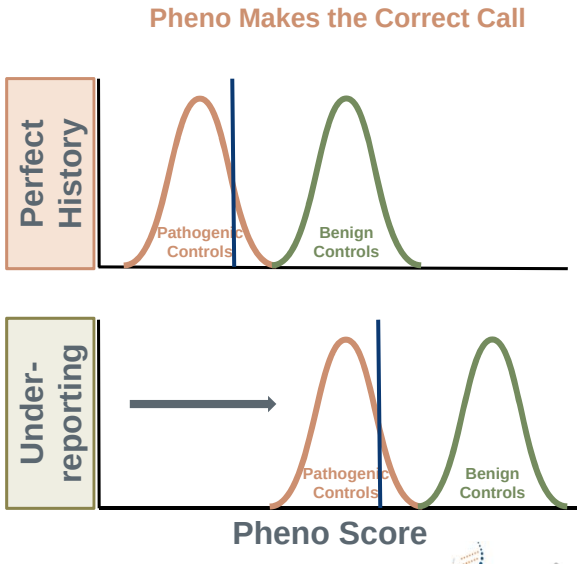


Pheno Score Based on Actual Cancer Histories

Pheno Makes the Correct Classification When Family History is Under-reported

Fact: TRF data is used for variant-carrying probands and probands used as pathogenic and benign controls

Result: If multiple patients under-report their family history, Pheno will make the correct classification call. All variant-specific and control scores shift together towards benign, but remain relatively similar



Pheno Makes the Correct Classification When Family History is Under-reported

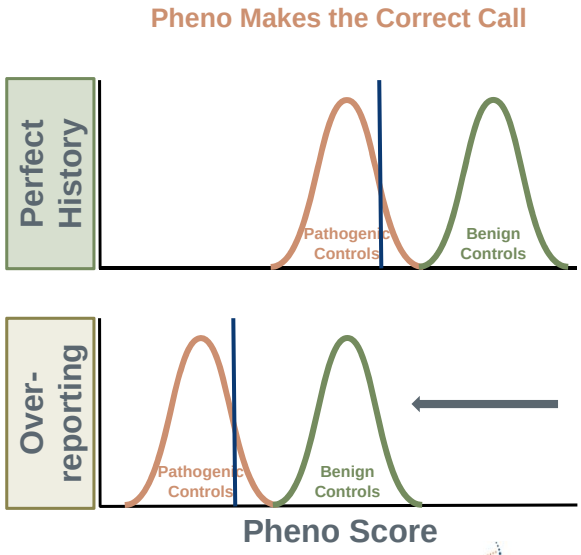
Family members (if present) were randomly deleted from a percentage of probands carrying the same variant and from control probands: ***50,000 benign and 25,000 pathogenic variants were tested through 2-fold cross-validations***

		% Probands: Two 2nd Degree Relatives Deleted			% Probands: One 1st and Two 2nd Degree Relatives Deleted		
Gene	Metric	10%	50%	100%	10%	50%	100%
BRCA1	PPV (%)	99.74	99.80	99.80	99.59	99.72	99.70
	NPV (%)	99.90	99.89	99.90	99.90	99.88	99.90
BRCA2	PPV (%)	99.65	99.81	99.78	99.84	99.88	99.85
	NPV (%)	99.90	99.89	99.90	99.88	99.90	99.90

Pheno Makes the Correct Classification When Family History is Over-reported

Fact: TRF data is used for variant-carrying probands and probands used as pathogenic and benign controls

Result: If multiple patients over-report their family history, Pheno will make the correct classification call. All variant-specific and control scores shift together towards pathogenic, but remain relatively similar.



Pheno Makes the Correct Classification When Family History is Over-reported

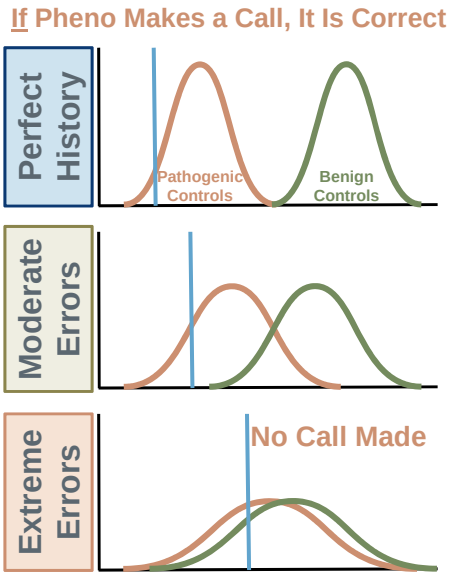
Affected sisters and/or aunts were randomly added to a percentage of probands carrying the same variant and to control probands: ***50,000 benign and 25,000 pathogenic variants were tested through 2-fold cross-validations***

