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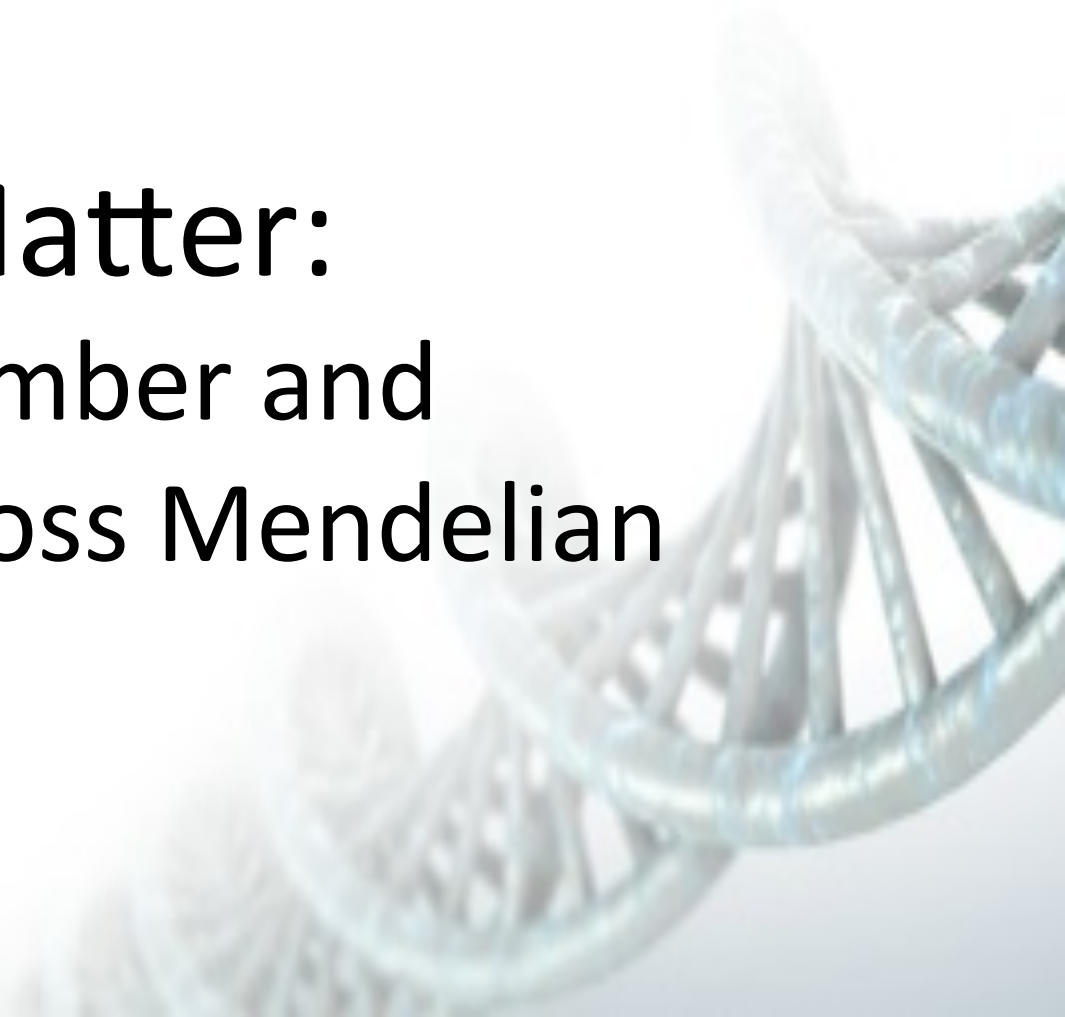
Tracing the Dark Matter:

Prevalence of Copy Number and Structural Variants Across Mendelian Disorders



Rebecca Truty
Invitae

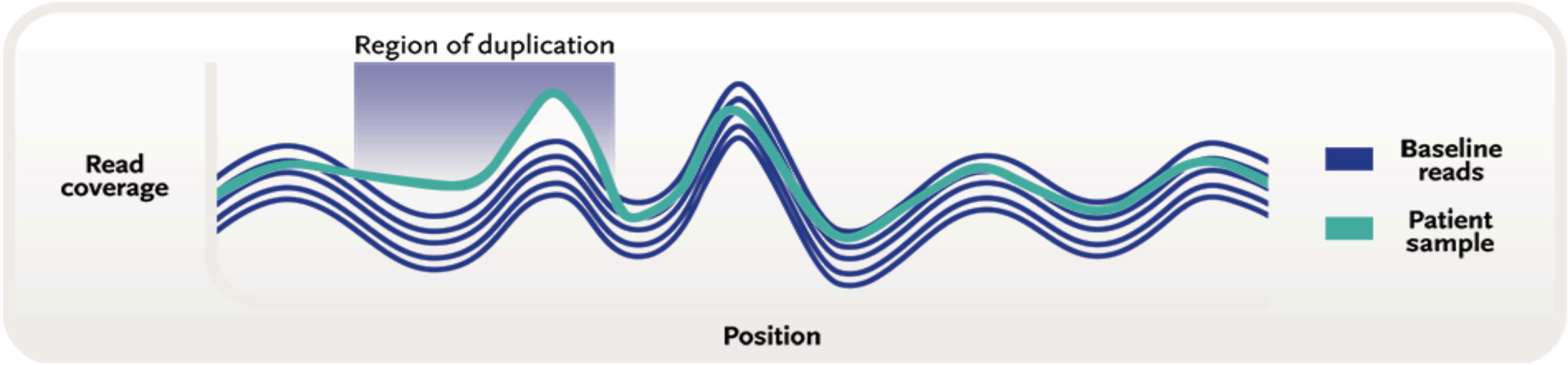
Outline

- Clinical prevalence of intragenic large variants is not well understood
  - Large variant detection has not been routinely applied for all tested genes, and traditional genetic testing involves separate methods to detect small variants and large variants
- We developed an NGS-based test to simultaneously capture small and large events with high sensitivity
- The prevalence of large variants, including intragenic CNVs, is higher than currently appreciated
  - ~10% of all positive molecular diagnoses in our cohort include large variants

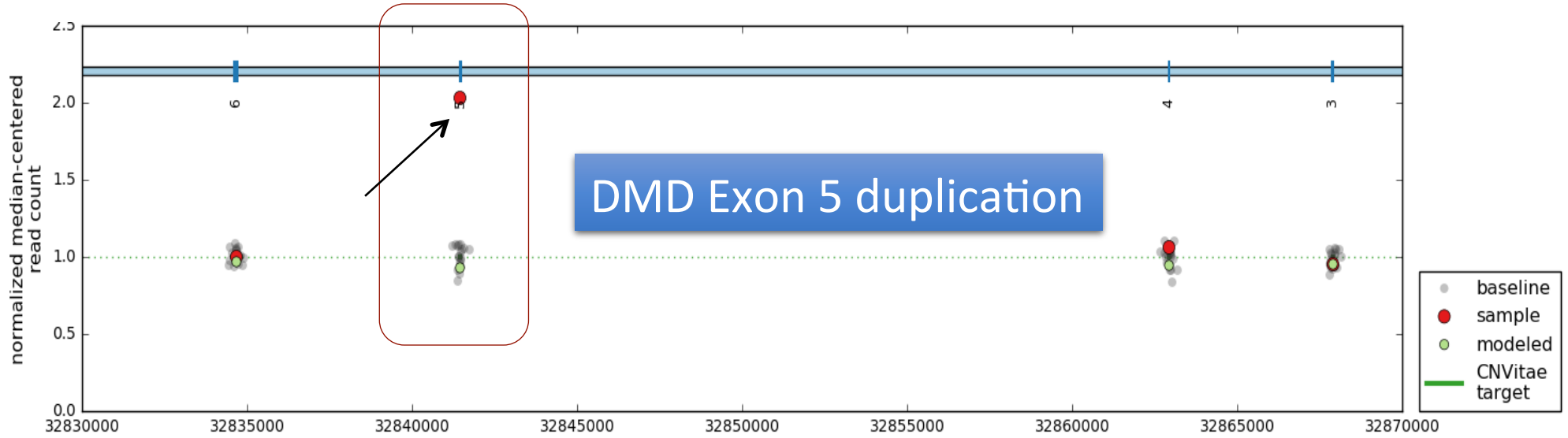
Methods

- We have developed a NGS-based test that detects large variants in addition to small variants (SNVs, indels<20bp):
  - Large variants include large indels, intragenic single and multi exonic CNVs, copy-neutral gene rearrangements
- Large variant detection has been validated in >100 positive samples (sensitivity) and >1000 variants (specificity)
- All clinically significant variants are confirmed by Sanger or PacBio sequencing or by array CGH

