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Agenda

- What's new at Invitae?
  Oncology Marketing Manager, Invitae
  Mona Varia

- Clinical Validity and Actionability of Multigene Tests for Hereditary Breast and Ovarian Cancer
  Head of Scientific Affairs, Invitae
  Stephen E. Lincoln

- A systematic and accurate approach to variant classification
  Head of Clinical Genomics, Invitae
  Scott Topper, PhD, FACMG

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The new Invitae test catalog: Building on our promise

Hundreds of new genes.

Dramatically expanded test catalog.

Same mission.

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Invitae's new test catalog

BROADER, DEEPER MENU WITH FLEXIBILITY TO FIT YOUR NEEDS

NEW: Common Hereditary Cancers Panel and Gastric Cancer Panel

EXPANDED: Panels for breast, gynecologic, colon, and pancreatic cancer

INCLUDES: Promoter regions for relevant genes, MSH2 inversion, and full PMS2 and CHEK2

MORE THAN 30 TESTS AND MANY NEW CONDITIONS

NEW: Invitae Arrhythmia and Cardiomyopathy Comprehensive Panel

NEW TESTING CATEGORIES: Familial hypercholesterolemia, aortopathies, pulmonary hypertension, and congenital heart disease

FLEXIBLE ORDERING: Combine genes and panels to build the right test

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Invitae's new test catalog

Pediatric genetics

MORE THAN 100 NEW CAREFULLY CURATED GENES

NEW: Tests for various congenital structural heart defects, a strong option for patients with negative or uncertain chromosomal microarray results

EXPANDED: Offerings for RASopathies and ciliopathies, including a comprehensive targeted panel for primary ciliary dyskinesia

Neurology

BROAD, AFFORDABLE MENU WITH PRE-CURATED PANELS AND FLEXIBLE ORDERING

NEW: Panels for Duchenne/Becker muscular dystrophy and dystonia

EXPANDED: Offerings for Charcot-Marie-Tooth and hereditary spastic paraplegia

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Invitae advantage remains the same

Select a pre-curated gene panel, customize with one click, or design your own.

Our pricing remains the same even with our newly expanded test catalog.

From clinical consultations to help with ordering, billing, and understanding results.

Proven results based on published data and backed by our team of genetic and medical experts.

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Clinical Validity and Actionability of Multigene Tests for Hereditary Breast and Ovarian Cancer

STEPHEN E. LINCOLN
Scientific Affairs, Invitae

September 2015

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- **Leif Ellisen**
- Andrea Desmond
- Michelle Gabree
- Kristen Shannon

- **Jim Ford**
- **Allison Kurian**
- Meredith Mills

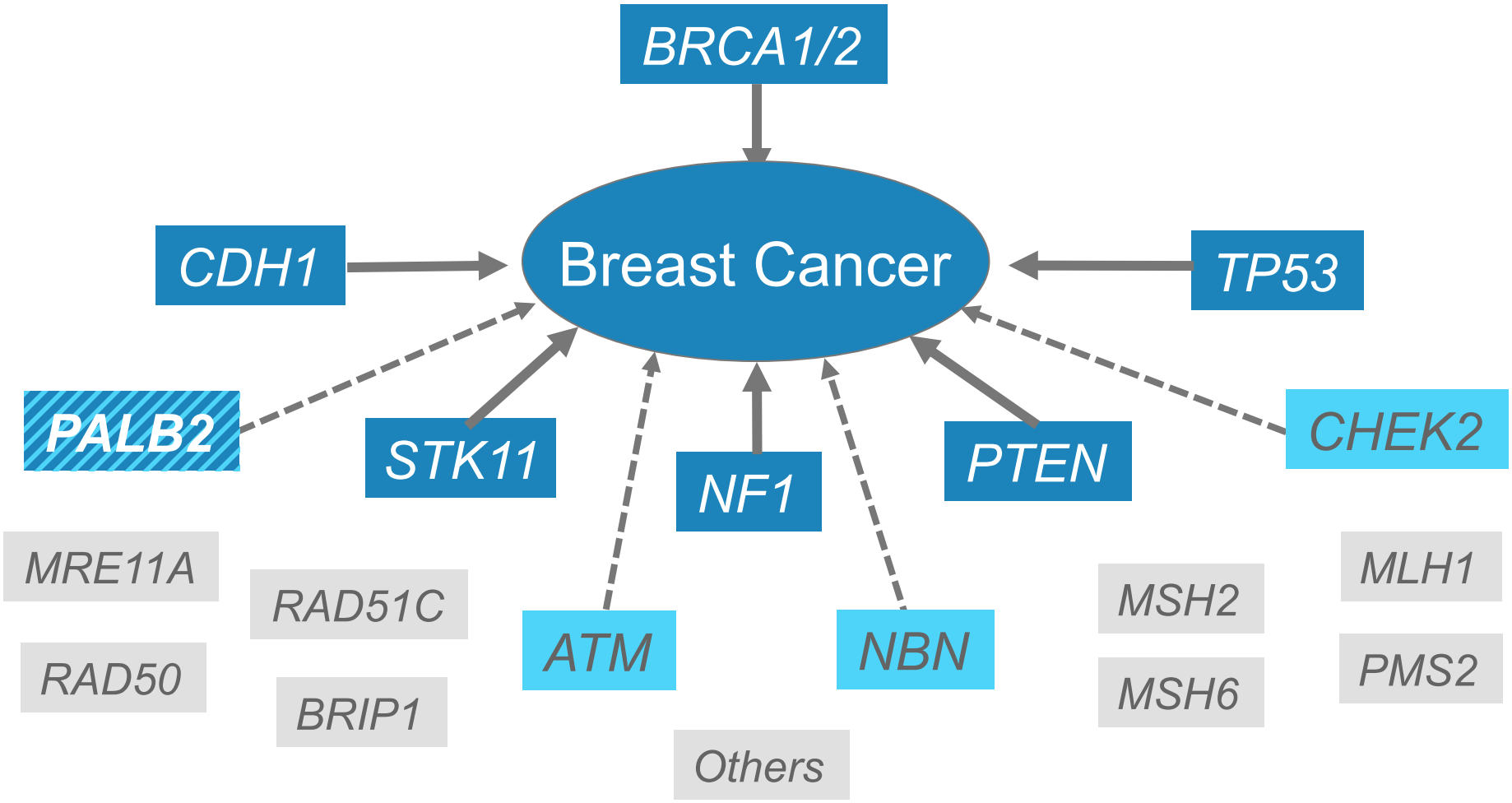
- **Nadine Tung**

- Steve Lincoln
- Shan Yang
- Yuya Kobayashi
- Michael Anderson
- Invitae Clinical Genomics Group

- Kevin Jacobs
- Geoff Nilsen
- Josh Paul
- Mike Kennemer
- Dan Kvitek

- Scott Topper
- Martin Powers
- Federico Monzon

Genetic Heterogeneity



Gene list from Easton *et al. NEJM* 2015
Figure adapted from Tuya Pal, Moffitt Cancer Center

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Clinical Heterogeneity

Genes	Elevated Cancer Risks
BRCA1, BRCA2	Breast, ovary, fallopian tube, peritoneal, prostate, pancreas, male breast
MLH1, MSH2, MSH6 PMS2, EPCAM	Colon, endometrium, ovary, stomach, urinary tract, small bowel
STK11	Breast, ovary, colon, stomach, pancreas, colon, lung
CHEK2	Breast, ovary, prostate, colon, thyroid, kidney
TP53	Bone, breast, brain, andrenocortical, colon, leukemia
PTEN	Breast, thyroid, endometrium, colon, kidney
CDH1	Stomach, breast, colorectal
Others…	Multiple…

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Cragun et al. Clinical Genetics 2014

NCI BRCA1/2 Fact Sheet, 2014

PNAS PNAS PNAS PNAS 2011

Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing

Tom Walsh[a], Silvia Casadei[a], Ming K. Lee[a], Christopher C. Pennil[b], Alex S. Nord[a], Anne M. Thornton[a], Wendy Roeb[a], Kathy J. Agnew[b], Sunday M. Stray[a], Anneka Wickramanayake[b], Barbara Norquist[b], Kathryn P. Pennington[b], Rochelle L. Garcia[c], Mary-Claire King[a,1], and Elizabeth M. Swisher[a,b,1]

- 360 patients with ovarian, fallopian, or peritoneal cancer
- Unselected for age of onset or family history
- 21-gene germline test

- **17.5% BRCA1/2** positive
- **6.1% non-BRCA1/2** positive
  - BARD1, BRIP1, CHEK2, MRE11A, MSH6,
  - NBN, PALB2, RAD50, RAD51C, TP53

Walsh *et al., PNAS* 2011

Cancer

Frequency of Mutations in Individuals With Breast Cancer Referred for *BRCA1* and *BRCA2* Testing Using Next-Generation Sequencing With a 25-Gene Panel

Nadine Tung, MD[1,2]; Chiara Battelli, MD[1]; Brian Allen, MS[3]; Rajesh Kaldate, MS[3]; Satish Bhatnagar, PhD[4]; Karla Bowles, PhD[5]; Kirsten Timms, PhD[6]; Judy E. Garber, MD[2,7]; Christina Herold, MD[1,2]; Leif Ellisen, MD, PhD[2,8]; Jill Krejdovsky, MS[9]; Kim DeLeonardis, MS[9]; Kristin Sedgwick, MS[9]; Kathleen Soltis, MA[9]; Benjamin Roa, PhD[5]; Richard J. Wenstrup, MD[10]; and Anne-Renee Hartman, MD[3]

- 2,158 patients with breast cancer referred for genetic testing
  - Some also had ovarian or pancreatic cancer
- 38% from high risk families; median age at Dx ≈ 46
- 25-gene germline test

- **9.3% BRCA1/2** positive
- **4.3% non-BRCA1/2** positive
  - ATM, BARD1, BRIP1, CHD1, CDKN2A, CHEK2, MSH2, MSH6,
  - MUTYH (biallelic), NBN, PALB2, PMS2, TP53

Tung *et al., Cancer* 2014

ORIGINAL RESEARCH ARTICLE | Genetics in Medicine

Prevalence of mutations in a panel of breast cancer susceptibility genes in *BRCA1/2*-negative patients with early-onset breast cancer

Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Inherited Mutations in 17 Breast Cancer Susceptibility Genes Among a Large Triple-Negative Breast Cancer Cohort Unselected for Family History of Breast Cancer

ARTICLE | European Journal of Human Genetics

Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes

Laurent Castéra[1,2], Sophie Krieger[1,2,3], Antoine Rousselin[1], Angélina Legros[1], Jean-Jacques Baumann[1],

Pilot clinical utility study

Stanford MEDICINE

JOURNAL OF CLINICAL ONCOLOGY | ORIGINAL REPORT

Clinical Evaluation of a Multiple-Gene Sequencing Panel for Hereditary Cancer Risk Assessment

Allison W. Kurian, Emily E. Hare, Meredith A. Mills, Kerry E. Kingham, Lisa McPherson, Alice S. Whittemore, Valerie McGuire, Uri Ladabaum, Yuya Kobayashi, Stephen E. Lincoln, Michele Cargill, and James M. Ford

Findings:

- Non-BRCA1/2 deleterious mutations were prevalent (4.5% - 9%)
- Many non-BRCA results were **potentially clinically actionable**

Limitations:

- Small patient population (n=198)
- Research panel included genes with varying levels of evidence
- Predates current interpretation and management guidelines

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Kurian *et al., J Clin Oncol* 2014

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Key questions about panel tests

- **Analytic Validity**
  - Can the new tests detect everything the old, gene by gene, tests did?
  - Including technically difficult genes and mutation types?
- **Clinical Validity**
  - Interpretation/classification of DNA alterations
  - Clinical relevance of findings
- **Clinical Utility**
  - What is the clinical impact of using these new tests?

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Laboratory technical study

the Journal of Molecular Diagnostics

Official Journal of the Association for Molecular Pathology

A systematic comparison of traditional and multi-gene panel testing for hereditary breast and ovarian cancer in more than 1000 patients

Stephen E. Lincoln[1], Yuya Kobayashi[1], Michael J. Anderson[1], Shan Yang[1], Andrea J. Desmond[2], Meredith A. Mills[3], Geoffrey B. Nilsen[1], Kevin B. Jacobs[1], Federico A. Monzon[1], Allison W. Kurian[3], James M. Ford[3], Leif W. Ellisen[2,4]

1. Invitae, San Francisco, CA
2. Massachusetts General Hospital Cancer Center, Boston, MA
3. Stanford University School of Medicine, Stanford, CA
4. Harvard Medical School, Boston, MA

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Lincoln *et al., J Mol Diag* 2015

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Analytic validity of NGS in N=1105 individuals

NGS vs. Traditional Methods In 1105 Individuals	
Sensitivity	**100.0%**
Specificity	100.0%

750 Comparable Variants (Pathogenic or Otherwise)	
Sequence Changes	721
Del/dups (CNVs)	**29**

Single Nucleotide	549
Small Indel	156
Large Indel*	**13**
Complex**	**6**

* Large Indel is deletion≥10bp, insertion≥5bp

** Complex includes homopolymer associated variants, indels in low-complexity sequence, short range haplotypes, etc.

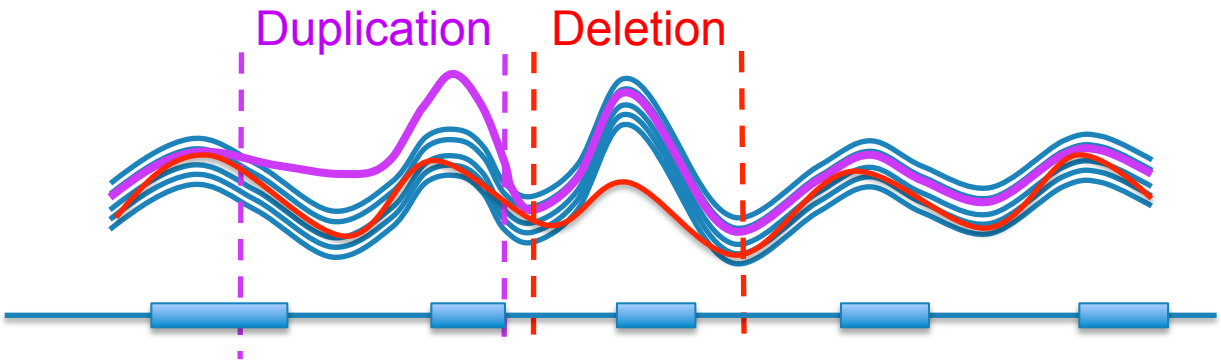
To achieve this, **specialized NGS methods,** biochemical and bioinformatics, are required.

The most challenging classes of variation tend to be **not well represented** in other validation studies.

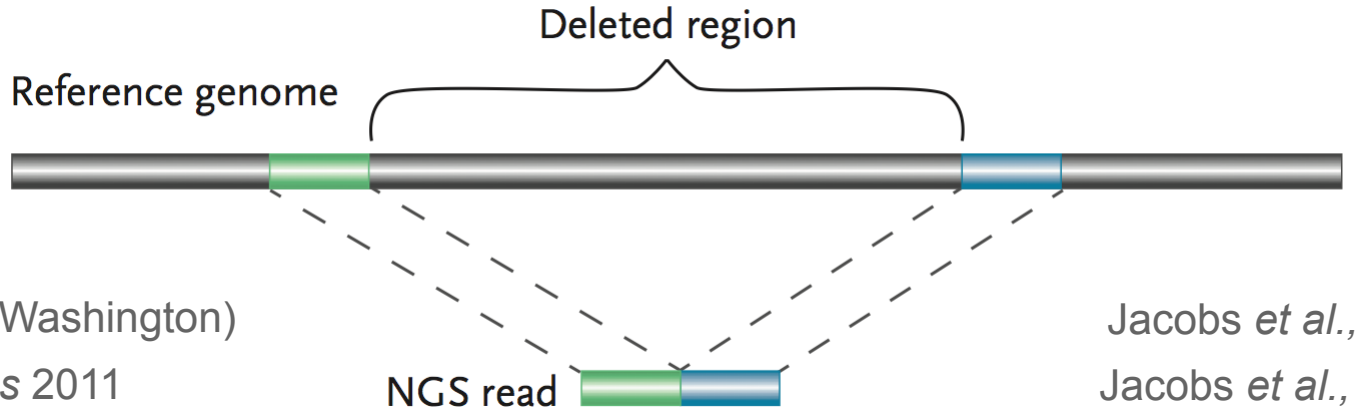
Lincoln *et al., J Mol Diag* 2015

Copy number del/dup detection by NGS

1. Biochemical methods to make data highly callable for CNVs
2. Read depth analysis



3. Combined with split-read analysis



Nord *et al.* (U. Washington)
BMC Genomics 2011

Jacobs *et al.*, CSHL 2013
Jacobs *et al.*, ASHG 2013

A different study: Analytic validity in 250 individuals

NGS vs. Traditional Methods in 250 Patients	
Sensitivity	100.0%
Specificity	99.99%

Note: These numbers assume that Table 2 includes all of the indels and del/dups, and that the remaining variants are benign SNPs. This could have been worded more clearly in the paper.

