Categorizing variants after whole genome sequencing:

Implementation of “binning” -- a structured algorithm for the identification of clinically relevant incidental findings

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Implementation of a next-generation clinical diagnostic test

- How do we find the clinically relevant variants?
  - PGx variants / common SNPs are easy, just a (somewhat difficult) question of which ones to use
  - Rare disease causing mutations are a challenge
- Three "sweeps" through the data:
  1. Diagnostic results (max. sensitivity)
  2. Incidental findings (max. specificity)
  3. Research (long-term analyses)

Context matters!

- Symptomatic patient
  - **Diagnostic assessment**
    - Needs to report full range of variants, including VUS (as with standard genetic testing)
- Asymptomatic patient
  - **Incidental assessment**
    - Prior probability of a genetic disorder approaches 0
    - Must maximize specificity so as to provide a clinically relevant posterior probability

Incidental analysis

- **Goal**: identify clinically relevant findings ***unrelated to the patient's presentation***
- **Premise**: the vast majority of genomic variants have no clinical relevance ***and must be ignored in a medical context***
  - Therefore imperative to maximize specificity and avoid reporting VUS
  - Set a "high bar" to ensure that variants reported to physicians/patients can be incorporated into clinical care in an evidence-based fashion

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COMMENTARY

Deploying whole genome sequencing in clinical practice and public health: Meeting the challenge one bin at a time

Jonathan S. Berg, MD, PhD[1], Muin J. Khoury, MD, PhD[2], and James P. Evans, MD, PhD[1]

- Current practices in medical genetics may not be suitable for genome-wide analysis
- Need for evidence-based, structured computational analysis for diagnostic and incidental results

Bins for incidental findings

- **<u>Strategy</u>**: Automated annotation of all variants
  1. *A priori* categorization of **genes** according to clinical utility and risk for harm
  2. *A priori* definition of the types of **variants** that should be reported

  – Sort variants into predetermined "bins," then review/report ***only those likely to be deleterious***
  – "Versioning" of analyses to allow one to know precisely what parameters were used

Bins for incidental findings

- Bin 1: Clinically actionable (Lynch, Long QT)
- Bin 2: Clinically valid but not directly actionable
  - 2a: low risk for harm (GWAS risk SNPs, PGx)
  - 2b: medium risk for harm (most Mendelian disorders)
  - 2c: high risk for harm (Huntington's, Presenilin)
- Bin 3: No known clinical significance
- Carrier status category*

Bins for incidental findings

- Informatics screening of OMIM genes
- 2016 genes “binned”
- Final bin decision was a judgment call

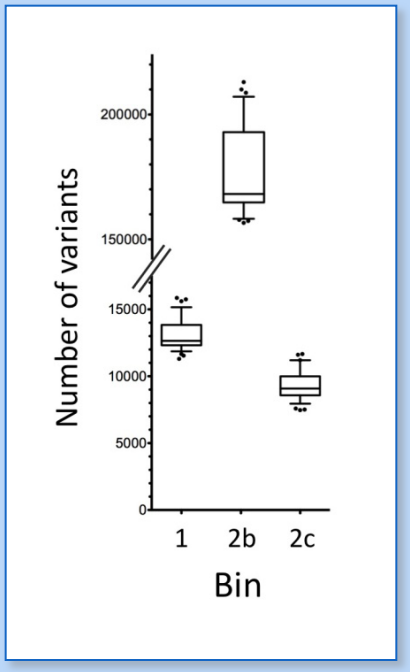
- Bin 1: 161 genes
- Bin 2:
  - 2b: 1798 genes
  - 2c: 57 genes

Theories are good, but data are better…

- 80 genomes sequenced by Complete Genomics
  - 19 patients with likely hereditary cancer susceptibility enrolled in a WGS study at UNC
  - 61 genomes made publically available by Complete Genomics
- 1000 Genomes Project allele frequency data
- Human Gene Mutation Database

Expectations

- The likelihood for any given person to have a disease-causing Mendelian mutation is LOW
  - Expect ***very few*** bin 1 or 2 findings per person
  - Most individuals will be carriers of heterozygous mutations in autosomal recessive genes



All variants in binned genes:

~13,000 variants in bin 1 genes

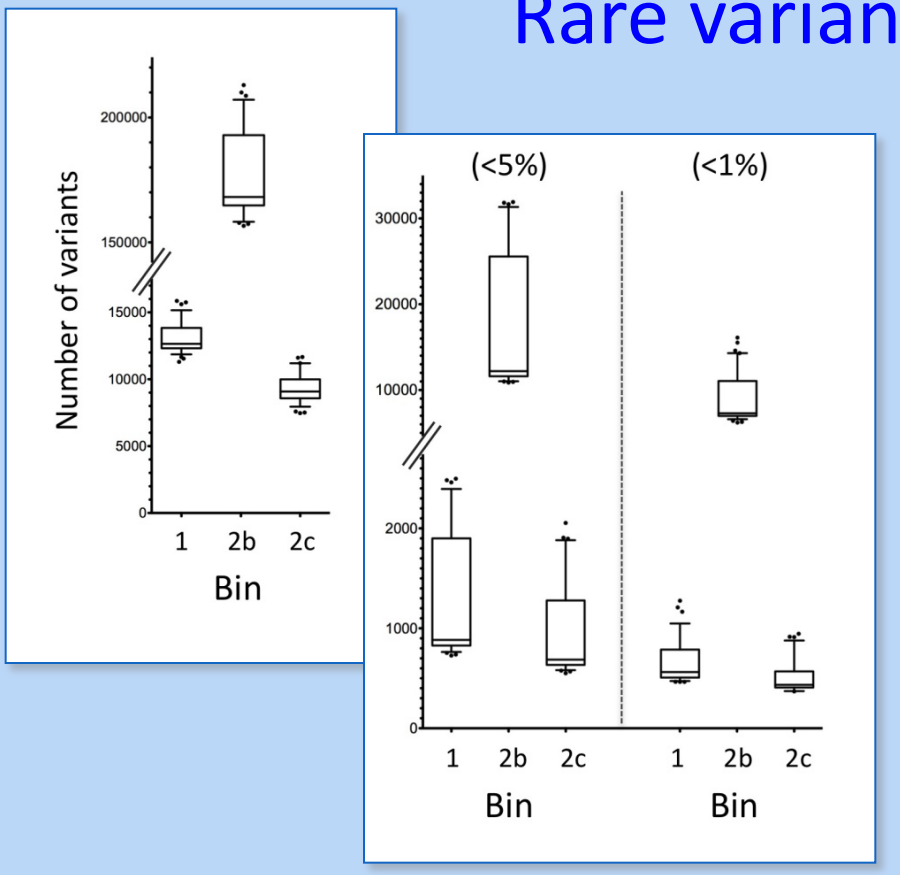
~175,000 variants in bin 2b genes

~9,200 variants in bin 2c genes

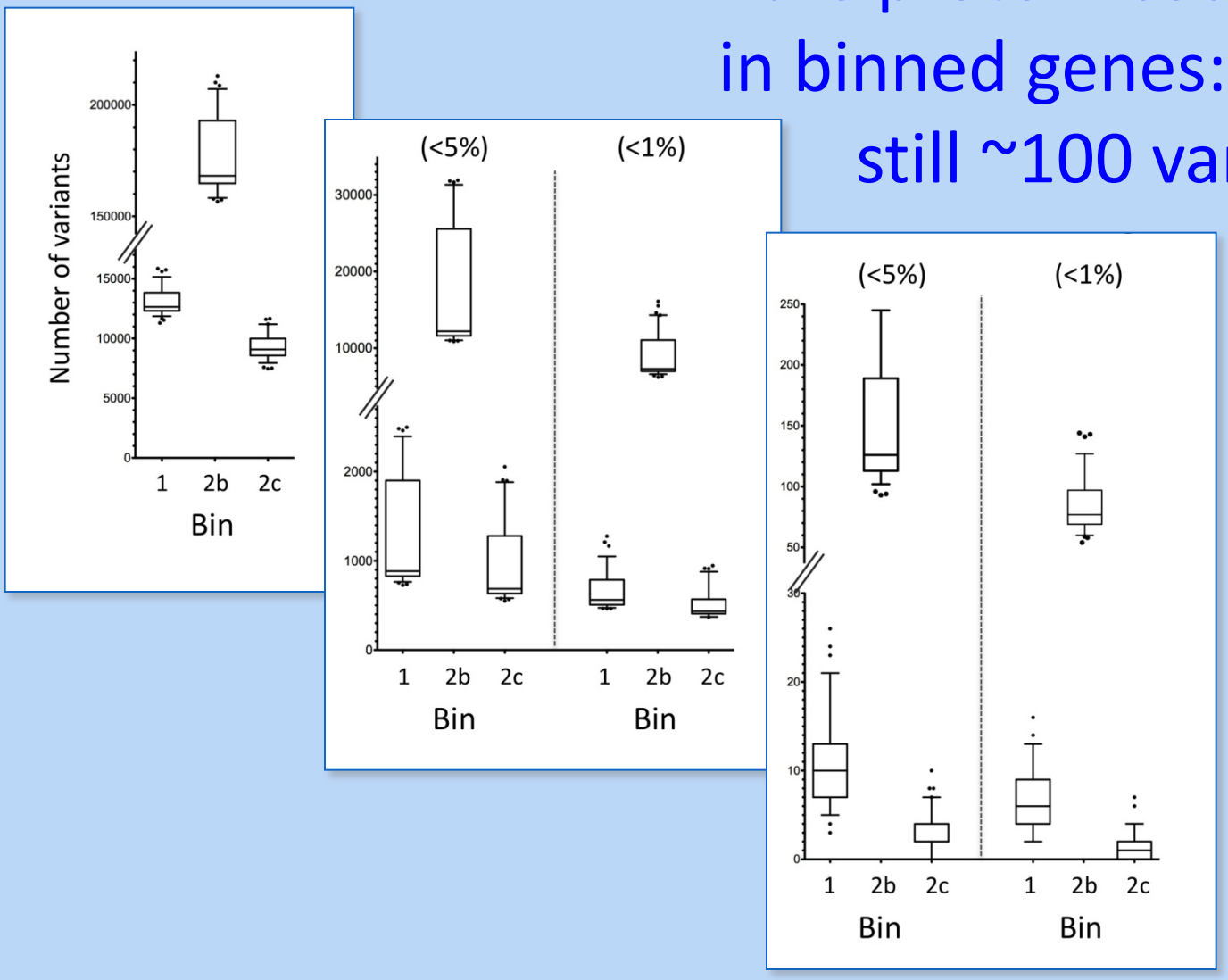
Rare variants in binned genes:

10-fold reduction (<5% AF)

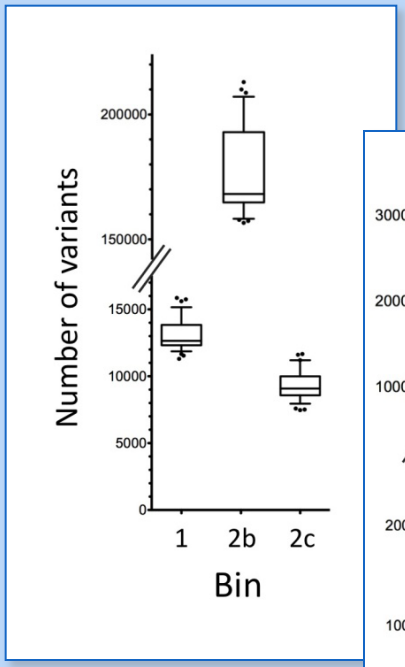
15-fold reduction (<1% AF)

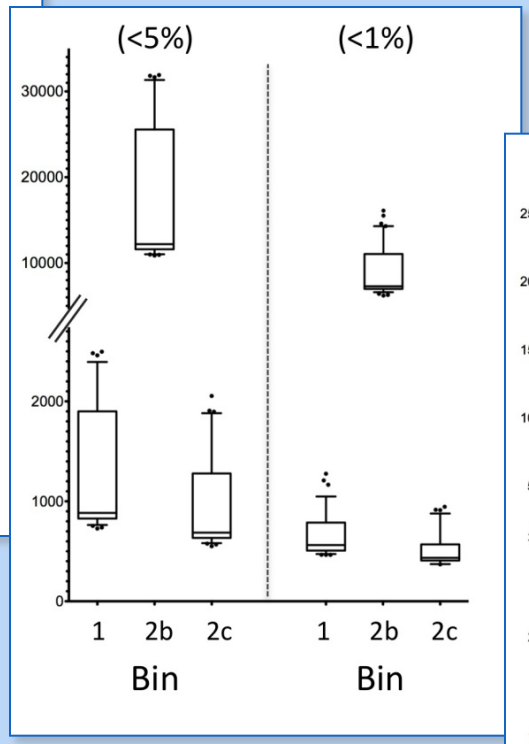


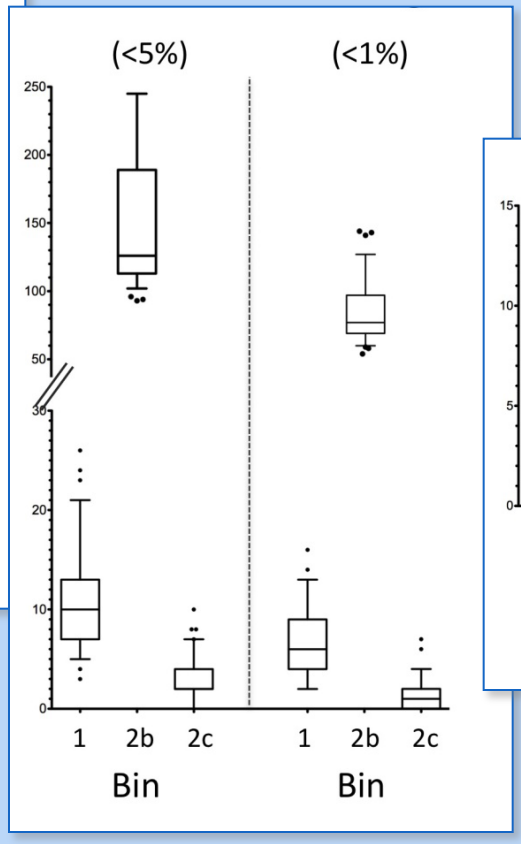
Rare protein-coding variants in binned genes:
still ~100 variants/person