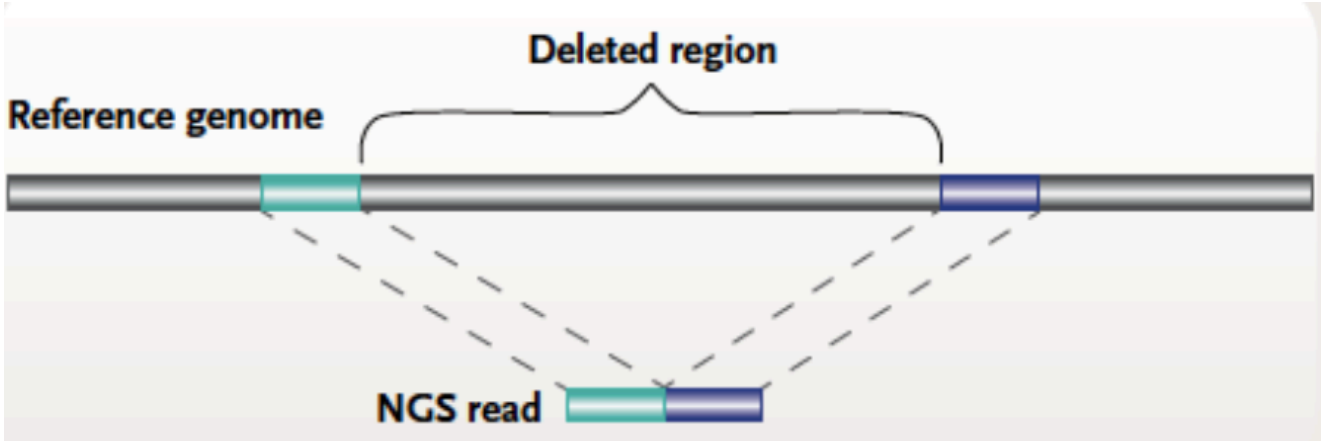
Methods: Read depth



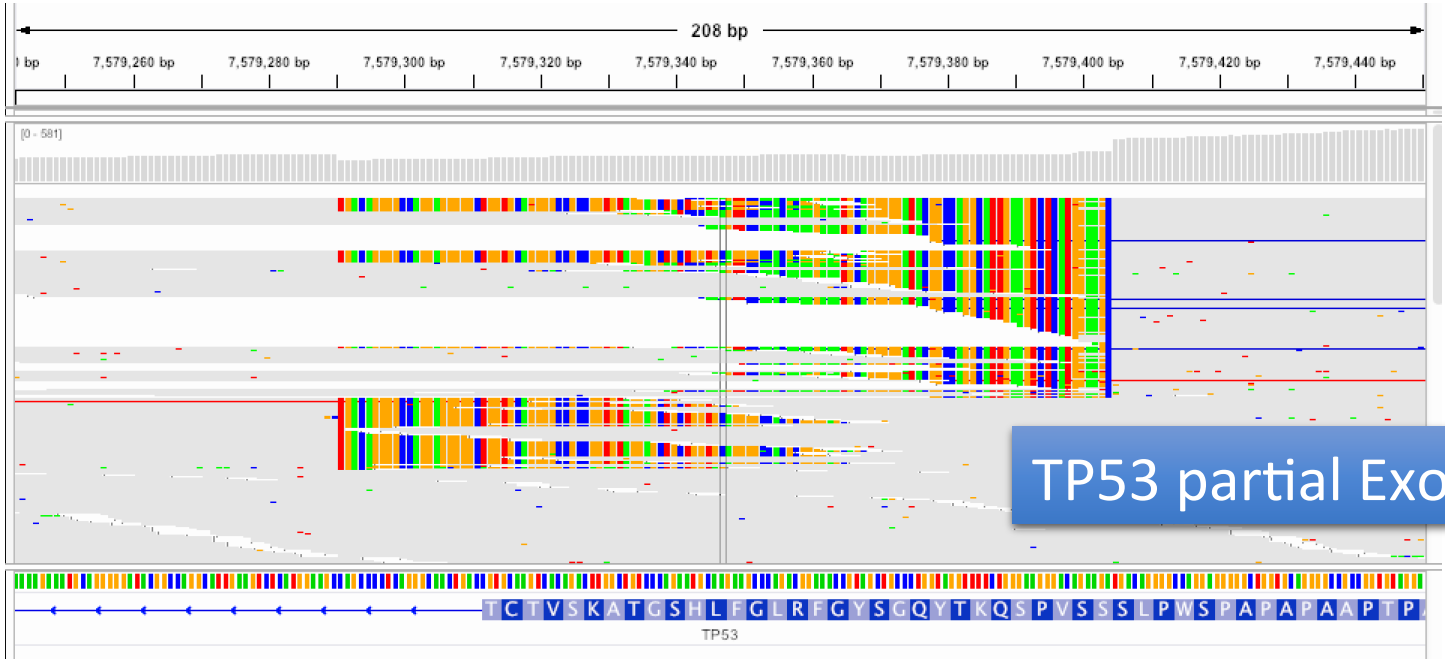
We use a carefully validated read-depth approach to identify exon-level CNVs at high sensitivity



Methods: Split read detection



We have validated detection of sub-exonic events, copy-neutral changes, and large insertions