		% Probands: Two Affected Aunts Added			% Probands: One Affected Sister and Two Affected Aunts Added		
Gene	Metric	10%	50%	100%	10%	50%	100%
BRCA1	PPV (%)	99.68	99.60	99.74	99.67	99.82	99.83
	NPV (%)	99.88	99.88	99.88	99.89	99.86	99.89
BRCA2	PPV (%)	99.54	99.59	99.80	99.63	99.75	99.76
	NPV (%)	99.90	99.89	99.89	99.91	99.87	99.90

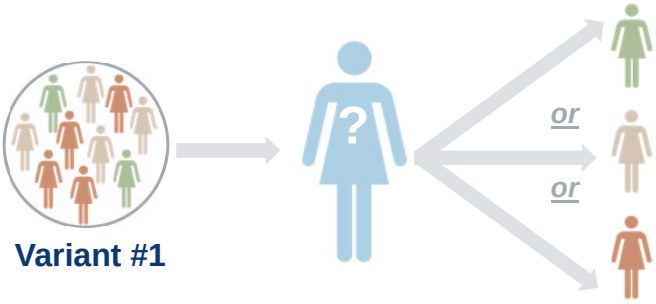
Pheno Makes the Correct Classification When Mixed History Errors are Present

Fact: TRF data is used for variant-carrying probands and probands used as pathogenic and benign controls

Result: If some patients over-report and others under-report family history, variant-specific Pheno scores look more like "an average" of pathogenic and benign. Pathogenic and benign control curves "slide together." Pheno calls are more difficult to make, but are still accurate.



We Tested the Effects of Mixed Family History Reporting Errors on Pheno Accuracy



Variant #1

1. Randomly select a proband, and replace that proband and all relatives with a random proband
2. Replace 1st and 2nd degree relatives without replacing the proband
3. Replace 2nd degree relatives without replacing the proband and 1st degree relatives

Pheno Makes the Correct Classification When Mixed History Errors are Reported

Relative histories were replaced for a percentage of random probands carrying the same variant and for control probands: ***50,000 benign and 25,000 pathogenic variants were tested through 2-fold cross-validations***

		% Probands: All 2nd Degree Relatives Replaced			% Probands: All 1st and 2nd Degree Relatives Replaced		
Gene	Metric	10%	20%	50%	10%	20%	50%
BRCA1	PPV (%)	99.74	99.64	99.75	99.70	99.79	99.68
	NPV (%)	99.89	99.89	99.88	99.90	99.89	99.91
BRCA2	PPV (%)	99.55	99.74	99.92	99.76	99.73	99.74
	NPV (%)	99.89	99.90	99.89	99.90	99.89	99.89

Pheno Makes the Correct Classification When Mixed History Errors are Reported

Proband and relative histories were replaced for a percentage of random probands carrying the same variant and for control probands: ***50,000 benign and 25,000 pathogenic variants were tested through 2-fold cross-validations***

		% Probands: Proband, 1st and 2nd Degree Relative Histories Replaced		
Gene	Metric	10%	20%	50%
BRCA1	PPV (%)	99.68	99.75	99.76
	NPV (%)	99.86	99.87	99.73
BRCA2	PPV (%)	99.81	99.70	Fail
	NPV (%)	99.90	99.89	Fail

Pheno Analysis is A Highly Accurate Algorithm

Rigorously Developed and Validated

- Optimized for each gene
- >99.5% PPV and NPV

Accurate in the Presence of Personal and/or Family History Errors

- Case-control design
- Will not make a call if data contains too many errors
- When Pheno makes a call, it is accurate

Critical for Variant Reclassification and Appropriate Patient Care

- 2016: 23,337 amended reports; ~55% from Pheno
- Patients and family members may receive appropriate medical management

Thank You!

BRCA1, BRCA2	• Pruss D, *et al.* Development and validation of a new algorithm for the reclassification of genetic variants identified in the *BRCA1* and *BRCA2* genes. Breast Cancer Res Treat. 2014 147(1):119-132.
MLH1, MSH2, MSH6	• Morris B, *et al.* Classification of genetics variants in genes associated with Lynch syndrome using a clinical history weighting algorithm. BMC Genet. 2016 17(1):99.
BRCA1, BRCA2	• Bowles KR, *et al.* A clinical history weighting algorithm accurately classifies *BRCA1* and *BRCA2* variants. Presented ASHG, 2013.
MLH1, MSH2, MSH6	• Bowles KR, *et al.* Development of a novel history weighting algorithm for the reclassification of genetic variants identified in genes associated with Lynch syndrome. Presented ACMG, 2015.
BRCA1, BRCA2, MLH1, MSH2, MSH6, ATM, CHEK2, PALB2	• Bowles KR, *et al.* Reclassification of uncertain variants identified in high and moderate cancer risk genes using history weighting analysis. Presented ACMG, 2016.
BRCA1, BRCA2, ATM, CHEK2, PALB2	• Bowles KR, *et al.* Enhancement of history weighting analysis to accurately classify variants in high and moderate risk cancer panel genes. International Symposium on HBOC, 2016.
BARD1	• Added summer 2017 – Data on file