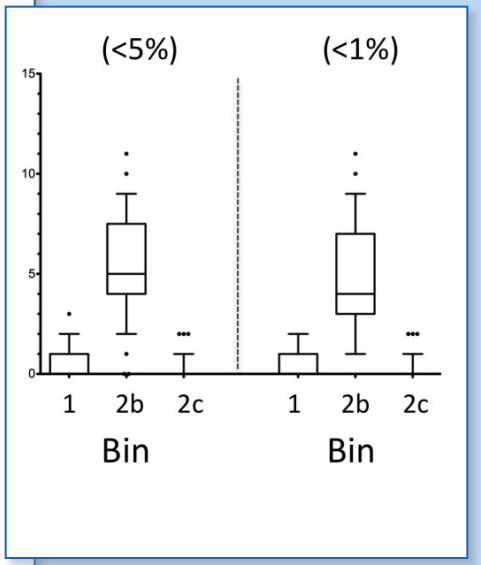


Rare truncating variants in binned genes: <10 variants/person





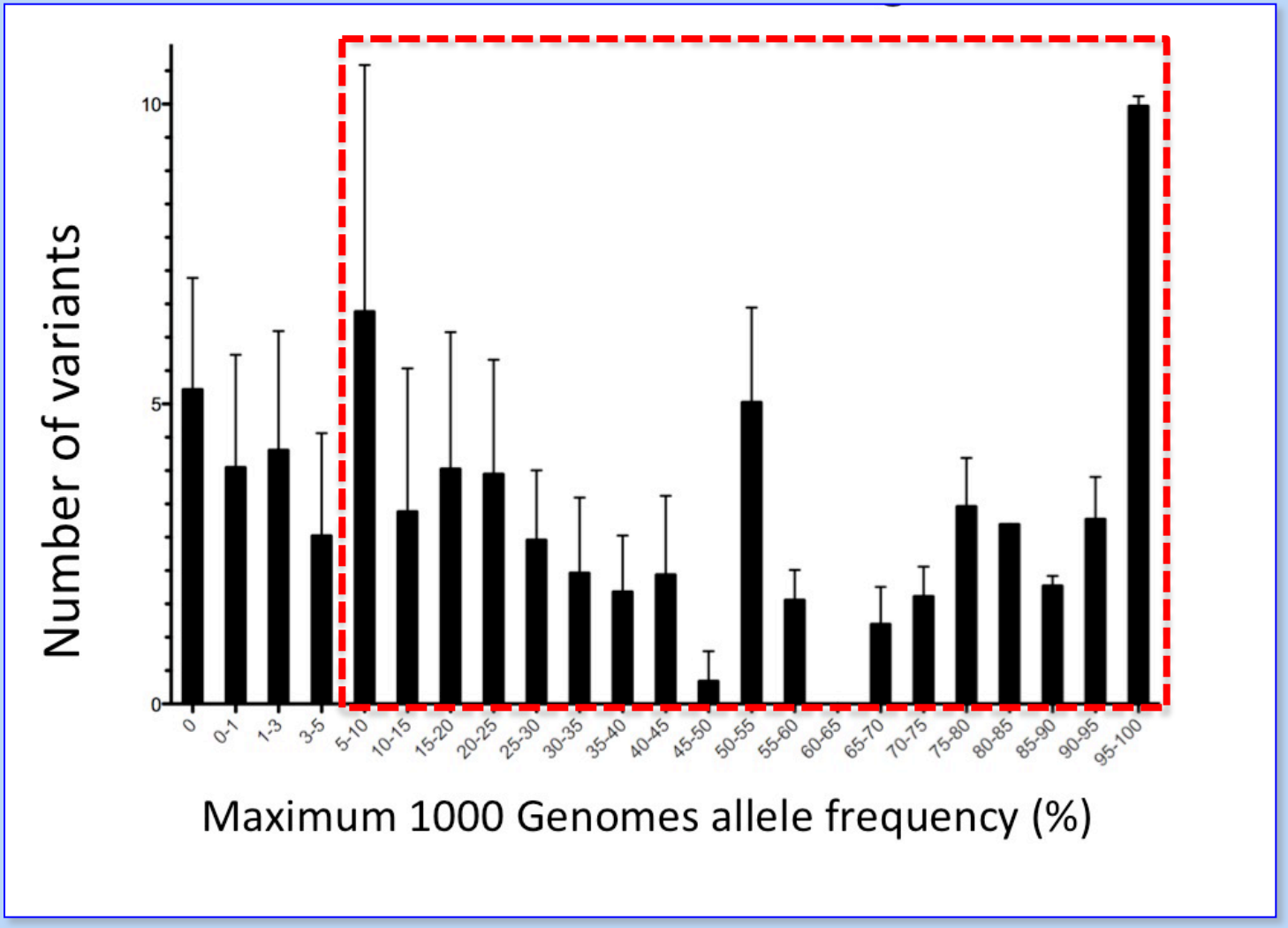




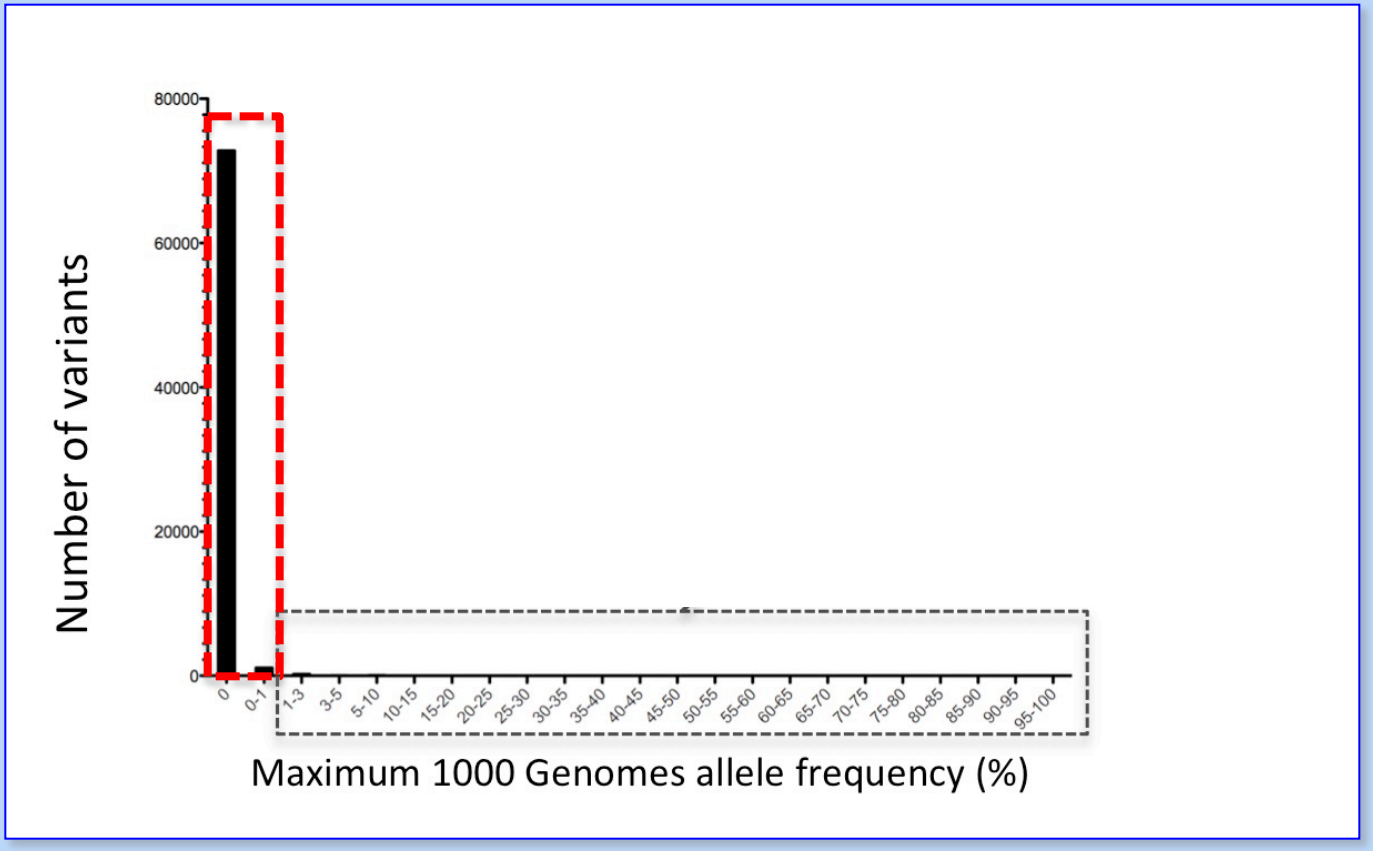
No missense variants?