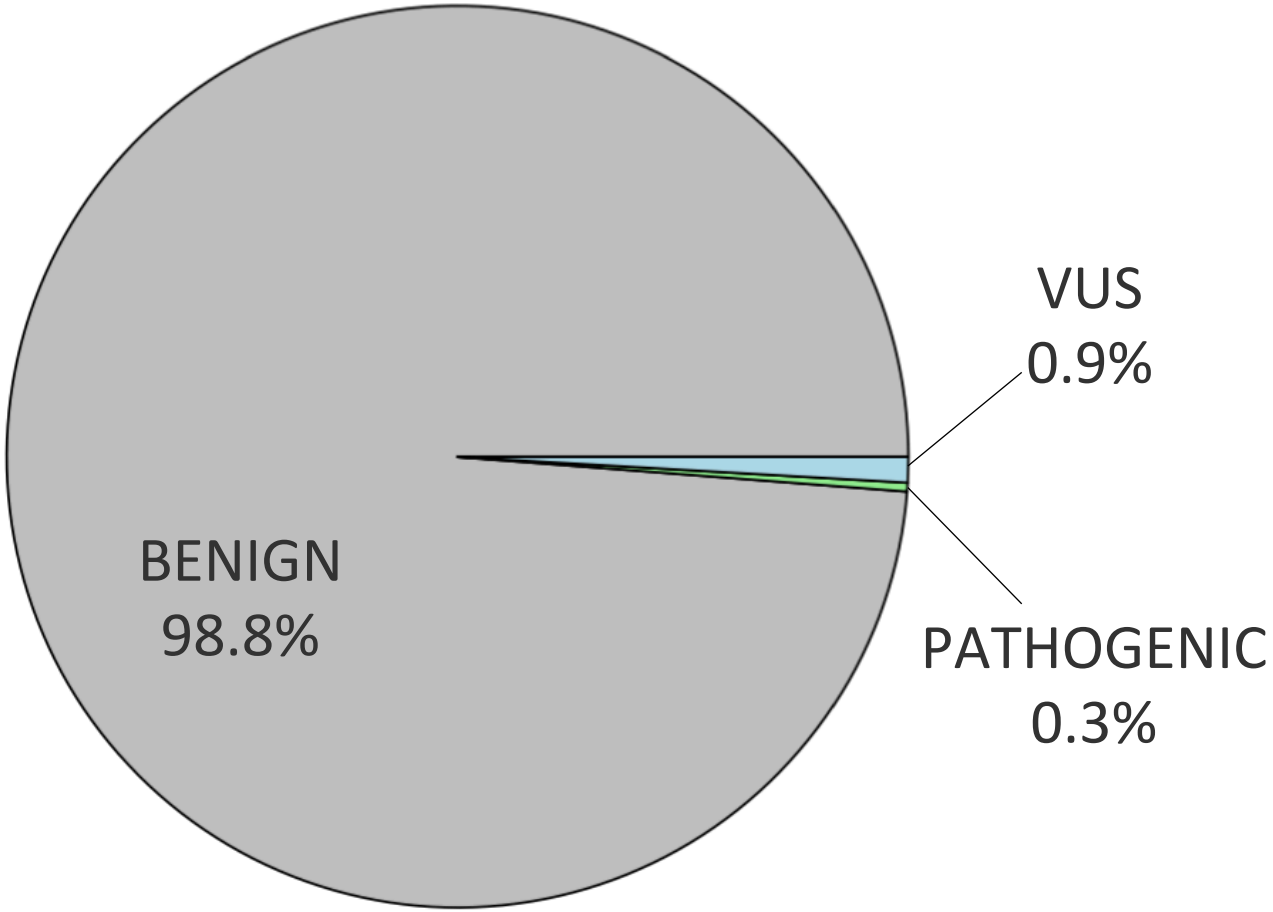
TP53 partial Exon 11 deletion

An extensive clinical NGS data set

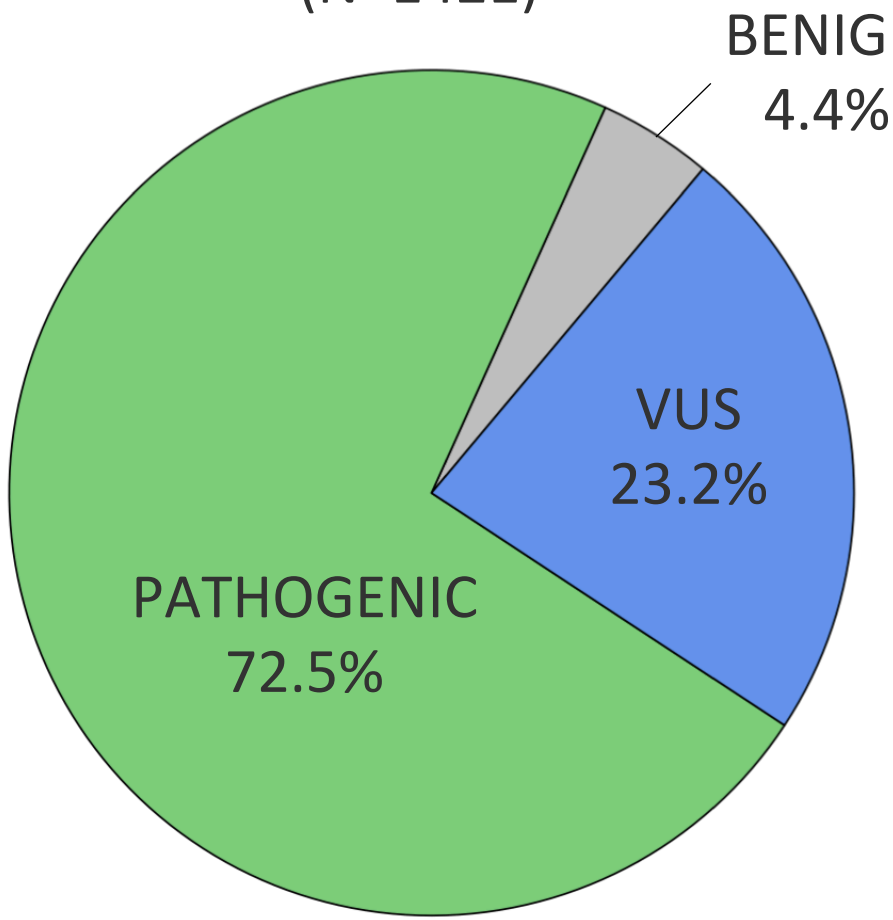
- >76000 unrelated patients tested (proband only)
  - Referred for diagnostic testing in a variety of clinical areas
- 1002 clinically relevant genes tested