3025 Variants Appropriate to Measure Sensitivity	
Sequence Changes	3021
Del/dups (CNVs)	4 ?

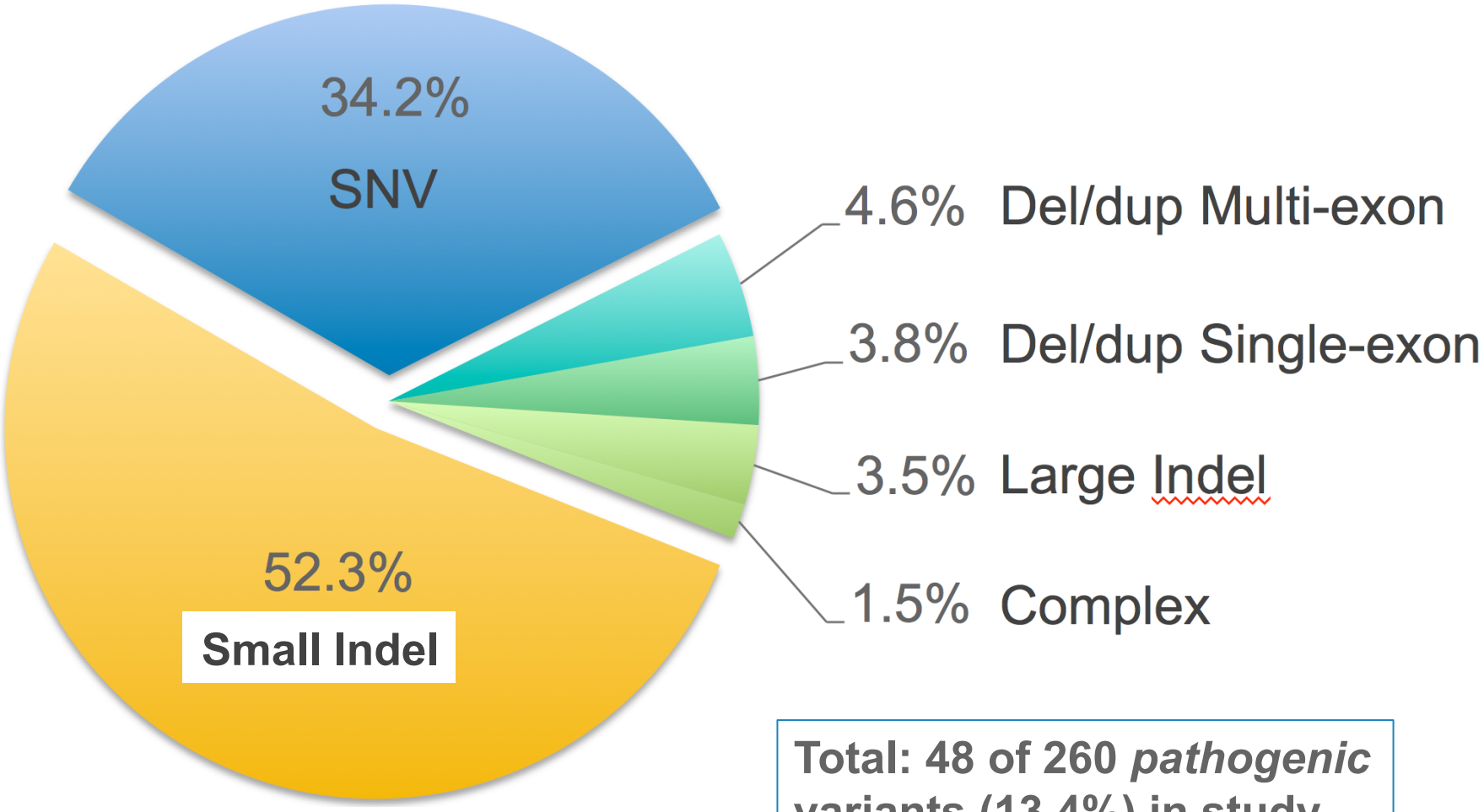
Single Nucleotide	3010
Small Indel	11 ?
Large Indel *	0
Complex **	0

* Large Indel is deletion≥10bp, insertion≥5bp

** Complex includes homopolymer associated variants, indels in low-complexity sequence, short range haplotypes, etc.

Chong *et al., PLOS One* 2014

A significant fraction of the **pathogenic** variants in clinical cases are technically challenging



Total: 48 of 260 *pathogenic* variants (13.4%) in study

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Lincoln *et al., J Mol Diag* 2015

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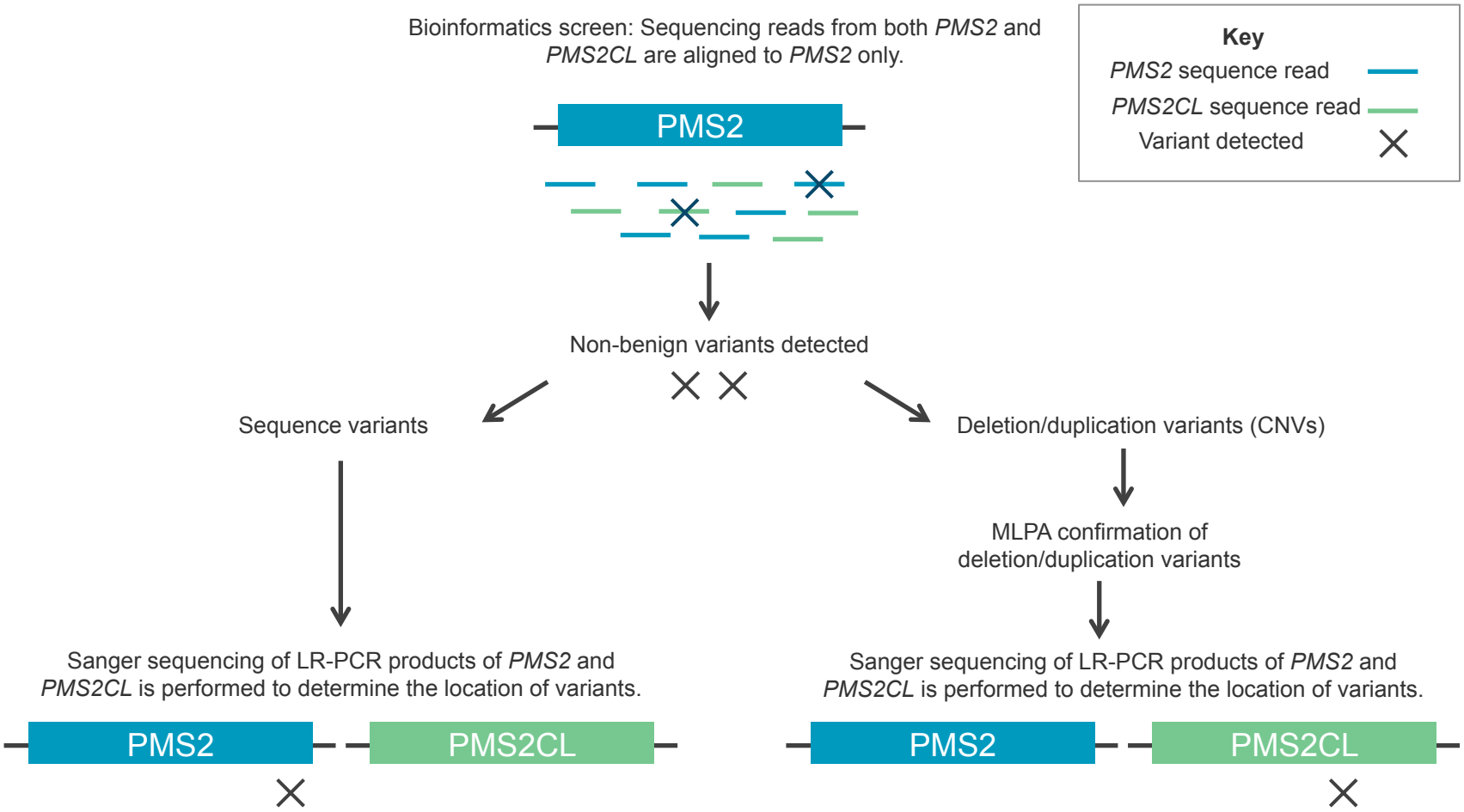
NGS methods for genes with pseudogenes (PMS2)



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BRCA1/2 classification concordance

Positive vs. not positive result for BRCA1/2	
Agree	99.8%
Disagree	0.2%

% of patients with one or more VUS in BRCA1/2	
Panel test	4.1%
Previous test	3.2%

Positive result = pathogenic or likely pathogenic variant

Not positive = VUS, likely benign, or benign variants only

VUS rate = % patients with any variant of uncertain significance, regardless of pathogenic variants present in the same patient

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Lincoln *et al., J Mol Diag* 2015

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Clinical actionability study

JAMA Oncology

Original Investigation

Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment

Andrea Desmond, BS; Allison W. Kurian, MD, MSc; Michele Gabree, MS, CGC; Meredith A. Mills, BA; Michael J. Anderson, PhD; Yuya Kobayashi, PhD; Nora Horick, MS; Shan Yang, PhD; Kristen M. Shannon, MS, CGC; Nadine Tung, MD; James M. Ford, MD; Stephen E. Lincoln, BS; Leif W. Ellisen, MD, PhD

Invited Commentary

Usefulness of Multigene Testing
Catching the Train That's Left the Station

Elizabeth M. Swisher, MD

Desmond *et al., JAMA Oncol.* 2015

Swisher, *JAMA Oncol.* 2015

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Clinical study patient population

- Multisite **prospective** recruitment

- N=1046 patients referred to Genetics who:
  - **Met NCCN criteria** for hereditary breast/ovarian cancer evaluation
  - Not BRCA1/2 positive
  - Not referred because of a mutation identified in a relative
  - Tested with a multigene panel (25 to 29 genes)
  - Representative cohort (in both demographics and indications)

➢ **63** patients with non-*BRCA1/2* mutations
  - 40 of 1046 (3.8%)
  - 23 additional patients from clinical practice (who met same criteria)

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Desmond *et al., JAMA Oncol* 2015

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Clinical relevance of 63 non-*BRCA1/2* findings

- **74%** of the cancer-affected probands have a **syndromic cancer** for the gene they were found to carry (26% do not)
- In **92%** of cases, the patient's **personal and/or family history was consistent** with the syndromic effects of the gene they carry
- Nevertheless, in most of these cases the proband **would not have been eligible for testing** for the gene they carry under indication-based guidelines

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Desmond *et al., JAMA Oncol* 2015

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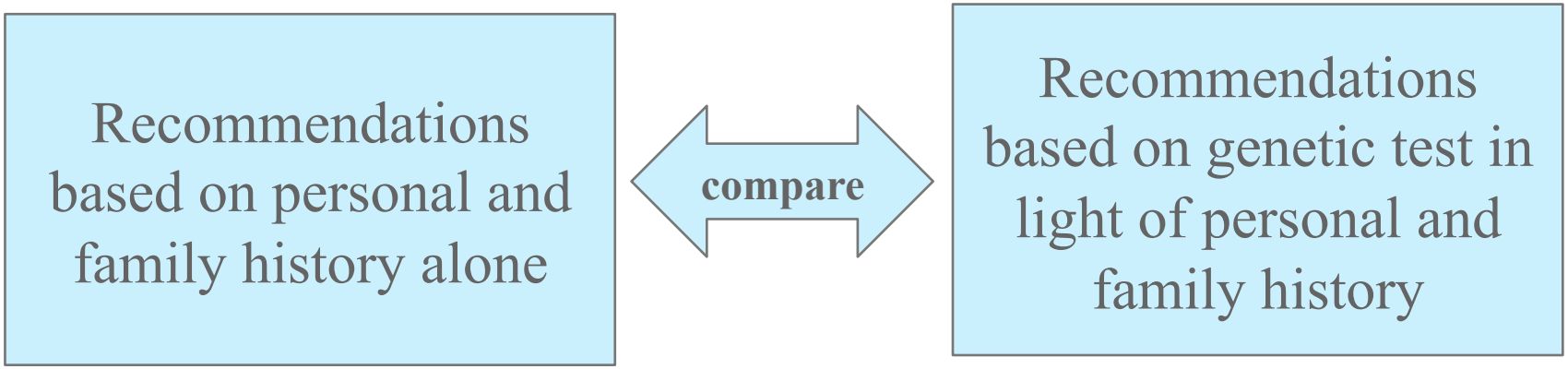
Clinical actionability of non-*BRCA1/2* findings

- For patients with non-*BRCA1/2* mutations we compared pre-test management recommendations to post-test recommendations, in both cases following current consensus management guidelines.



- Similar review of first-degree female family members

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Desmond *et al., JAMA Oncol* 2015

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	Recommend MRI[c] (>20% risk of breast cancer[d])	Recommend RRSO	Discuss Option of RRM
Intervention Warranted based on gene and/or risk level	***ATM*** ***BRCA1*** ***BRCA2*** ***CDH1*** ***CHEK2*** ***PALB2*** ***PTEN*** ***STK11*** ***TP53***	***BRCA1*** ***BRCA2*** **Lynch syndrome[e]**	***BRCA1*** ***BRCA2*** ***CDH1*** ***PTEN*** ***TP53***
Insufficient evidence for intervention[b]	***BARD1*** ***BRIP1***	***BARD1*** ***BRIP1*** ***PALB2*** ***RAD51C*** ***RAD51D***	***ATM*** ***BARD1*** ***CHEK2*** ***PALB2*** ***STK11***

[b]Intervention may still be warranted based on family history or other clinical factors.

[a]Other genes may be included in multi-gene testing.
[b]Intervention may still be warranted based on family history or other clinical factors.
[c]See NCCN Guidelines for Breast Cancer Screening and Diagnosis.
[d]May be modified based on family history or specific gene mutation.
[e]See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.

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Genes with established links to breast cancer

The NEW ENGLAND JOURNAL of MEDICINE

SPECIAL REPORT

Gene-Panel Sequencing and the Prediction of Breast-Cancer Risk

Douglas F. Easton, Ph.D., Paul D.P. Pharoah, Ph.D., Antonis C. Antoniou, Ph.D., Marc Tischkowitz, M.D., Ph.D., Sean V. Tavtigian, Ph.D., Katherine L. Nathanson, M.D., Peter Devilee, Ph.D., Alfons Meindl, Ph.D., Fergus J. Couch, Ph.D., Melissa Southey, Ph.D., David E. Goldgar, Ph.D., D. Gareth R. Evans, M.D., Georgia Chenevix-Trench, Ph.D., Nazneen Rahman, M.D., Ph.D., Mark Robson, M.D., Susan M. Domchek, M.D., and William D. Foulkes, M.B., B.S., Ph.D.

Easton *et al.*, *NEJM* 2015

Eleven genes with established links to breast cancer

The NEW ENGLAND JOURNAL of MEDICINE

High-Risk Panels✚	Moderate-Risk Panels ✚
BRCA1	*ATM*
BRCA2	*CHEK2*
TP53	*NBN* °
CDH1	*NF1* °
PALB2	
PTEN *	
STK11 *	

° Not mentioned in 1.2015 NCCN HBOC guidelines

* Relative risk is not yet precisely estimated (per Easton *et al.*)

✚ Typically included on panels offered by multiple laboratories

Easton *et al.*, *NEJM* 2015 (Table 3)

Clinical management recommendations changed

Criteria	Positive genes	Potential change	Patients	Family members
High-risk genes 1.2015 NCCN management guidelines	*CDH1*, *TP53*, *PTEN*, *MLH1*, *MSH2*, *MSH6*, *PMS2*, *APC*, *BMPR1A*, *MUTYH* (biallelic)	Guidelines-based surveillance/ prevention	**20 / 20**	**19 / 19**
≥40% breast cancer risk (and <40% pre-test risk)	*PALB2*	Surgical prevention candidate	**5 / 8**	**7 / 7**
>20% breast cancer risk (<20% pre-test risk) NCCN 1.2015 guidelines	*ATM*, *CHEK2*, *NBN*, *RAD51C*, *BRIP1*	Enhanced breast screening candidate	**5 / 32**	**13 / 29**
Other cancer risks (pancreas, melanoma)	*CDKN2A*	Pancreas screening candidate	**3 / 3**	**3 / 3**

- **52%** of non-*BRCA1/2* positive probands (33/63)
- **72%** of family members (42/58) if found to also be positive

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Desmond *et al. JAMA Oncology* 2015

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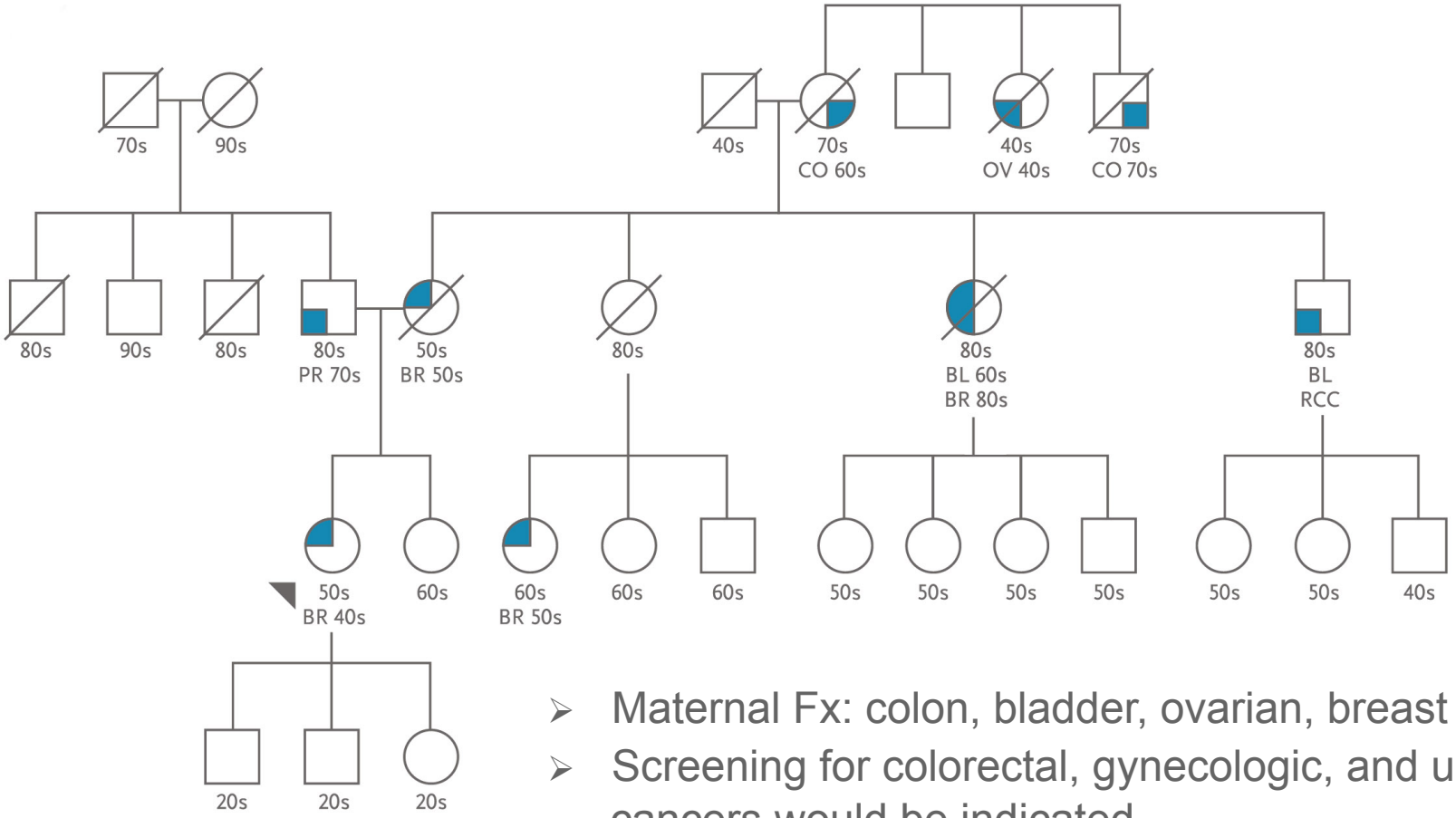
Case study: MSH6 positive



- Maternal Fx: colon, bladder, ovarian, breast cancers
- Screening for colorectal, gynecologic, and urologic cancers would be indicated.
- RR gynecologic surgery could be considered.
- Additional family member testing recommended.