- Limiting to truncating variants sacrifices sensitivity, excludes known disease causing missense mutations
- Possible solution: query the Human Gene Mutation Database for “DM” variants
  - Identified 871 unique variants, 771 missense
  - Average 74 (61-106) per person
  - Surprisingly little overlap with rare missense variants

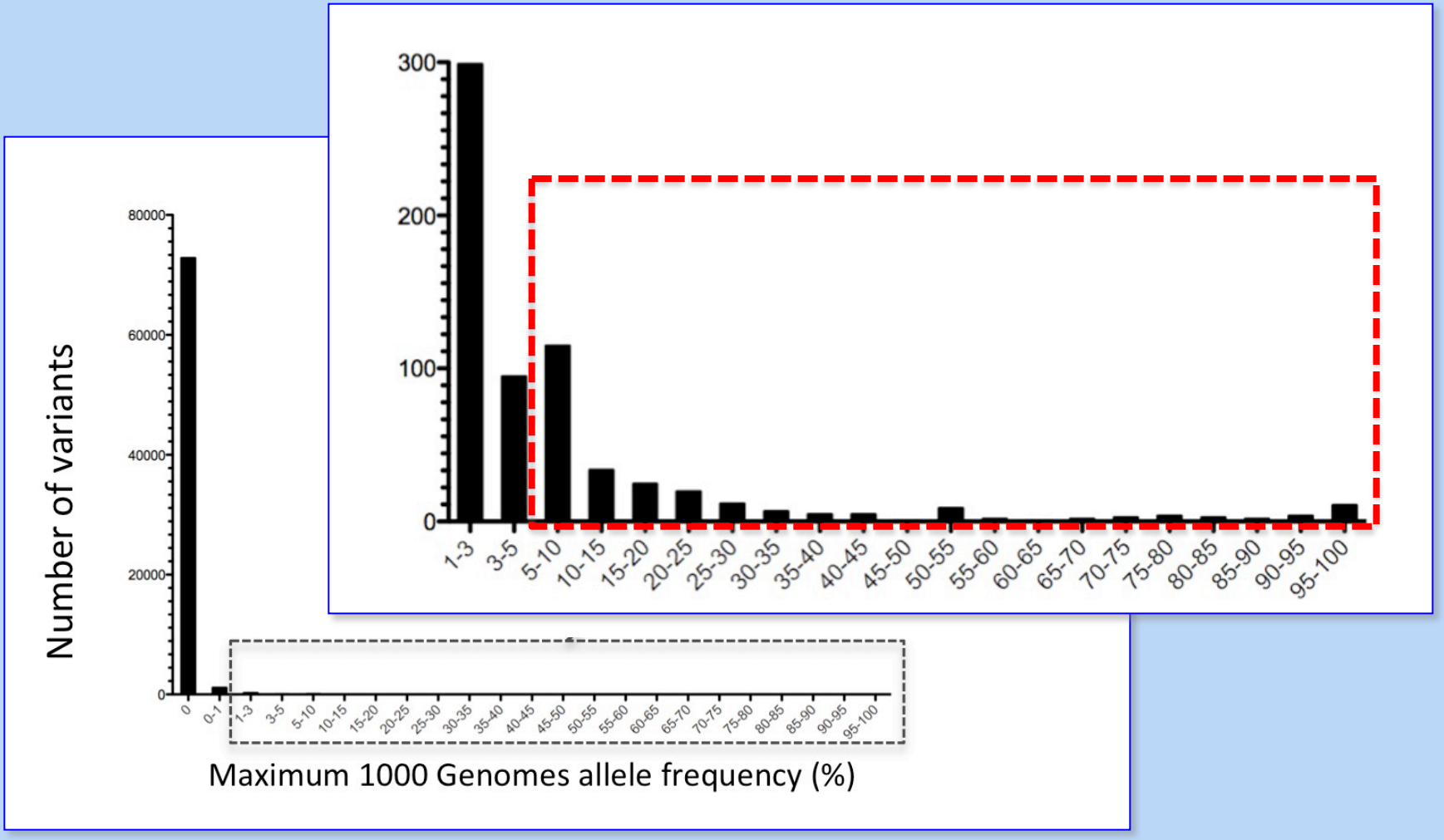
~80% of HGMD “DM” variants identified have >5% allele frequency



Most HGMD “DM” variants are rare…



…but too many are common



Final binning algorithm

- Variant annotated within “binned” gene

and

- <5% allele frequency

and

  - “DM” in HGMD

  OR

  - Protein truncating

Final binning algorithm
Variants per genome

	Bin 1	Bin 2b	Bin 2c	Carrier
Binned variants	1.5 (0-5)	6.4 (2-14)	0.2 (0-2)	9.2 (0-17)