- The equivalent of 2.2M single-gene tests
- Identified 1421 CNV and split-read detected events
  - 745 unique variants across 227 genes

Most large variants are pathogenic

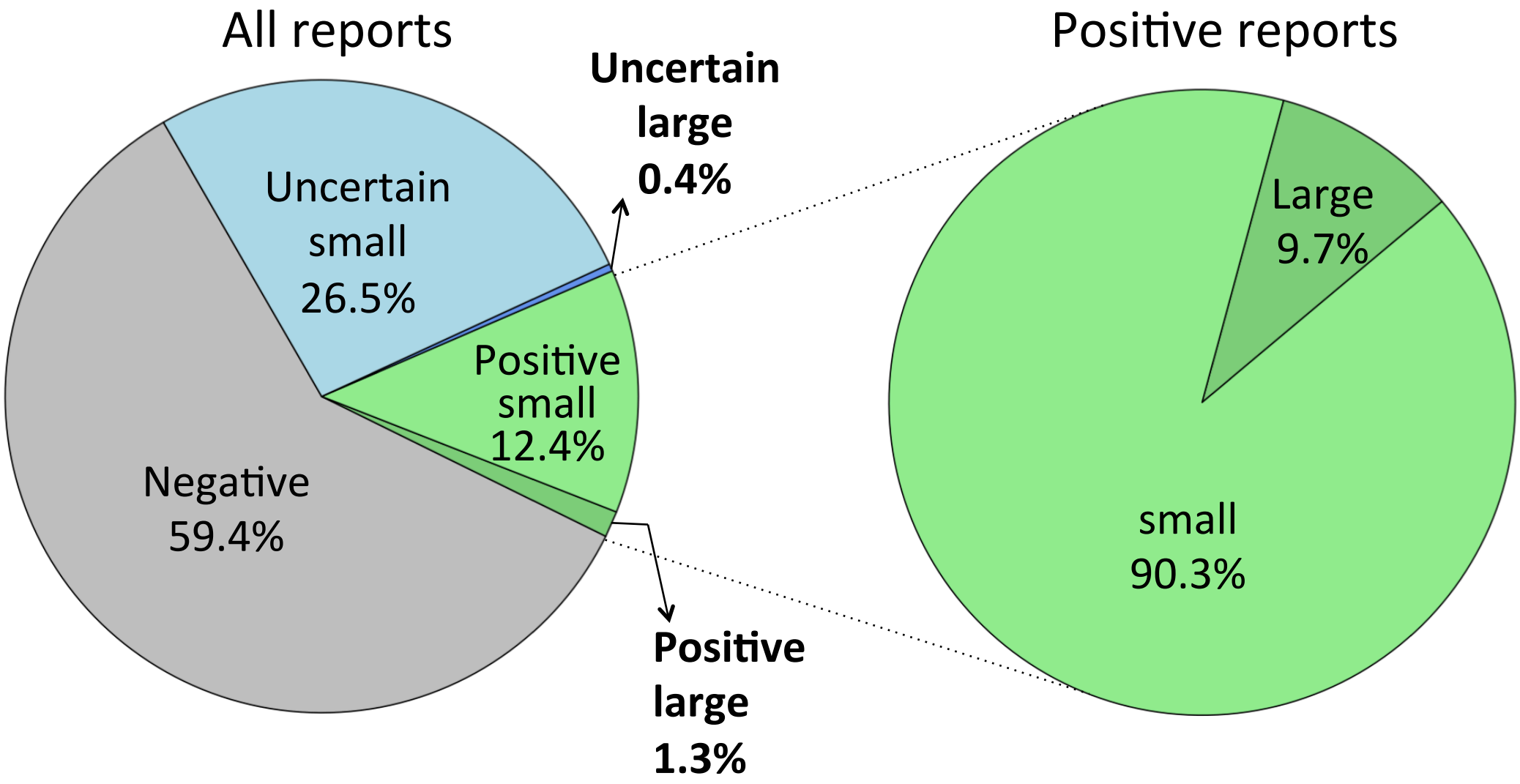
Small variants (SNVs and small indels)
(N=4 million)