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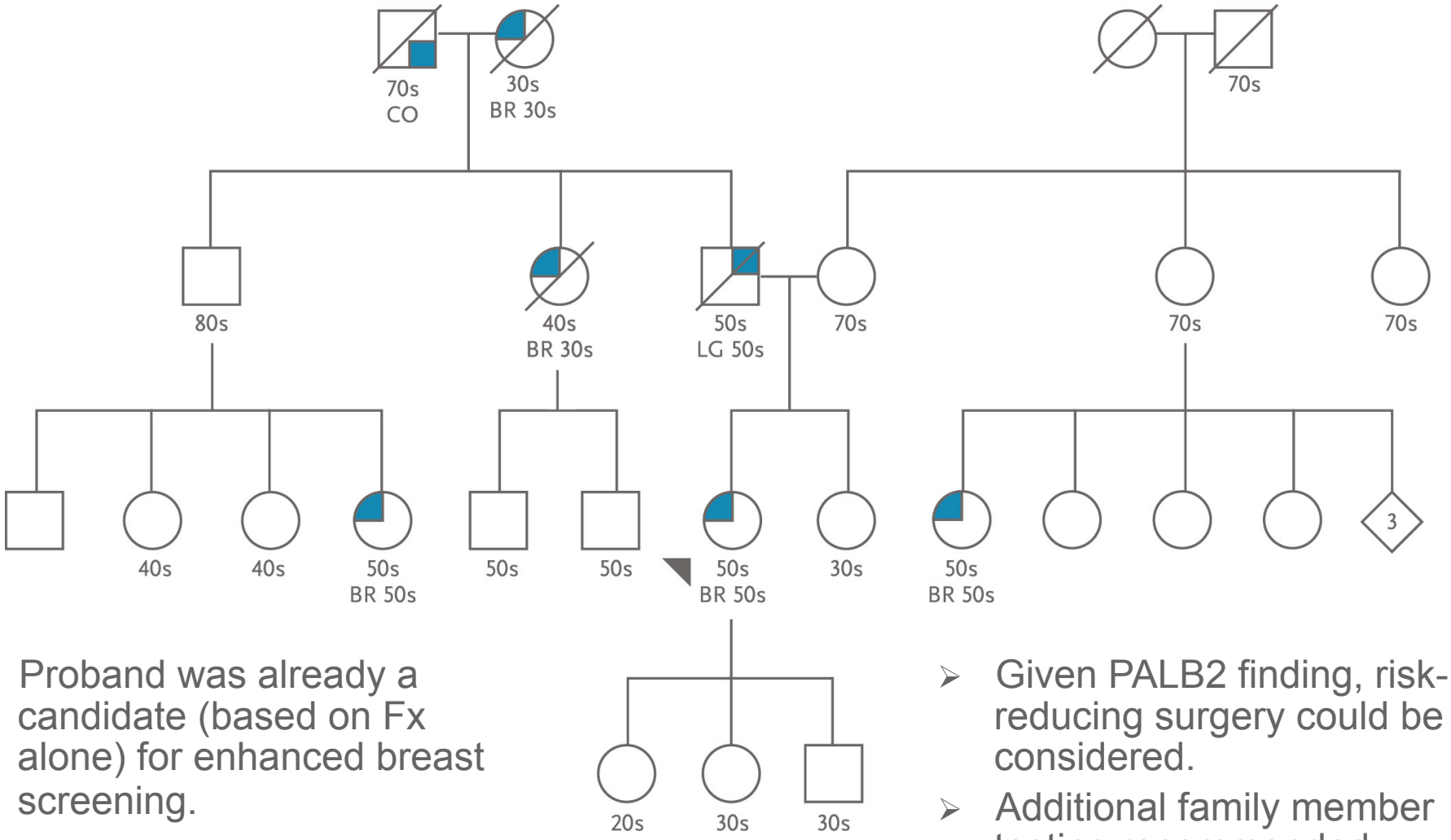
Desmond *et al. JAMA Oncology* 2015

Case study: PALB2 positive



- Proband was already a candidate (based on Fx alone) for enhanced breast screening.

- Given PALB2 finding, risk-reducing surgery could be considered.
- Additional family member testing recommended.

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Desmond *et al. JAMA Oncology* 2015

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Patients with no management change in this study

1. Patient has or had breast cancer
   - Breast screening recommendations were not considered
2. Patient already had bilateral mastectomy
   - RRM recommendation was not considered
3. Action would already be indicated based on personal or family history alone
4. Lifetime risk was not likely to be >20%

- We also did not evaluate management changes for patients with negative results (potentially downgrading risk)
- *Thus, we likely underestimated actionability in this study*

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Desmond *et al. JAMA Oncology* 2015

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Important notes on this study

1. We used current gene-specific risk estimates.
2. We used current consensus guidelines.
3. We had extensive family histories on all patients.
4. We did not examine which of these management changes were or would be implemented.
5. We measured actionability in appropriately referred patients meeting current guidelines for evaluation.

Desmond *et al. JAMA Oncology* 2015

Conclusions

- **Analytic Validity**
  - NGS-based tests can provide **equivalent performance** to traditional genetic tests
    - *Includes CNVs (del/dups) and complex classes of sequence variation*
    - *But only with specialized NGS biochemistry and bioinformatics methods*
- **Clinical Validity**
  - Interpretations for BRCA1/2 can be **highly concordant**
    - *Following ACMG 2015 ISV guidelines and using public resources*
- **Clinical Utility**
  - Findings from gene panels are often **clinically actionable**
    - *Under current guidelines and over and above any actions based on personal and family history alone*

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JAMA Oncology

- Desmond *et al.*, 2015 (open access)
- Swisher, 2015 (commentary)

the Journal of Molecular Diagnostics

- Lincoln *et al.*, 2015 (open access)

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  - steve.lincoln@invitae.com

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- **Leif Ellisen**
- Andrea Desmond
- Michelle Gabree
- Kristen Shannon

Stanford MEDICINE

- **Jim Ford**
- **Allison Kurian**
- Meredith Mills

Beth Israel Deaconess Medical Center

- **Nadine Tung**

A systematic and accurate approach to variant classification

Scott Topper, PhD, FACMG
Head of Clinical Genomics, Invitae

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Variant Classification

- Variant classification is the first pillar of clinical genetics.
- Variants are either **Pathogenic** or **Benign.**
- Sometimes the nature of the variant is **Unknowable** given the current state of information.
- Goals:
  - **Accurately**, **efficiently,** and **transparently** assess pathogenicity
  - When there is insufficient information, **confidently** conclude that the nature of the variant is unknowable

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Challenge 1: Abundance

- Many variants
- Many labs
- Many opinions

- Extensive literature
- Conflicting literature
- Redundant literature
- Extensive direct and indirect evidence

ClinVar submissions

This page summarizes the number of genes and distinct variant locations currently represented in ClinVar from in submissions. A gene is reported if a variant in ClinVar is either found within, or includes, that gene. Thus the nun as it is because of the structural variants in the database that span many genes.

This page lists all submitters and the summary of their contributions. We acknowledge their support.

Submission overview

Category of analysis	Current total (Apr 27, 2015)
Total accessioned submissions	172043
Total genes represented	26391
Total genes, in submissions with assertions, with variants in one gene	7406
Total genes, in submissions with assertions, with variants in multiple genes	26204
Total variations represented	145314
Total variations, in submissions with assertions	118174
Total submitters	312

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Challenge 2: Too little, too late

REPORTS

Mutation of MEF2A in an Inherited Disorder with Features of Coronary Artery Disease

Lejin Wang,[1,2] Chun Fan,[1,2] Sarah E. Topol,[1] Eric J. Topol,[1,3]* Qing Wang[1,2]*

28 NOVEMBER 2003 VOL 302 SCIENCE www.sciencemag.org

PMID: 14645853

- 21bp in-frame deletion; conserved AAs
- Segregates in one large family (LOD 4.1)
- del7 AA disrupts protein function in vitro
- Variant is not present in 119 normal individuals

Possible Conclusion: causes disease

Research article Related Commentary, page 831

PMID: 15841183

2005

Lack of *MEF2A* mutations in coronary artery disease

Li Weng,[1] Nihan Kavaslar,[2] Anna Ustaszewska,[1] Heather Doelle,[2] Wendy Schackwitz,[1] Sybil Hébert,[2] Jonathan C. Cohen,[3] Ruth McPherson,[2] and Len A. Pennacchio[1,4]

[1]US Department of Energy, Joint Genome Institute, Walnut Creek, California, USA. [2]Division of Cardiology and the Lipoprotein and Atherosclerosis Research Group, University of Ottawa Heart Institute, Ottawa, Ontario, Canada. [3]Center for Human Nutrition and McDermott Center for Human Growth and Development, University of Texas Southwestern Medical Center, Dallas, Texas, USA. [4]Genomics Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA.

- 0/300 CAD patients had any likely causative variants in MEF2A
- 3/1821 (0.2%) controls had the MEF2A del7 variant
- No segregation with disease in 3 families with del7 variant

Possible Conclusion: does NOT cause disease

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Challenge 3: Passion

- We **want** to find an answer, and sometimes the answer isn't there

- Reasonable people disagree

What we need:

- A system and infrastructure that…
  - Encourages different people to come to the same conclusion
  - Protects against the overuse of types of evidence
  - Supports effective discussion, disagreement and debate

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Standardization efforts

ACMG/AMP ISV Guidelines

Draft

Aug 2013

The Scoring Rules for Classification

Pathogenic

a) 1 Very Strong (PVS1) *AND*
   a. 1 Strong (PS1-PS4) *OR*
   b. ≥1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) *OR*
   c. ≥ 2 Supporting (PP1-PP5)

b) 2 Strong (PS1-PS4)

c) 1 Strong (PS1-PS4) *AND*
   a. ≥ 3 Moderate (PM1-PM6) OR
   b. ≥2 Moderate (PM1-PM6) and 2 Supporting (PP1-PP5) *OR*
   c. ≥1 Moderate (PM1-PM6) and 4 Supporting (PP1-PP5)

Likely Pathogenic

a) 1 Strong (PS1-PS4) *AND*
   a. ≥ 1 Moderate (PM1-PM6) *OR*
   b. ≥ 2 Supporting (PP1-PP5)

b) ≥3 Moderate (PM1-PM6)

c) ≥2 Moderate(PM1-PM6) *AND* 2 Supporting (PP1-PP5)

d) ≥1 Moderate (PM1-PM6) *AND* 4 Supporting (PP1-PP5)

Benign

a) 1 Stand-Alone (BA1) *OR*

b) ≥ 2 Strong (BS1-BS4)

Likely Benign

a) 1 Strong (BS1-BS4) and ≥1 Supporting (BP1-BP6) *OR*

b) > 2 Supporting (BP1–BP6)

***Variants should be classified as Uncertain Significance if other criteria are unmet.**

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Expertly trained doctorate-level scientists

Keith Nykamp, PhD

Michael Anderson, PhD

A large team of doctorate-level scientists with years of research training and a deep understanding of molecular and human genetics, led by **Keith Nykamp** and **Michael Anderson**

John Garcia, PhD

Yuya Kobayashi, PhD

Jody Westbrook, PhD

Nila Patil, PhD

Janita Thusberg, PhD

Blanca Herrera, PhD

Yuan-Yuan Ho, PhD

Brandon Bunker, PhD

Hio Chung Kang, PhD

Daniel Beltran, PhD

Michelle Zeman, PhD

Ali Entezam, PhD

Sarah McCalmon, PhD

Rachel Lewis, MS, MB(ASCP)

Laura Murillo, PhD

Karl Erhard, PhD

Carolina Pardo, PhD

Rita Quintana, PhD

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A strong genetic counseling team

Amy Fuller
M.S., CGC

Christy Hartshorne
M.S., LCGC

A large team of board-certified genetic counselors with years of experience in both laboratories and the clinic, led by **Amy Fuller** and **Christy Hartshorne**

Darlene Riethmaier
M.S., CGC

Tali Ekstein
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Marina Rabideau
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Sc.M., LCGC

Stephanie Gandomi
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Renowned medical and clinical genetics experts

Robert Nussbaum, M.D.
Chief Medical Officer

Strong lineup of board-certified professionals and genomics scientists, led by chief medical officer **Dr. Robert Nussbaum**, a pioneer in medical genetics

Adam Rosendorff
M.D.

Swaroop Aradhya
Ph.D., FACMG

Scott Topper
Ph.D., FACMG

Martin Powers
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Karen Ouyang
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Eden Haverfield
Ph.D., FACMG

Britt Jphnson
Ph.D., FACMG

Tom Winder
Ph.D., FACMG

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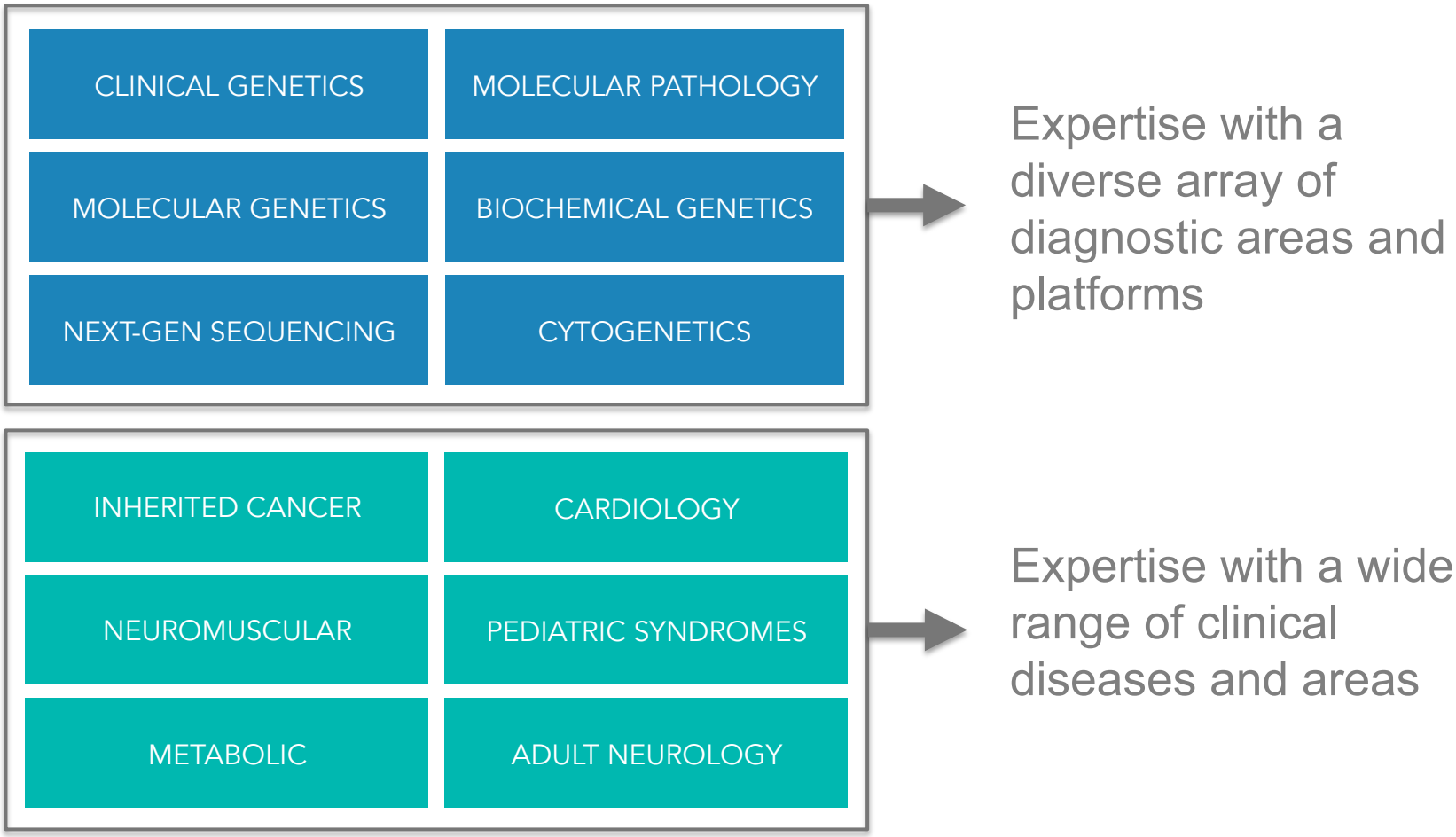
We have an outstanding team