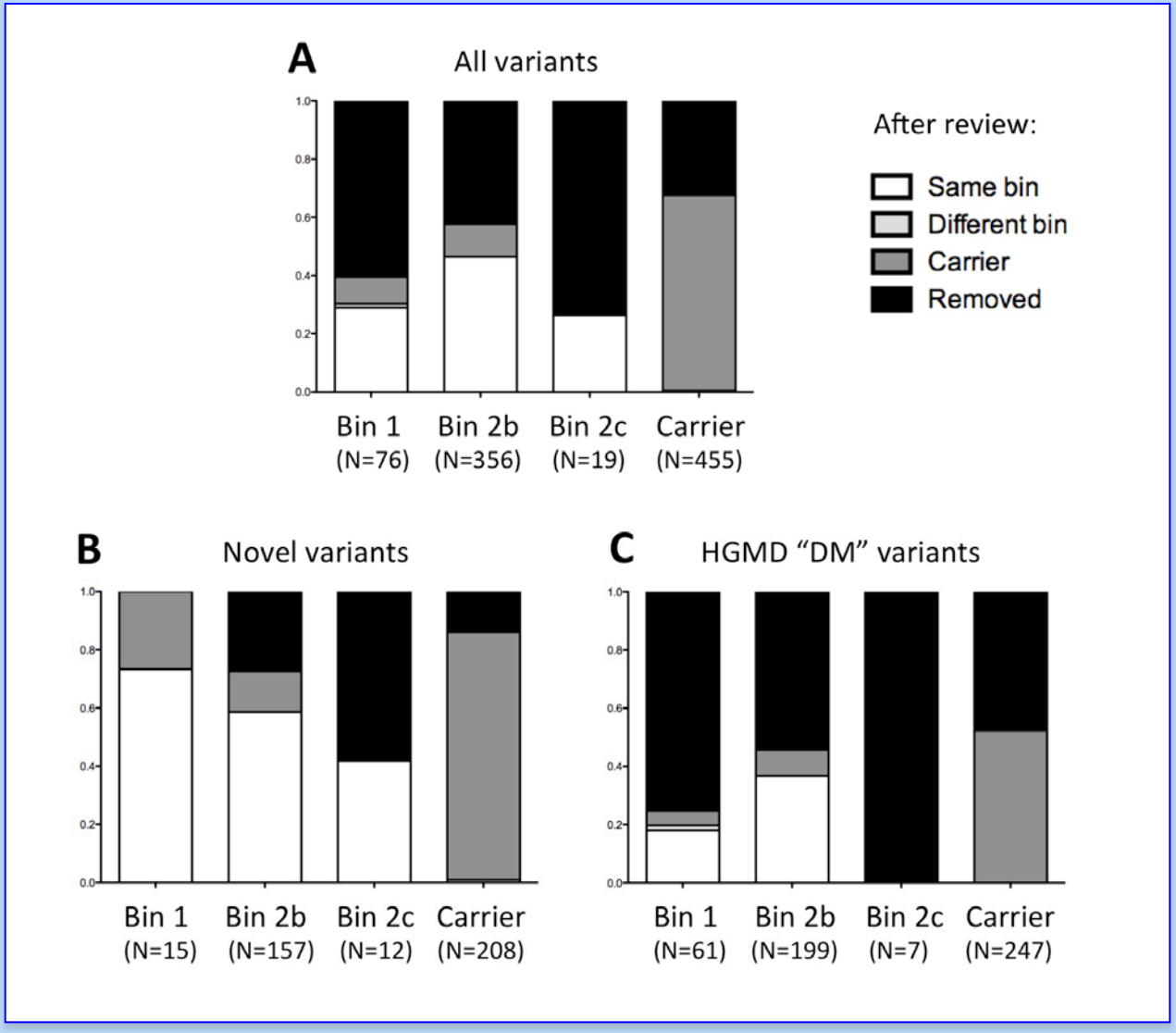
- A very tractable number for a human to review on a per person basis
- Close to expected numbers, but still too many
- Needed to perform manual curation of 1391 variants to remove or reassign

Final binning algorithm
Variants per genome

	Bin 1	Bin 2b	Bin 2c	Carrier
Binned variants	1.5 (0-5)	6.4 (2-14)	0.2 (0-2)	9.2 (0-17)
Upon review, ~50% were removed from consideration, 5% moved to carrier status				

- Used "Goldilocks" approach to reviewing variants (not too harsh, not too lenient)
  - Reviewed literature
  - Assessed type/location of variant
  - Used allele frequency information, especially in dominant disorders