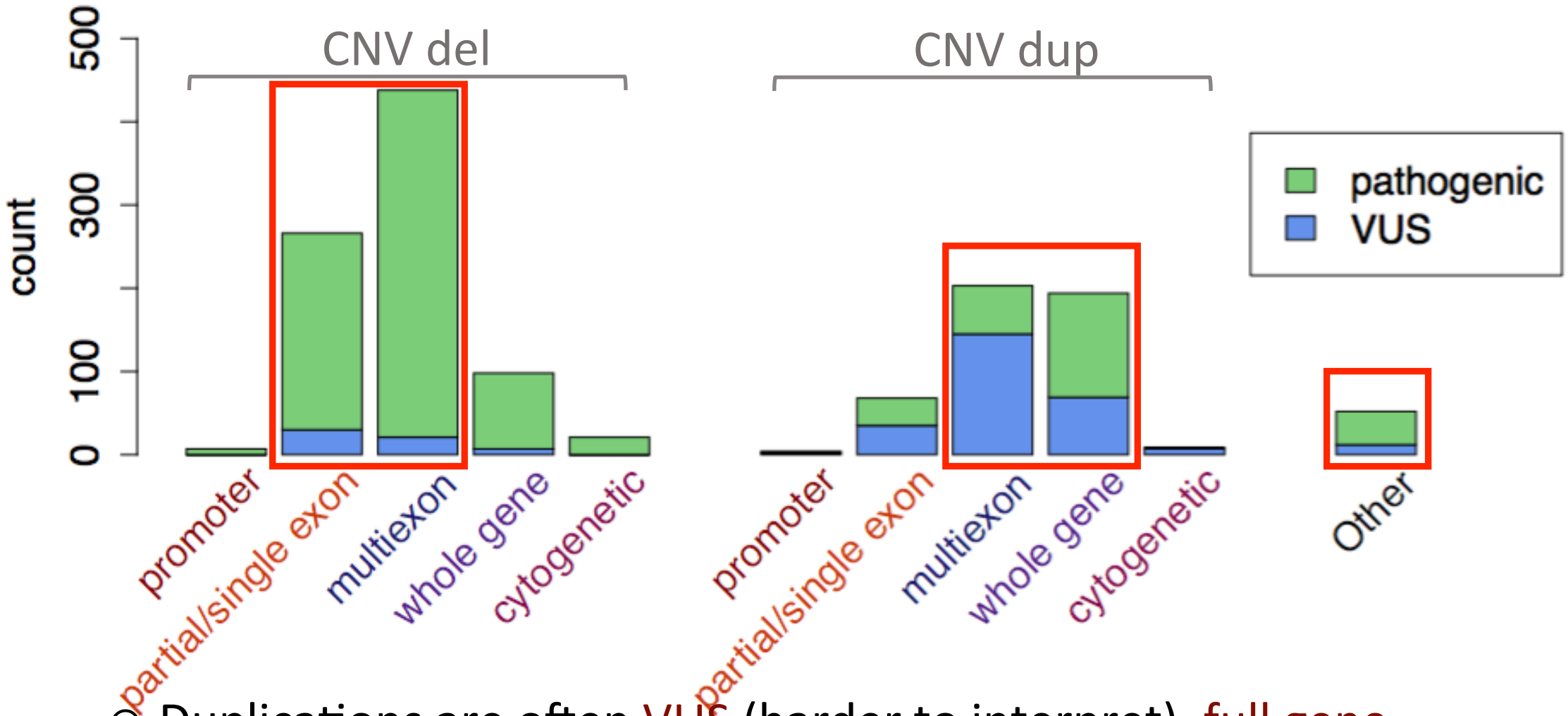
Large variants (large indels, CNVs)
(N=1421)