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Standardization efforts

ACMG/AMP ISV Guidelines

Draft

Aug 2013

The Scoring Rules for Classification

Pathogenic

a) 1 Very Strong (PVS1) *AND*
   a. 1 Strong (PS1-PS4) *OR*
   b. ≥1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) *OR*
   c. ≥ 2 Supporting (PP1-PP5)

b) 2 Strong (PS1-PS4)

c) 1 Strong (PS1-PS4) *AND*
   a. ≥ 3 Moderate (PM1-PM6) OR
   b. ≥2 Moderate (PM1-PM6) and 2 Supporting (PP1-PP5) *OR*
   c. ≥1 Moderate (PM1-PM6) and 4 Supporting (PP1-PP5)

Likely Pathogenic

a) 1 Strong (PS1-PS4) *AND*
   a. ≥ 1 Moderate (PM1-PM6) *OR*
   b. ≥ 2 Supporting (PP1-PP5)

b) ≥3 Moderate (PM1-PM6)

c) ≥2 Moderate(PM1-PM6) *AND* 2 Supporting (PP1-PP5)

d) ≥1 Moderate (PM1-PM6) *AND* 4 Supporting (PP1-PP5)

Benign

a) 1 Stand-Alone (BA1) *OR*

b) ≥ 2 Strong (BS1-BS4)

Likely Benign

a) 1 Strong (BS1-BS4) and ≥1 Supporting (BP1-BP6) *OR*

b) > 2 Supporting (BP1–BP6)

***Variants should be classified as Uncertain Significance if other criteria are unmet.**

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Standardization efforts

ACMG/AMP ISV Guidelines

Draft → Final

Aug 2013

Mar 2015

“consensus document”

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a) ≥3 Moderate (PM1–PM6) *OR* (b) 2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

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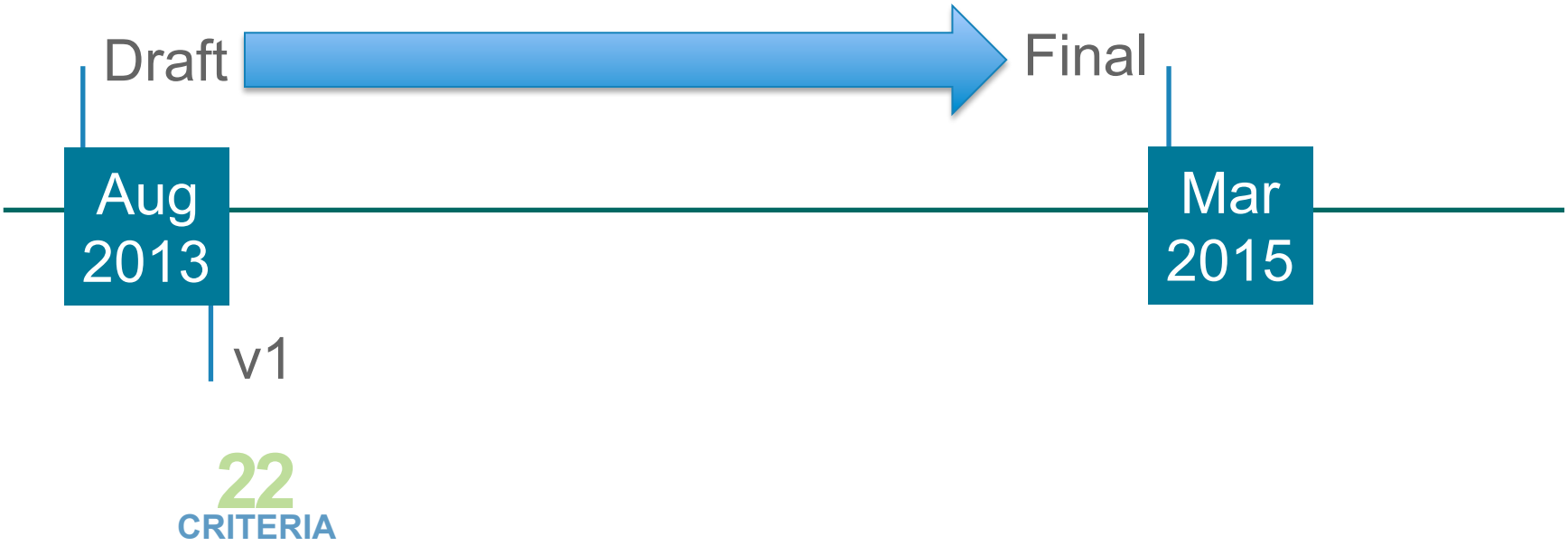
Implementation, expert review and evolution

ACMG/AMP ISV Guidelines



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Truncating Variants

PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3′ end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts

Truncating Variant, Presumed loss of protein	*Only use for genes with known loss-of-function mechanisms. Do not use if the premature stop codon is in the last exon or within the last 15 codons of the penultimate exon, unless there are proven LOF mutations farther downstream. Instead use "Truncating mutation, may escape NMD." Do not use for start codon mutations if an alternate methionine may be present nearby.*
Truncating Variant, may escape NMD	*Only use for genes with known loss-of-function mechanisms. Used for truncating mutations when complete loss of protein function cannot be presumed. Examples include a premature stop codon in the last exon or within the last 15 codons of the penultimate exon, a truncating variant seen at a relatively high frequency in the general population, or a start codon substitution with a nearby alternative initiator methionine.*
Truncating Variant, Molecular mechanism unknown	*Use this if the molecular mechanism is unknown or complicated.*
Truncating Variant, GOF mechanism	*Use if gain-of-function has been established as the only disease mechanism*

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Case Reports

PP1 Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

Note: May be used as stronger evidence with increasing segregation data

PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance.

Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

- 4 unrelated case reports, w/ pathognomonic features
- STRONG segregation w/ disease - (LOD>3, 2+ families)
- Homozygous or hemizygous in an unaffected adult, recessive or X-linked, HIGH penetrance,
- Co-occurrence in an affected individual w/ an alternate cause of disease

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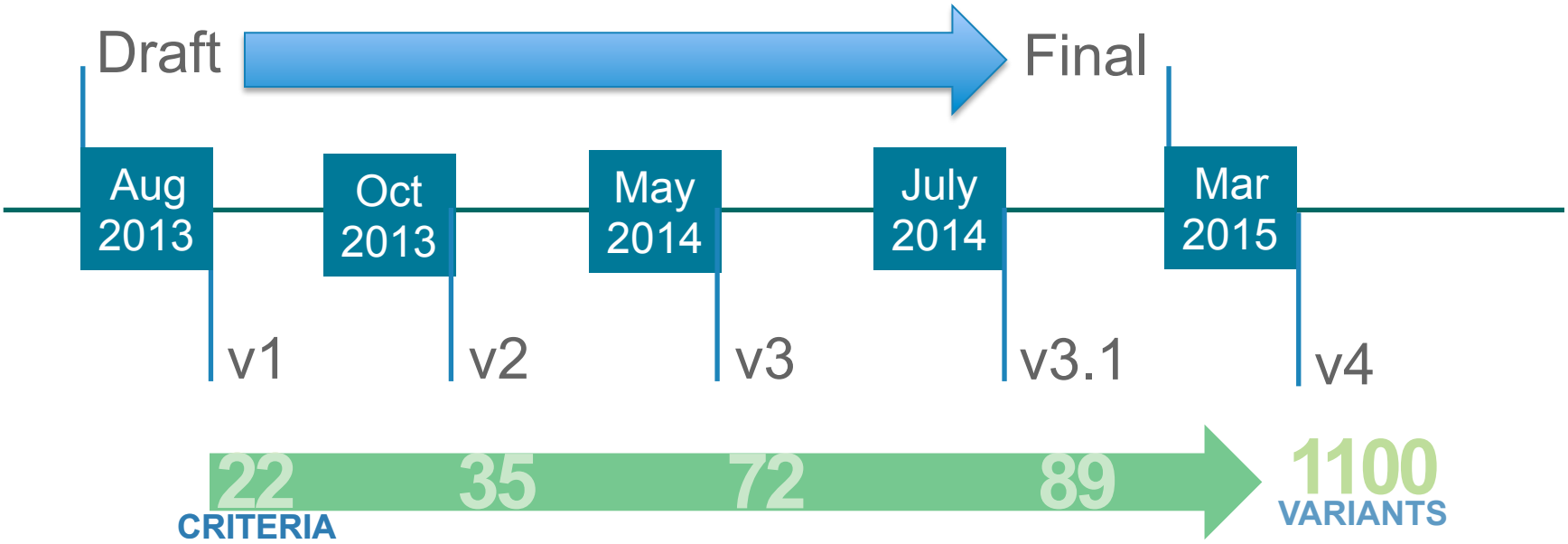

Invitae Clinical Genomics Team

- Add/remove criteria
- Change scores, adjust LB threshold
- Create priority groups
- Add usage notes

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Scientific hierarchical evidence regimen for variant locus classification

SHERLOC

5 basic organizing principles in the schema

- **Points:** each EV criteria is given a preset number of points – meant to reflect the relative importance of each evidence type
- **Pathogenic vs. Benign:** each EV criteria supports either Pathogenic or Benign, and sums in both directions are assessed independently
- **Groups**: certain types of evidence contribute to the same basic argument, and these are grouped together with only the highest priority evidence contributing to classification
- **Additive:** multiple instances of the same evidence type, in some cases, can contribute to the final classifications – other types of evidence do not
- **Thresholds:** the number of points required for a classification is preset

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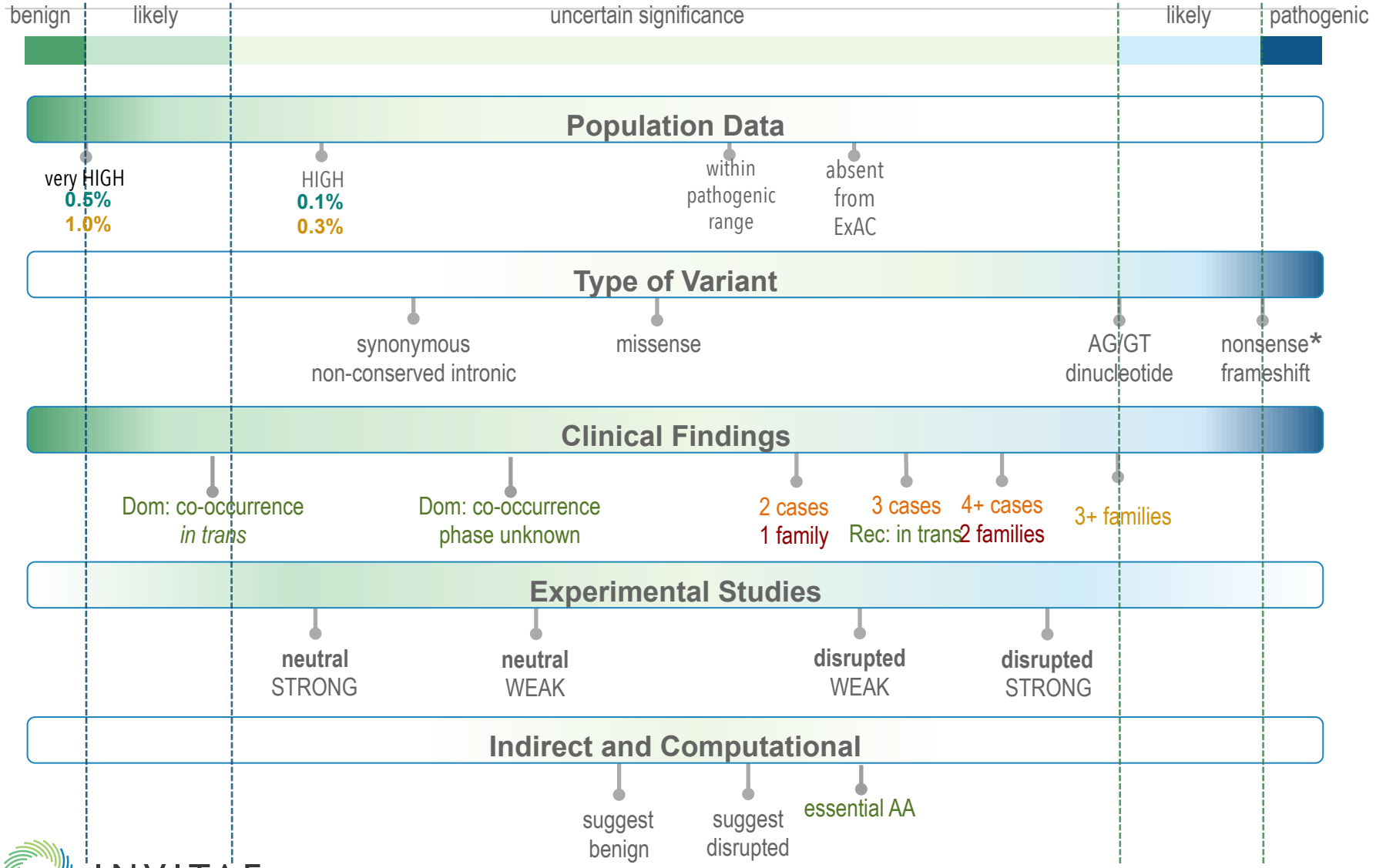
Evidence Types



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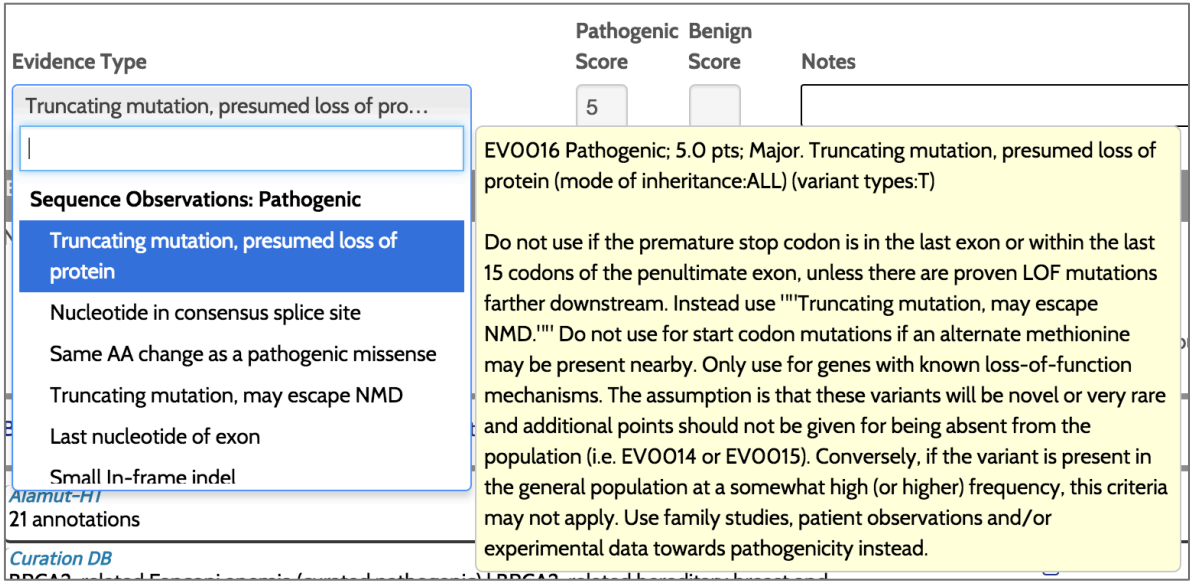
…with detailed usage rules

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Variable name	Path/Benign	Strength	Category	path rules	variant type	score	description			Add points if you use same criterion >1x		
2	Variable type	Binary	Ordinal	Nominal	Nominal	Nominal	Continuous	Free TEXT	Group	Priority (	Addit	NOTES (ID)	ACMG group
3	UID												
19	EV0076	Benign	Major	Population	Dominant	VI/S	5	Population data: BRCA1/2 - Very high MAF 0.4%	Z0001	4	N	N0059, N0055	STAND ALONE
20	EV0077	Benign	Major	Population	Dominant	VI/S	4	Population data: BRCA1/2 - High MAF 0.1%	Z0001	5	N	N0059, N0048, N0055	STRONG
21	EV0089	Benign	Major	Population	Dominant	VI/S	1	Population data: BRCA1/2 - Somewhat high, 3 repor	Z0001	6	N	N0059, N0055	
22	EV0013	Benign	Major	Population	ALL	ALL	4	3+ homozygotes in public databases HIGH	Z0002	2	N	N0047, N0066	Strong
23	EV0090	Benign	Major	Population	ALL	ALL	2	3+ homozygotes in public databases MODERATE	Z0002	1	N	N0047, N0045	Strong
24	EV0082	Pathogenic	Major	Individual Observations	Recessive	CNV/MS/ID	4	Novel homozygous variant in a classically affected in	Z0003	1	Y	N0063, N0053	
25	EV0014	Pathogenic	Major	Individual Observations	ALL	ALL	2.5	Novel variant detected in an individual with expecte	Z0003	2	N	N0026, N0025	Moderate
26	EV0015	Pathogenic	Minor, direct	Population	ALL	ALL	1	Variant absent from general population	Z0003	3	N	N0026, N0056	
27	EV0016	Pathogenic	Major	Sequence Observations	ALL	T	5	Truncating mutation, presumed loss of protein	Z0004	1	N	N0035, N0001	Very Strong
28	EV0017	Pathogenic	Major	Sequence Observations	ALL	CSS	4	Nucleotide in consensus splice site	Z0004	2	N	N0029, N0001	Very Strong
29	EV0018	Pathogenic	Major	Sequence Observations	ALL	MS/ID	4	Same AA change as a pathogenic missense	Z0004	3	N	N0031, N0054	Strong