Reclassification of variants after review



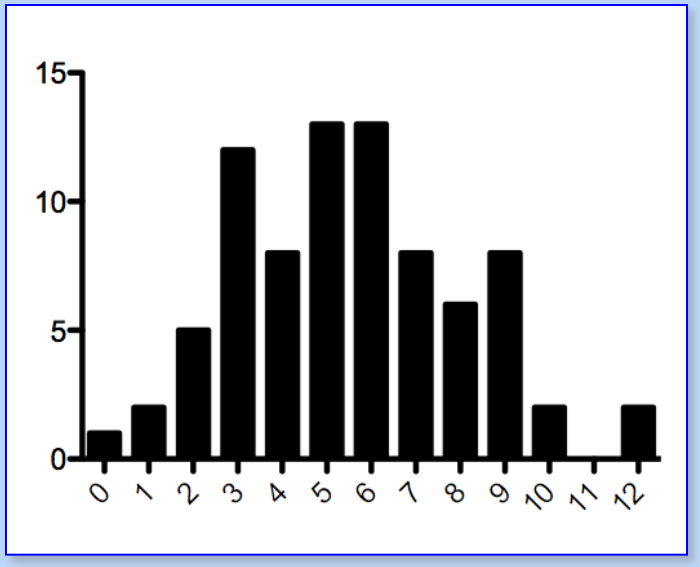
Final binning algorithm
Variants per genome

	Bin 1	Bin 2b	Bin 2c	Carrier
Binned variants	1.5 (0-5)	6.4 (2-14)	0.2 (0-2)	9.2 (0-17)
~50% removed from consideration, 5% moved to carrier status				
After review	0.3 (0-2)	2.6 (0-8)	0.06 (0-1)	5.5 (0-12)
~8.5 variants to confirm/report per sample				

- Still more variants than expected
- Sequencing artifacts? False positive reports in the literature? Incomplete penetrance?

Incidental carrier status findings

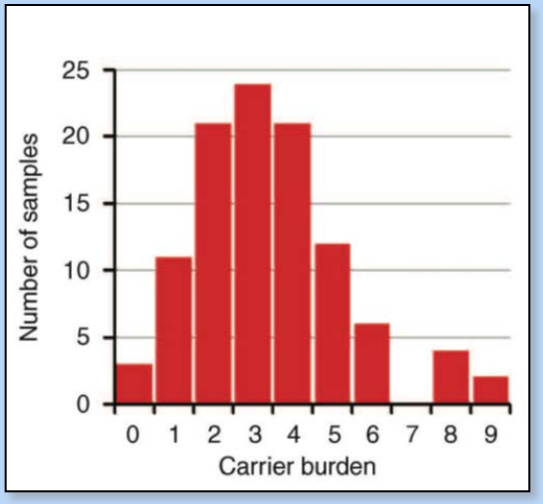
- 79/80 were “carriers” for at least one recessive condition
  - Range 0 – 12



RESEARCH ARTICLE
www.ScienceTranslationalMedicine.org 12 January 2011 Vol 3 Issue 65 65ra4
HUMAN GENOMICS

Carrier Testing for Severe Childhood Recessive Diseases by Next-Generation Sequencing

Callum J. Bell,[1]* Darrell L. Dinwiddie,[1,2]* Neil A. Miller,[1,2] Shannon L. Hateley,[1] Elena E. Ganusova,[1] Joann Mudge,[1] Ray J. Langley,[1] Lu Zhang,[3] Clarence C. Lee,[4] Faye D. Schilkey,[1] Vrunda Sheth,[4] Jimmy E. Woodward,[1] Heather E. Peckham,[4] Gary P. Schroth,[3] Ryan W. Kim,[1] Stephen F. Kingsmore[1,2]†



Summary

- A structured framework permits consistent analysis of WGS data, critical for clinical work
  - Every report will be linked to a “version” of the analytic scheme for reproducibility and future updating
- Clinical WGS analysis for incidental findings **can be** a tractable problem
  - High quality variant database critical
  - Predefined “rules” for automated annotation

Future Directions

- Refinement of bins
  - Expect debate over the genes in each category
  - Anticipate changes with advances in medical genetics
- Refinement of “rules” for reporting variants
  - More nuanced, gene- and disease-specific criteria
  - Development and utilization of clinical-grade databases
- Validation and deployment of risk prediction models with proven clinical utility

Future Directions

- Best practices for informed consent, return of results, integration with medical record
- ELSI issues related to clinical use of NGS
  - The field is in a state of equipoise regarding return of incidental findings
    - Should all results be divulged automatically?
    - How can we best enable patient preferences?
  - Need to study patient decision-making and outcomes from return of incidental findings
  - Incidental findings in infants/children?

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