Large variants significantly contribute to positive reports



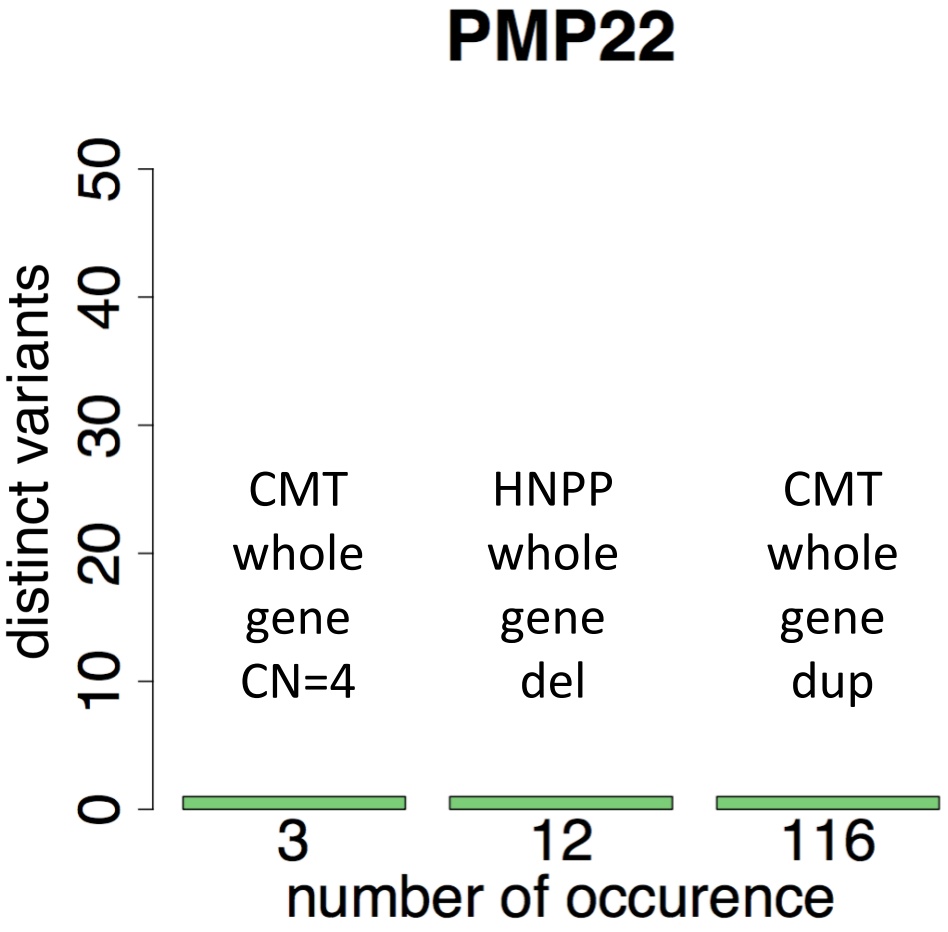
Variants vary in size and interpretability