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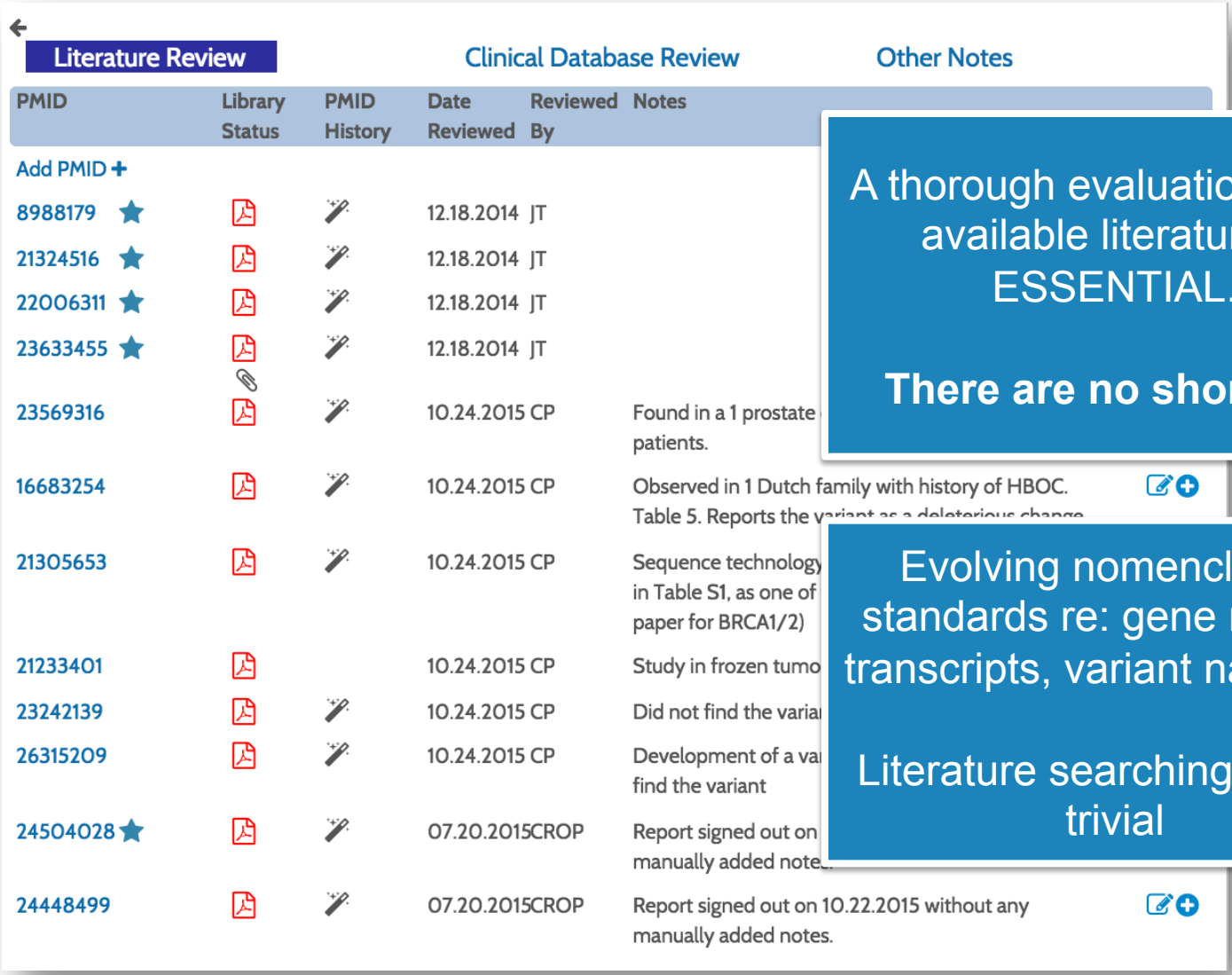
Comprehensive literature searching



A thorough evaluation of the available literature is ESSENTIAL.

There are no shortcuts

Evolving nomenclature standards re: gene names, transcripts, variant names….

Literature searching is non-trivial

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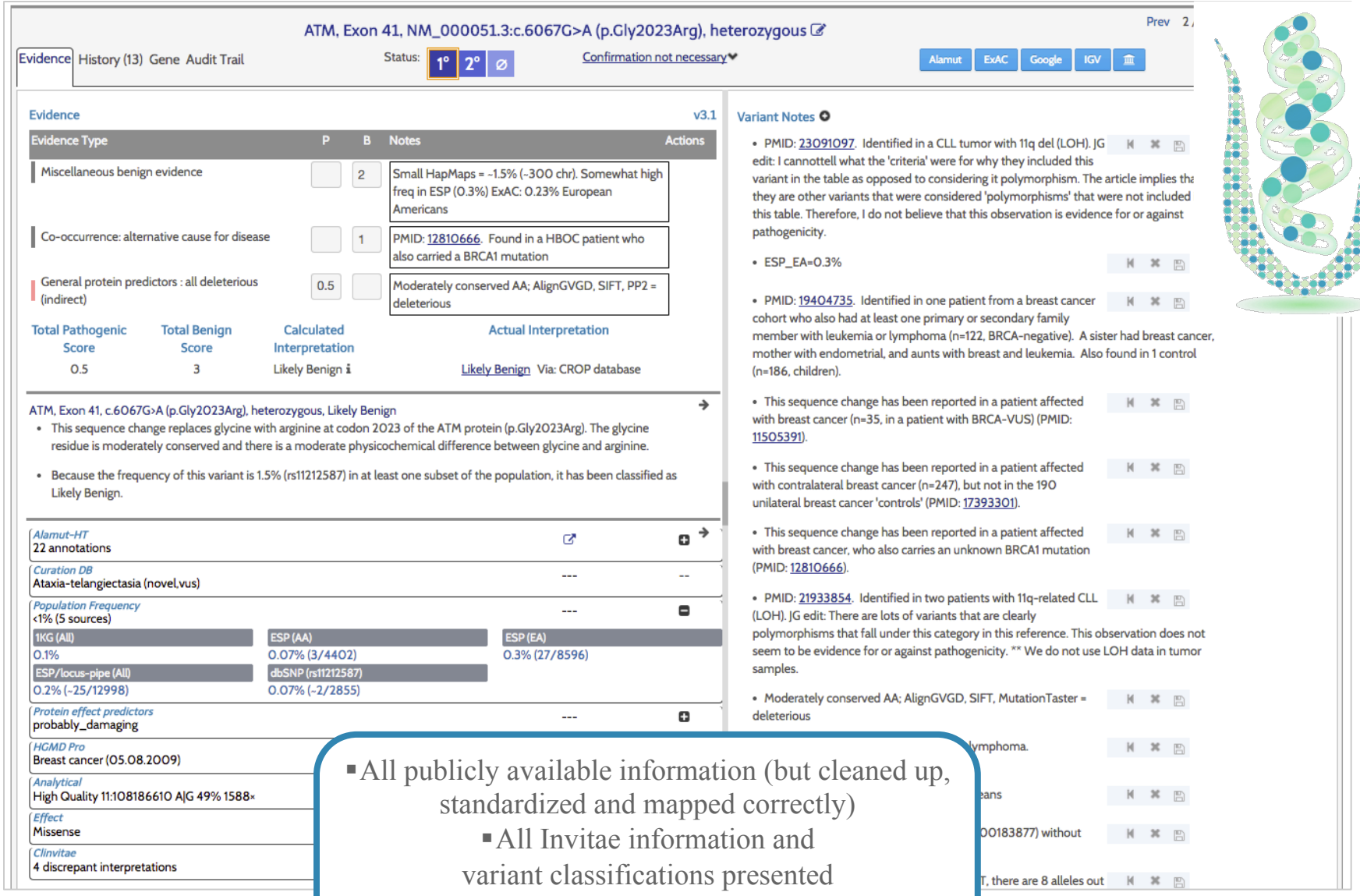
Pulling all available information into focus



- All publicly available information (but cleaned up, standardized and mapped correctly)
- All Invitae information and variant classifications presented
- Variant history
- Links to quality threshold data

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Evaluating the validity of our variant classification approach

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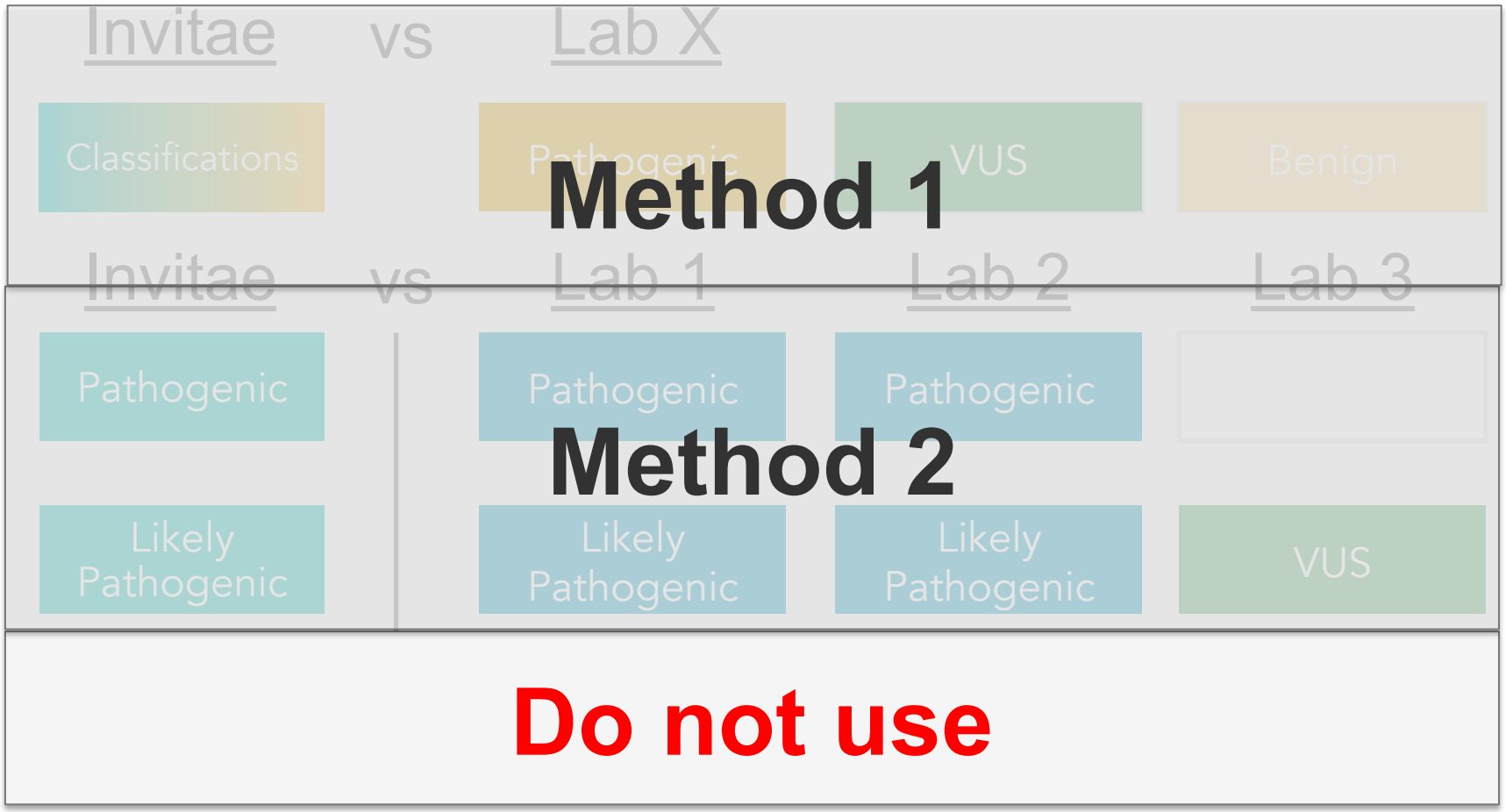
There is no gold-standard data set for comparison



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Method 1: We compared our results to Myriad's

- Compared Invitae's NGS test to traditional BRCA1/BRCA2 tests in more than 1000 patients
- 95% classification concordance along the 5-tier scale for all variants
- 100% analytic sensitivity and specificity for sequencing and CNV detection
- 99.8% report concordance (Pos, VUS, Neg) with Myriad Genetics reports

The Journal of Molecular Diagnostics,

ELSEVIER

MGH 1811 MASSACHUSETTS GENERAL HOSPITAL

Stanford MEDICINE

the Journal of Molecular Diagnostics

jmd.amjpathol.org

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Method 2: Sherloc interpretations are consistent with ClinVar

Sherloc vs ClinVar Consensus

- 92.2% concordance with the consensus majority
- Almost all mismatches were P-LP or **VUS-LB-B** differences
- The single exception was a Sherloc VUS compared to a **Consensus LP**

Consensus Interpretation

Sherloc	Pathogenic	LP	VUS	LB	Benign	Total	#Variants
Pathogenic	99.1%	0.9%	0.0%	0.0%	0.0%	100%	117
LP	50.0%	50.0%	0.0%	0.0%	0.0%	100%	10
VUS	0.0%	0.4%	93.6%	5.6%	0.4%	100%	249
LB	0.0%	0.0%	11.0%	78.0%	11.0%	100%	82
Benign	0.0%	0.0%	2.5%	2.2%	95.2%	100%	356
						Total	814

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…more so than the average clinical testing lab

Sherloc vs ClinVar Consensus

- 92.2% concordance with the consensus majority
- Almost all mismatches were P-LP or **VUS-LB-B** differences
- The single exception was a Sherloc VUS compared to a **Consensus LP**

Other Labs vs ClinVar Consensus

- Other **laboratories had on average an** 85.7% concordance
- The average lab had **more clinically significant** discordances

Consensus Interpretation

Other Labs	Pathogenic	LP	VUS	LB	Benign	Total	#Interpretations
Pathogenic	97.8%	1.3%	0.6%	0.3%	0.0%	100%	312
LP	13.3%	66.7%	20.0%	0.0%	0.0%	100%	15
VUS	0.6%	0.2%	93.1%	3.0%	3.0%	100%	494
LB	0.4%	0.0%	12.1%	49.8%	37.7%	100%	281
Benign	0.0%	0.0%	2.0%	4.4%	93.6%	100%	798
						Total	1900

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In conclusion…

> ***"Nothing clears up a case so much as stating it to another person."***
>
> *-Silver Blaze,* **Sir Arthur Conan Doyle**

- Sherloc is adaptable, efficient and transparent.
- Variant classifications are a direct result of thorough and reproducible evaluation of evidence.
- Your clients need to know the logic behind your conclusions.

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Thank you
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Appendix

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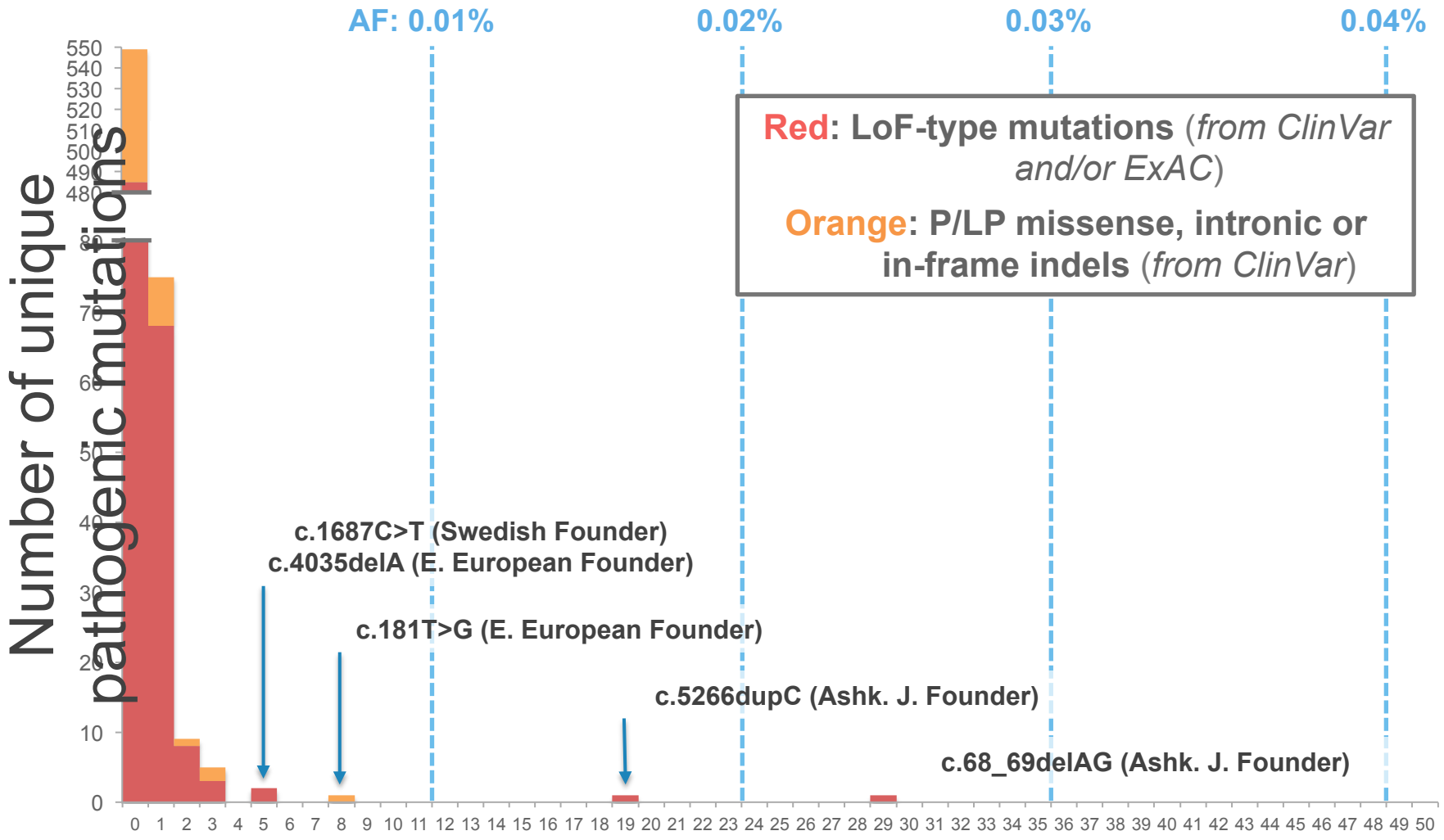
BRCA1 pathogenic mutation frequency spectrum



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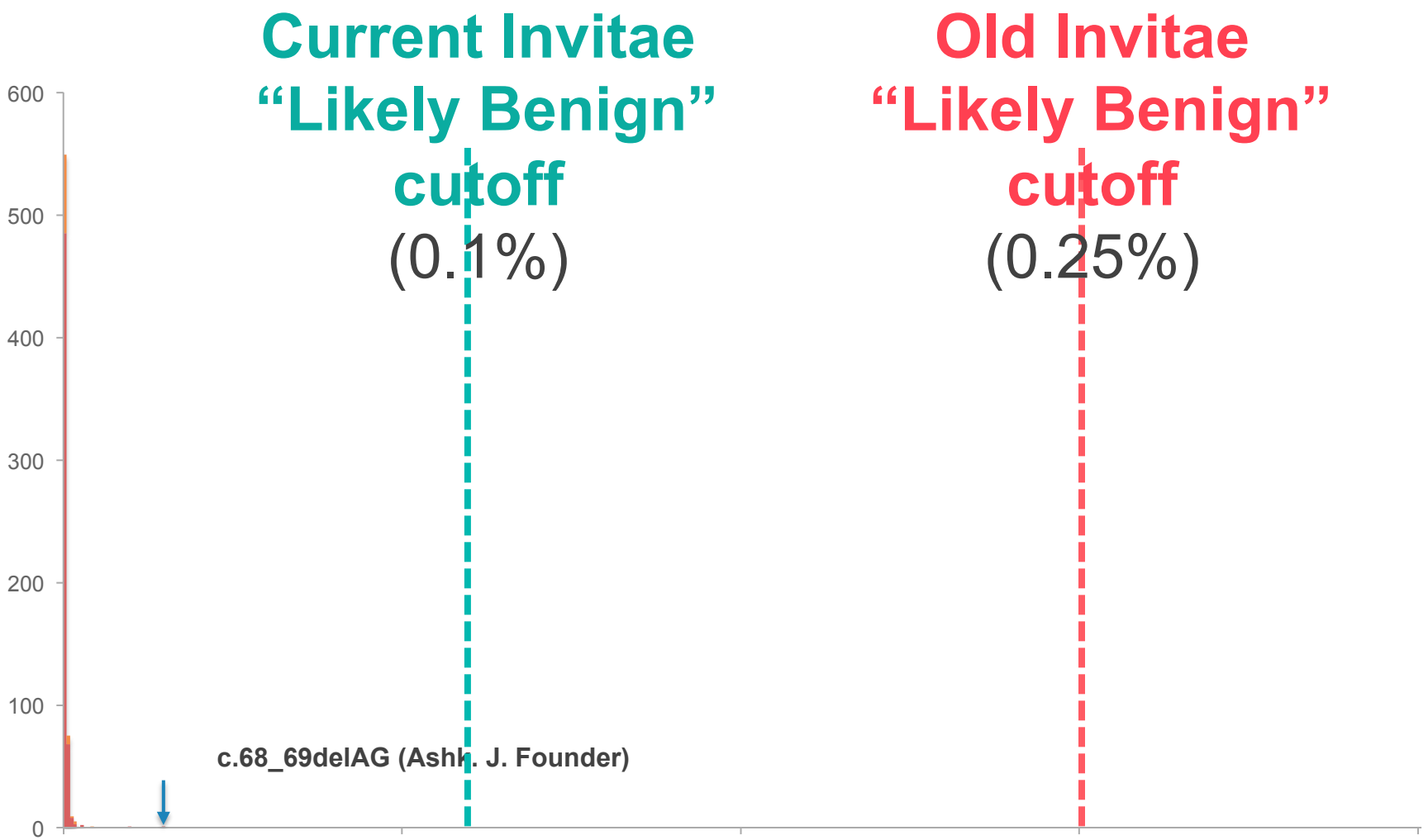
BRCA1 pathogenic mutation frequency spectrum



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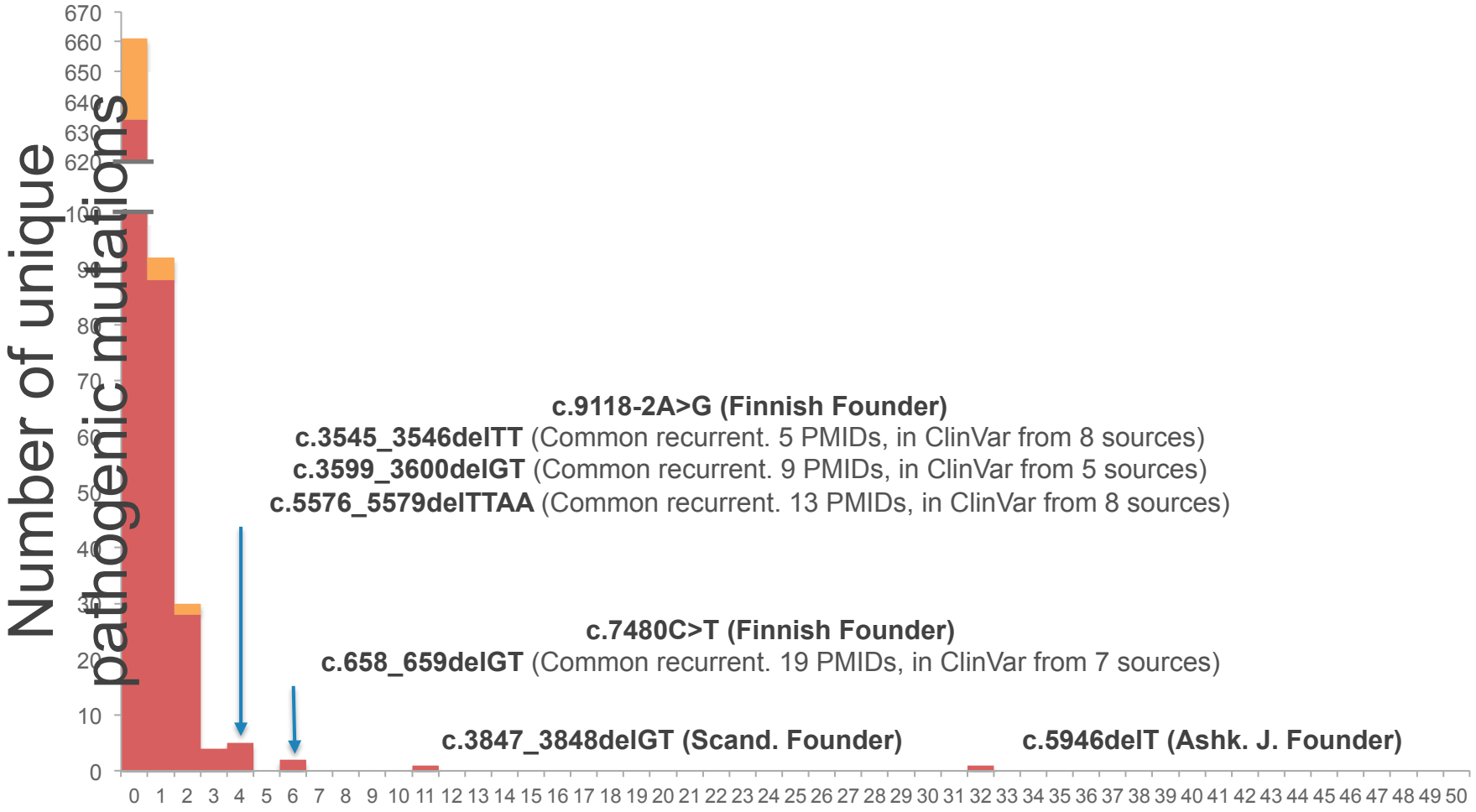
BRCA2 pathogenic mutation frequency spectrum



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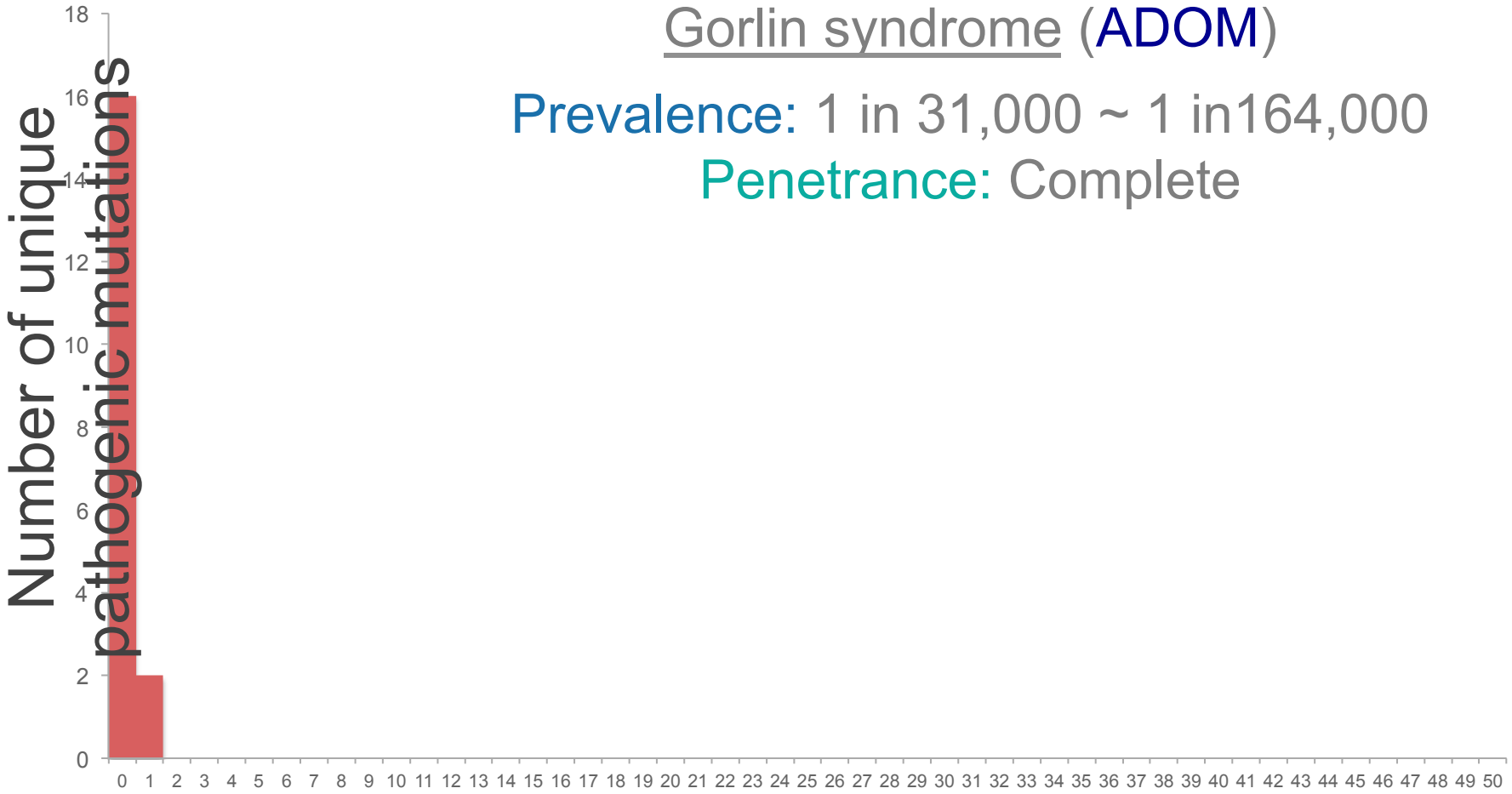
PTCH1 pathogenic mutation frequency spectrum



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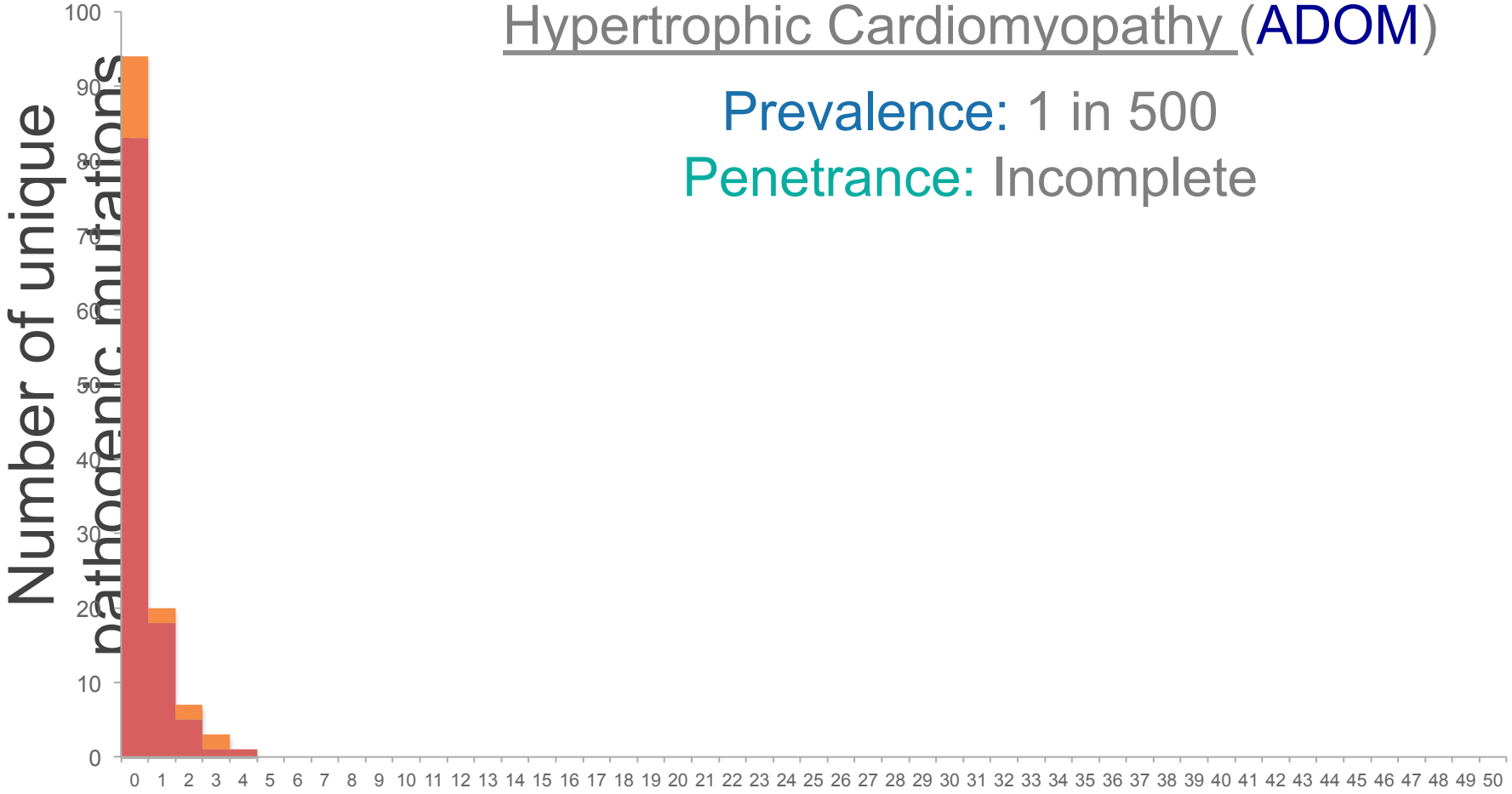
MYBPC3 pathogenic mutation frequency spectrum



Hypertrophic Cardiomyopathy (ADOM)

Prevalence: 1 in 500

Penetrance: Incomplete

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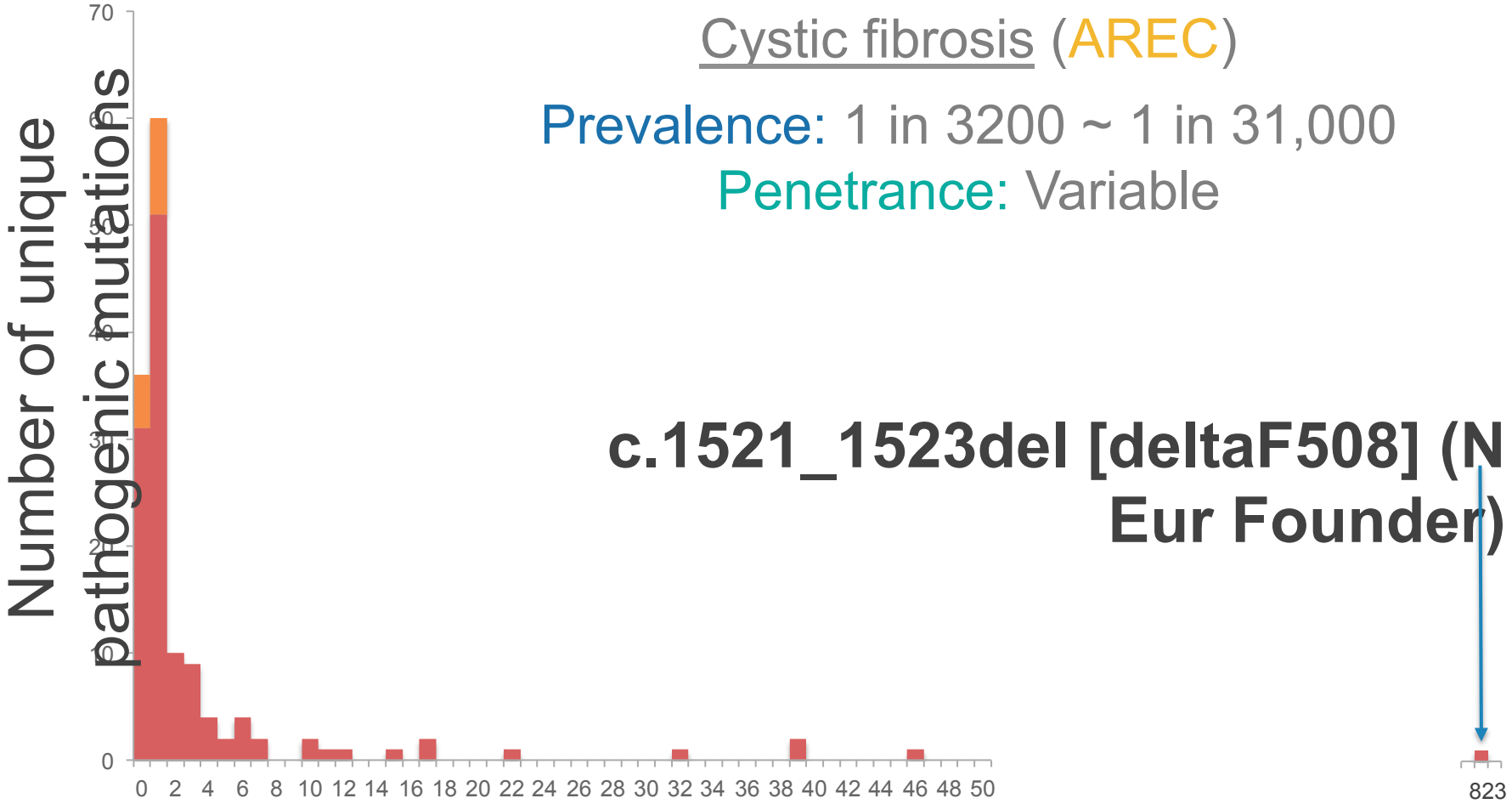
CFTR pathogenic mutation frequency spectrum



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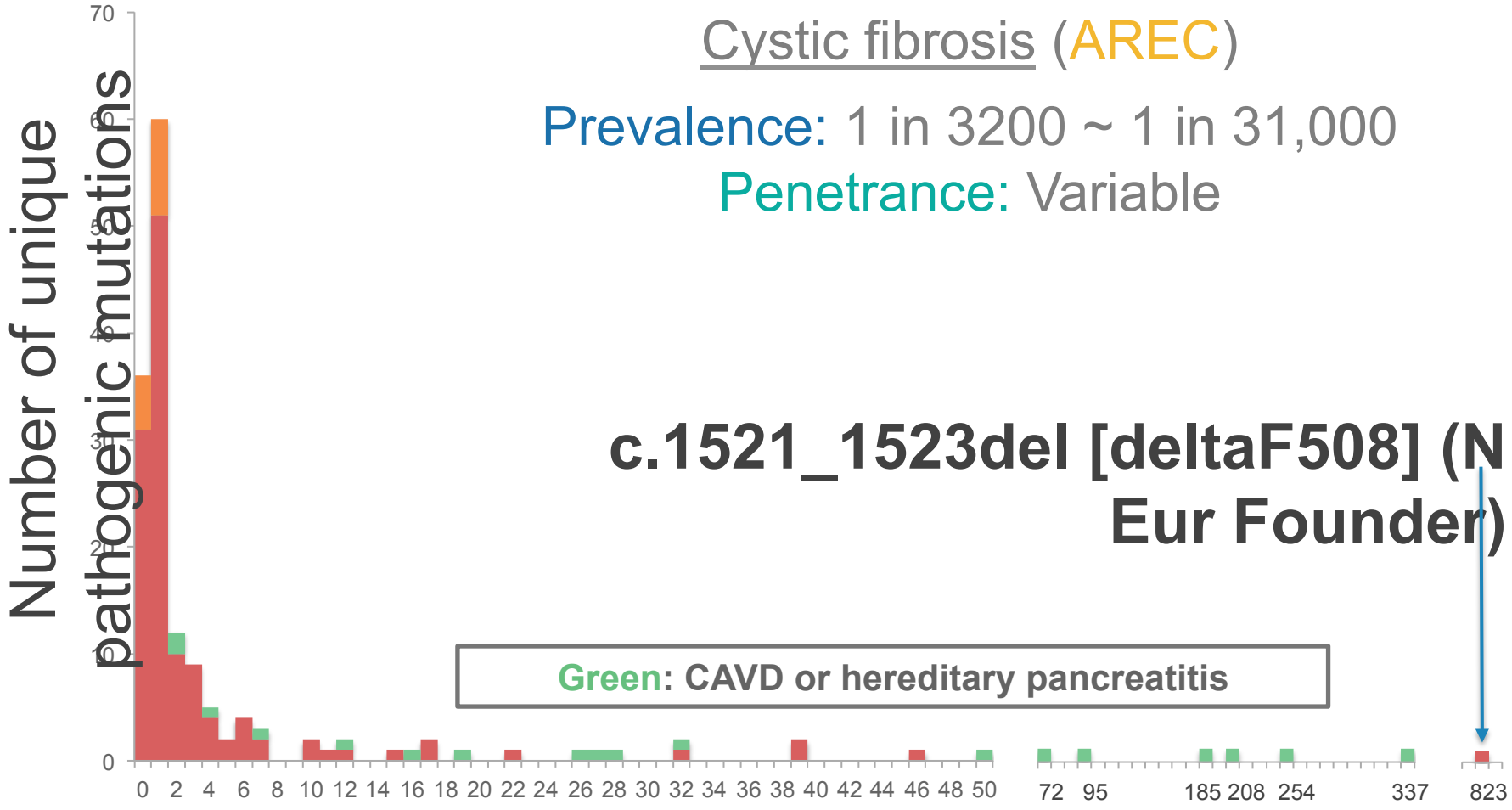
CFTR pathogenic mutation frequency spectrum



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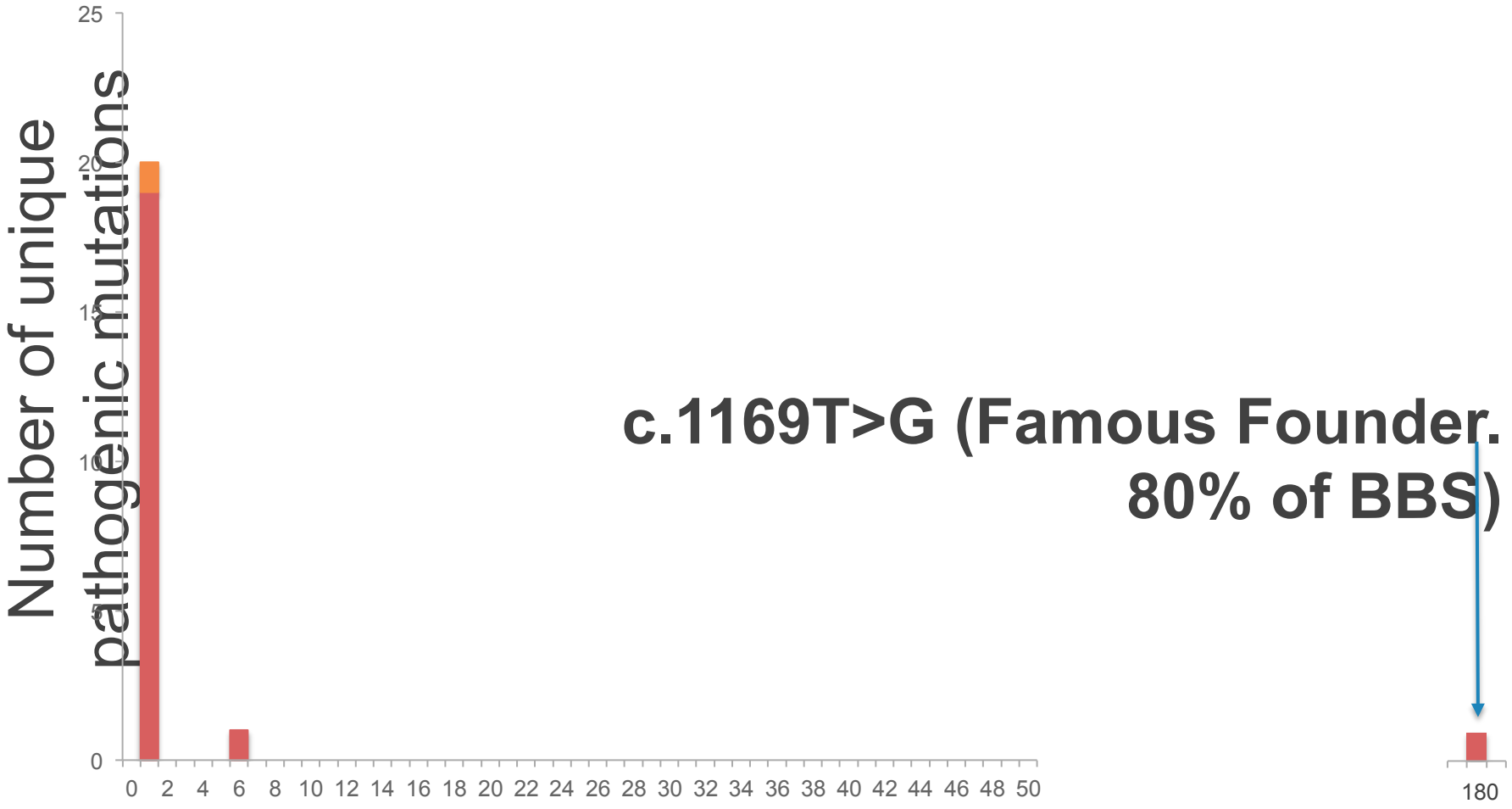
BBS1 pathogenic mutation frequency spectrum



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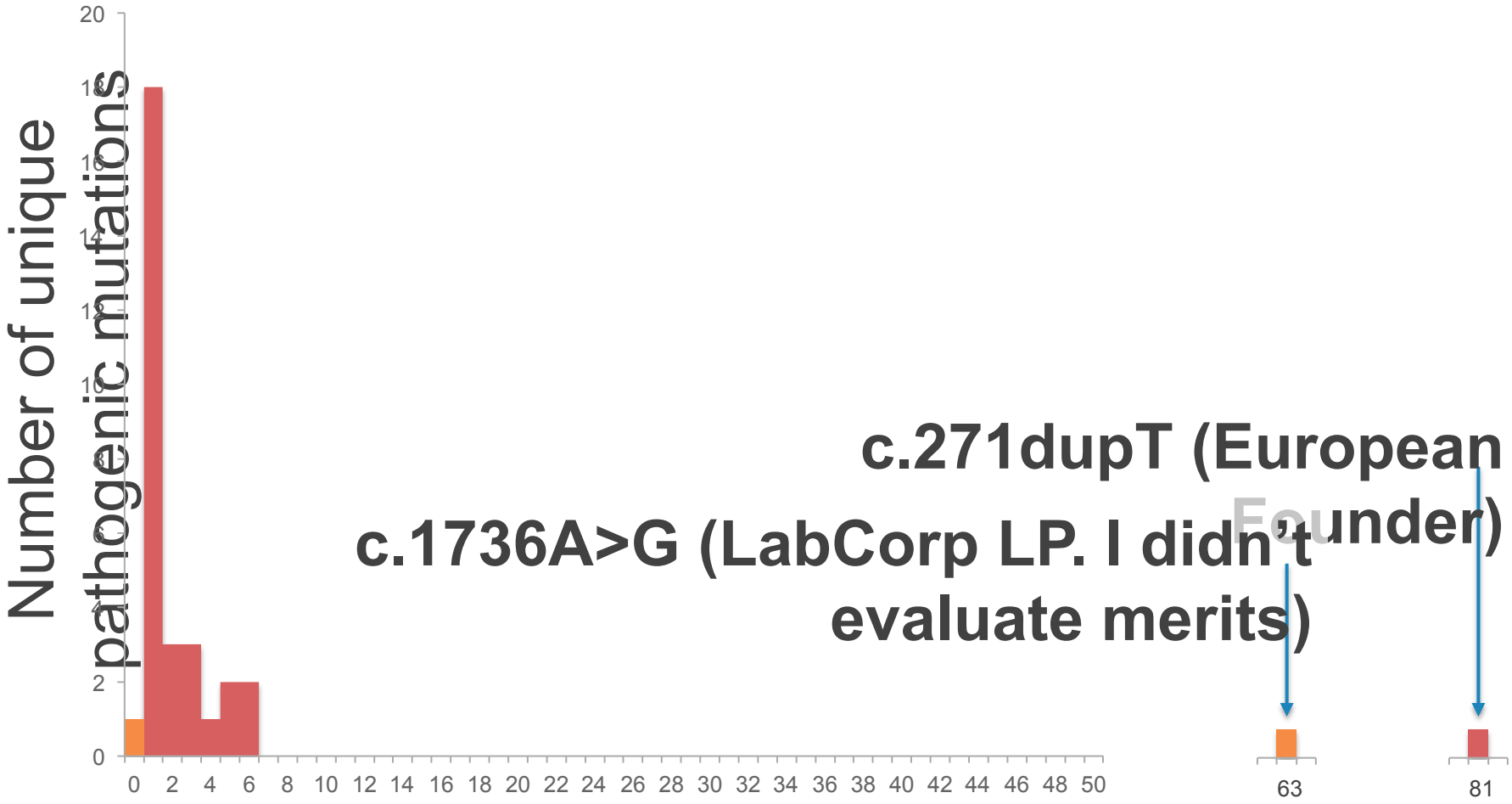
BBS1 pathogenic mutation frequency spectrum



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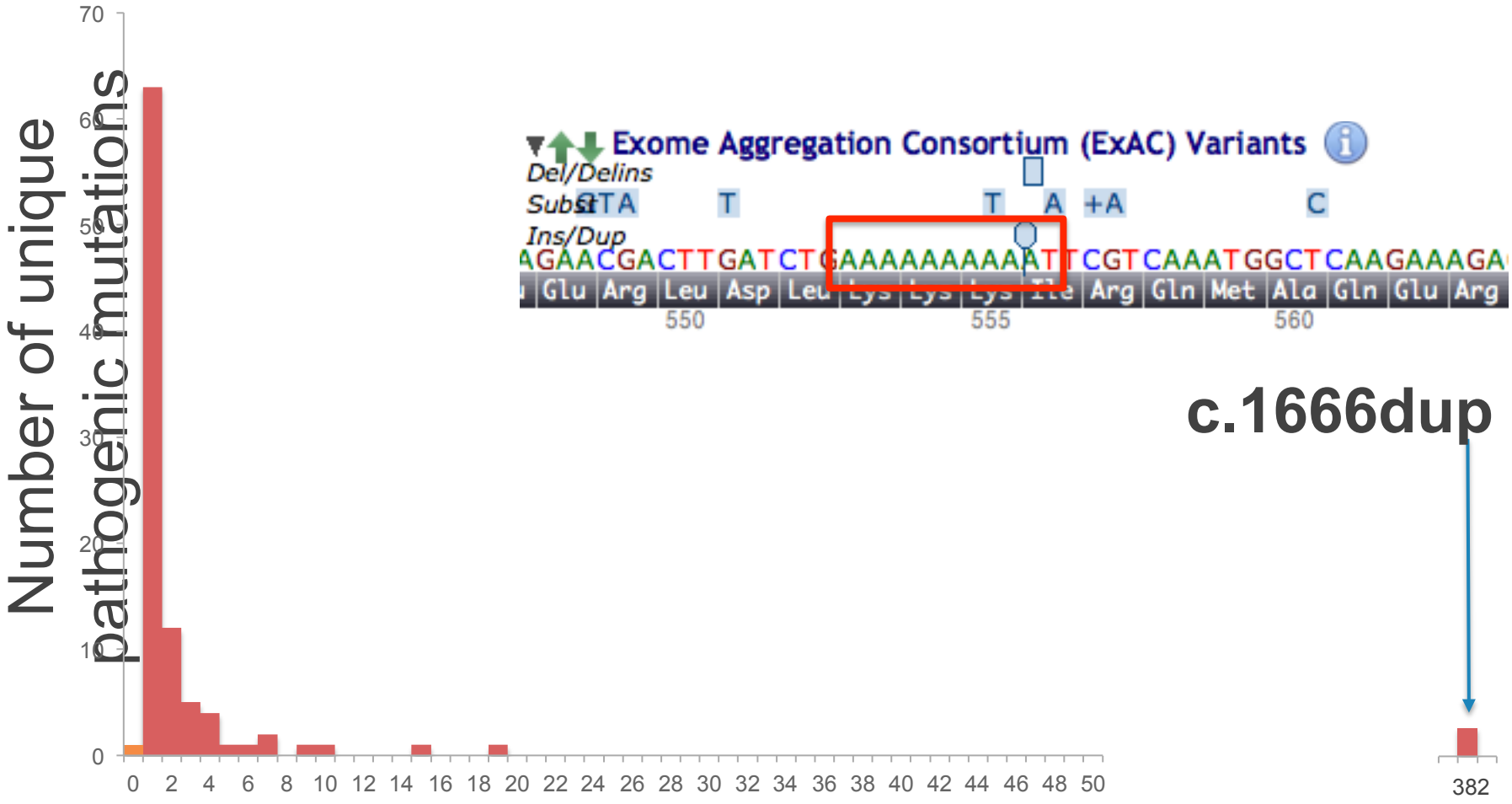
CEP290 pathogenic mutation frequency spectrum



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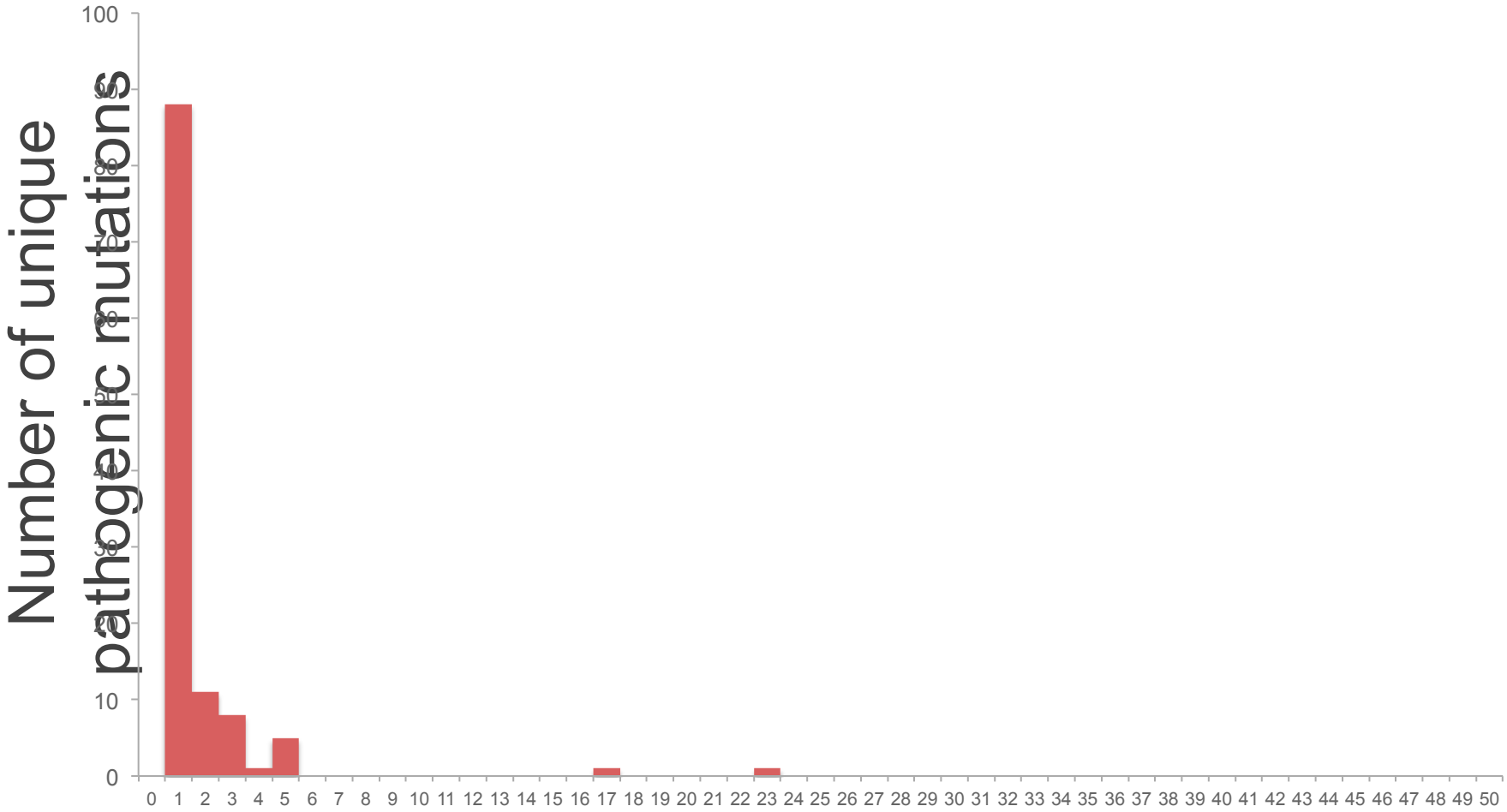
DNAH5 pathogenic mutation frequency spectrum



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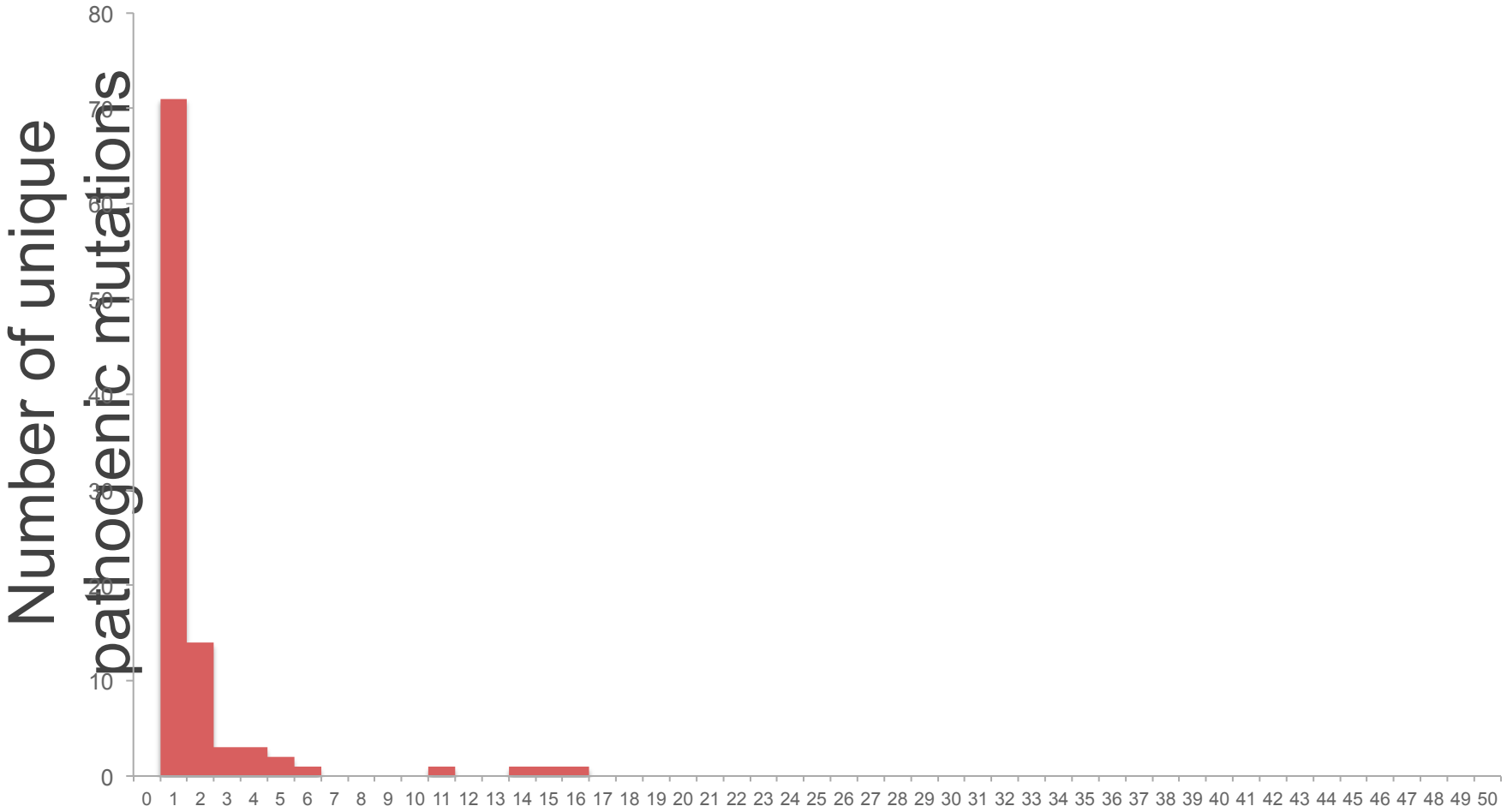
DNAH11 pathogenic mutation frequency spectrum



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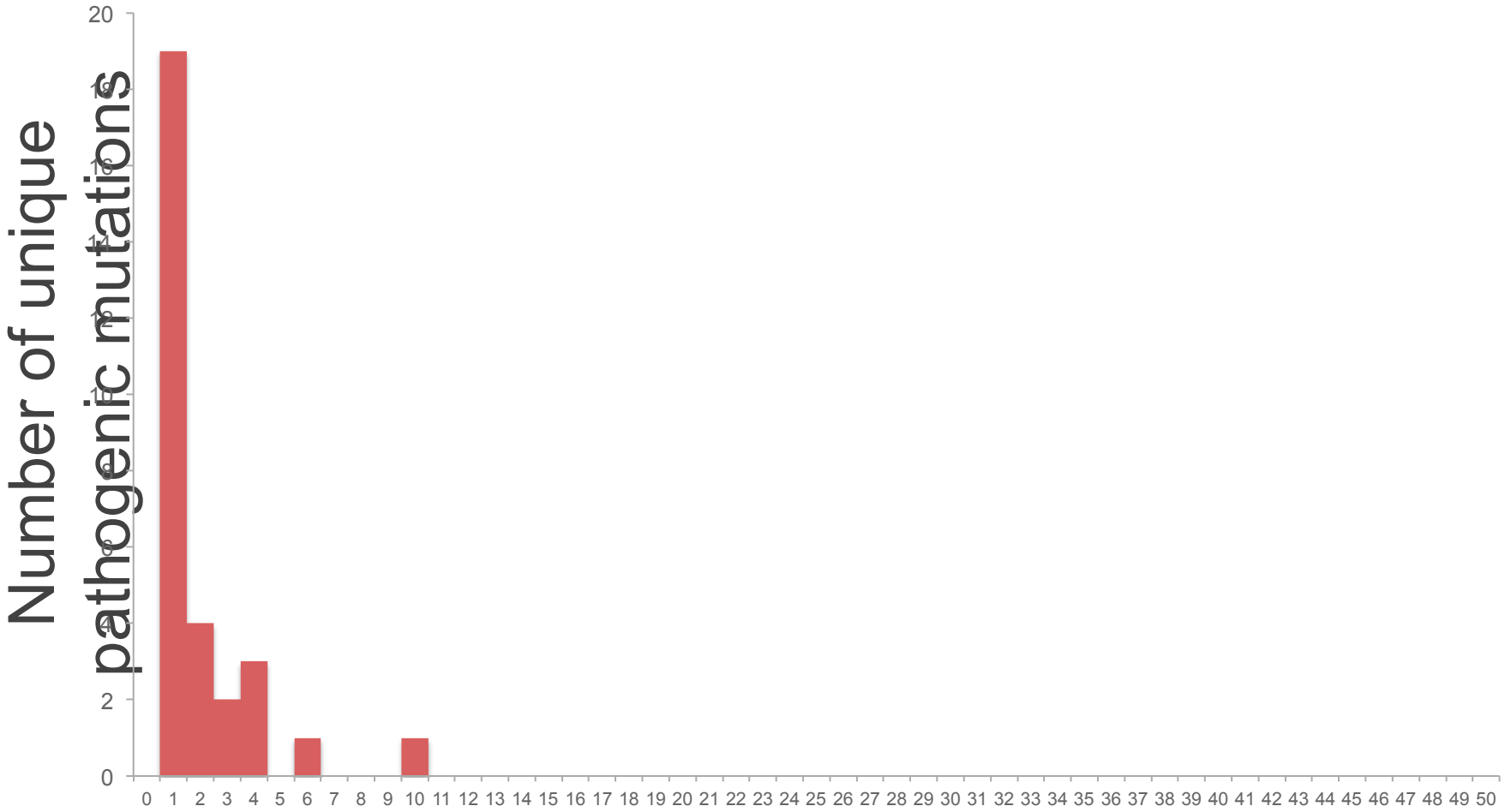
INVS pathogenic mutation frequency spectrum



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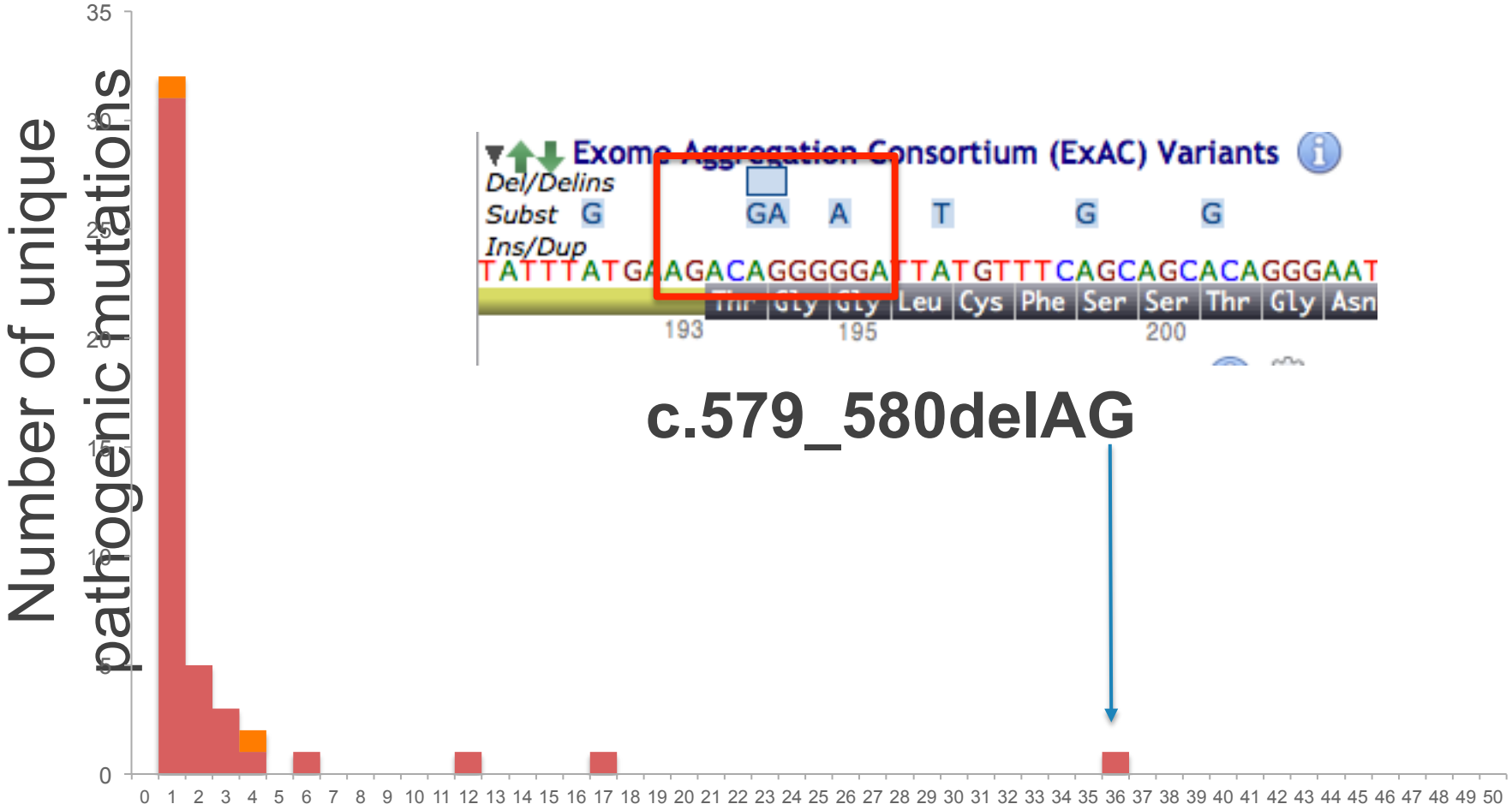
TMEM67 pathogenic mutation frequency spectrum



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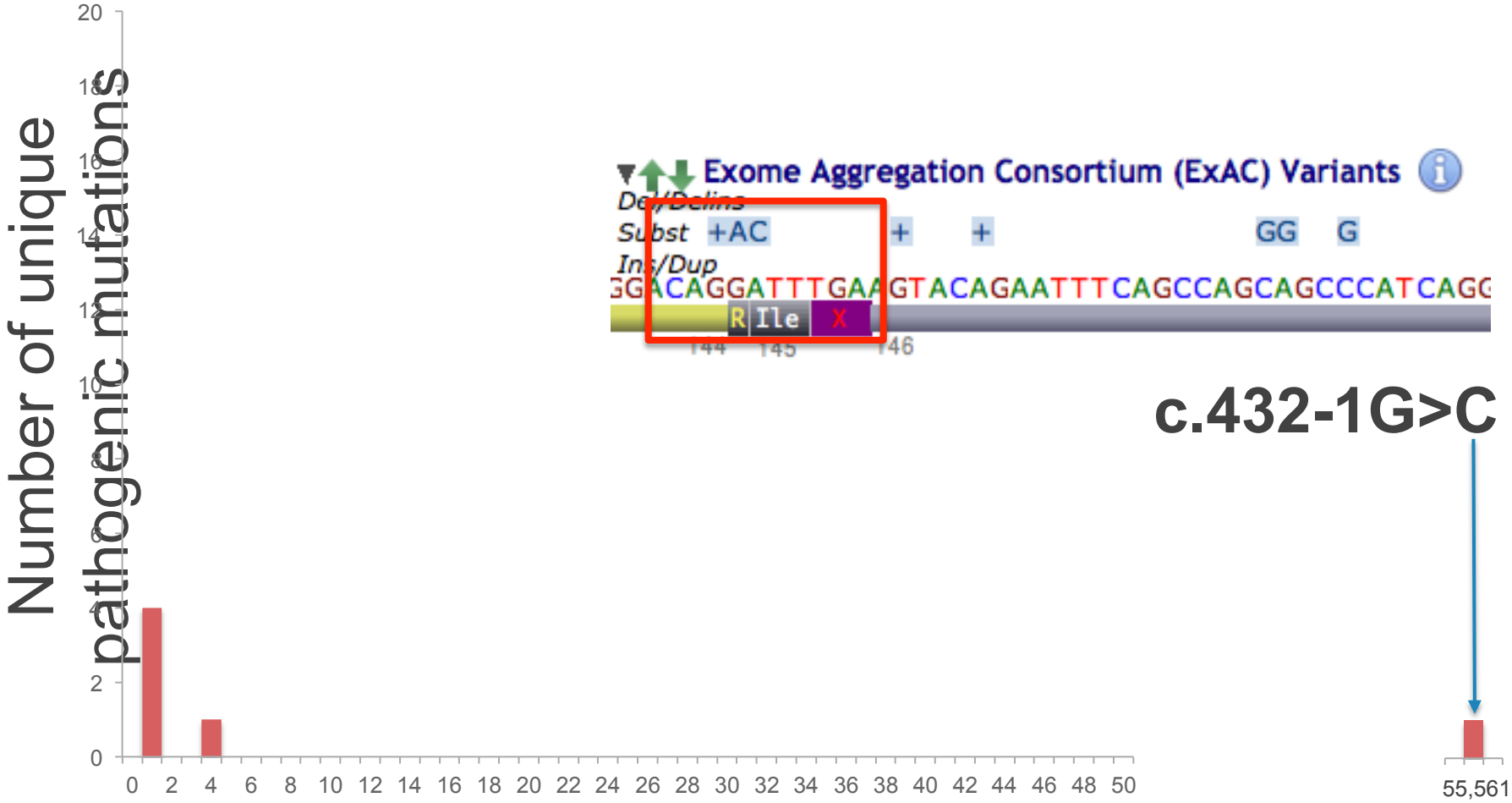
TMEM216 pathogenic mutation frequency spectrum



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