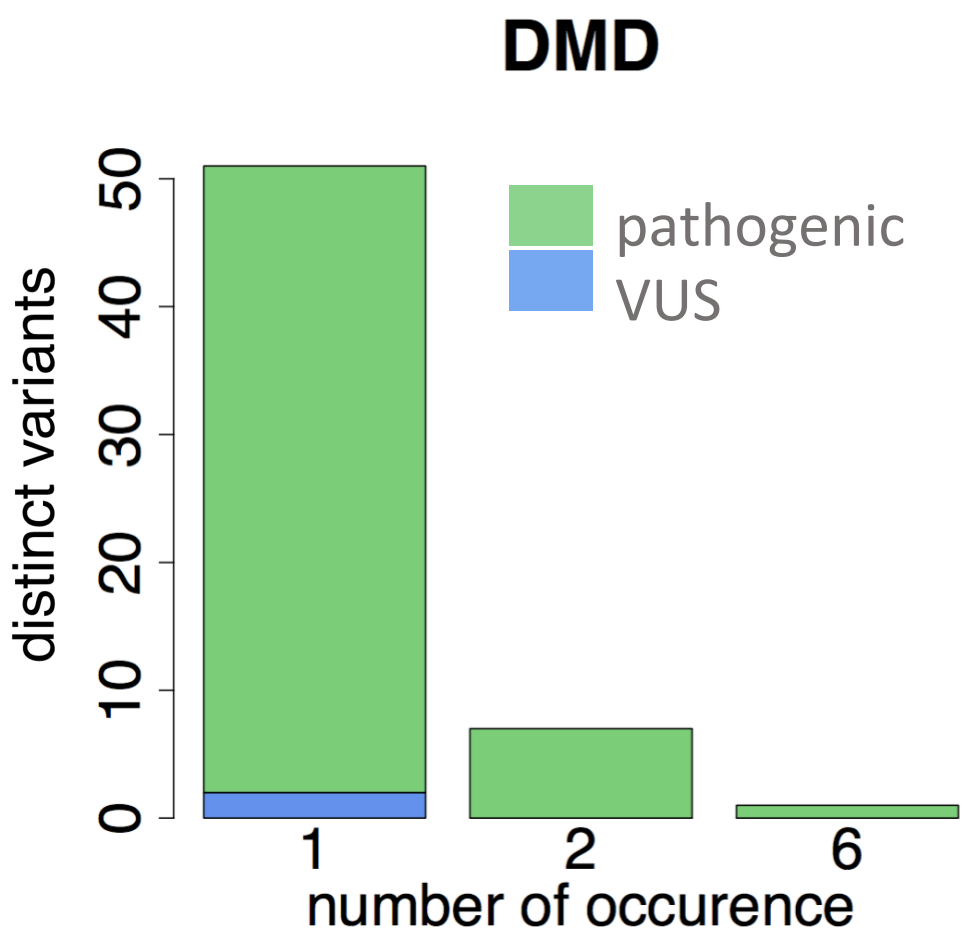


- Duplications are often VUS (harder to interpret), full gene
- Deletions are prevalent, small, pathogenic
  - Could be missed by traditional methods (both small and large)

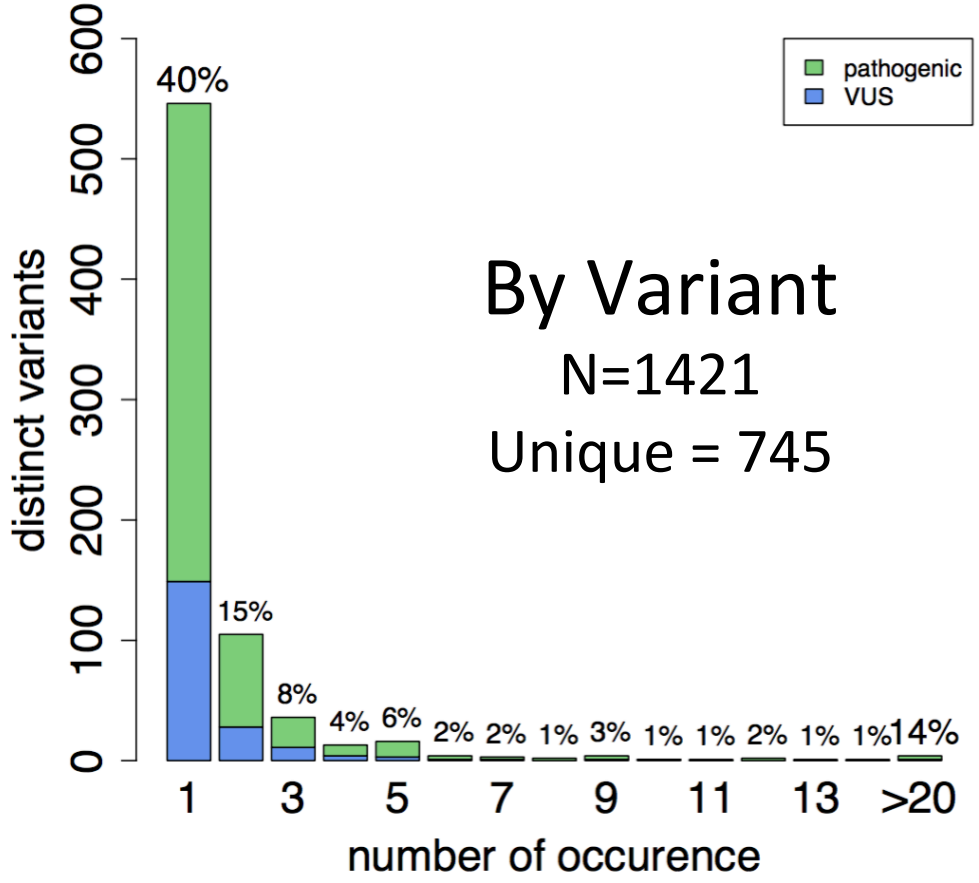
Few common variants vs many rare variants

- 131 patients with *PMP22* large events, only 3 distinct variants
- 71 patients with *DMD* large events, 59 distinct variants





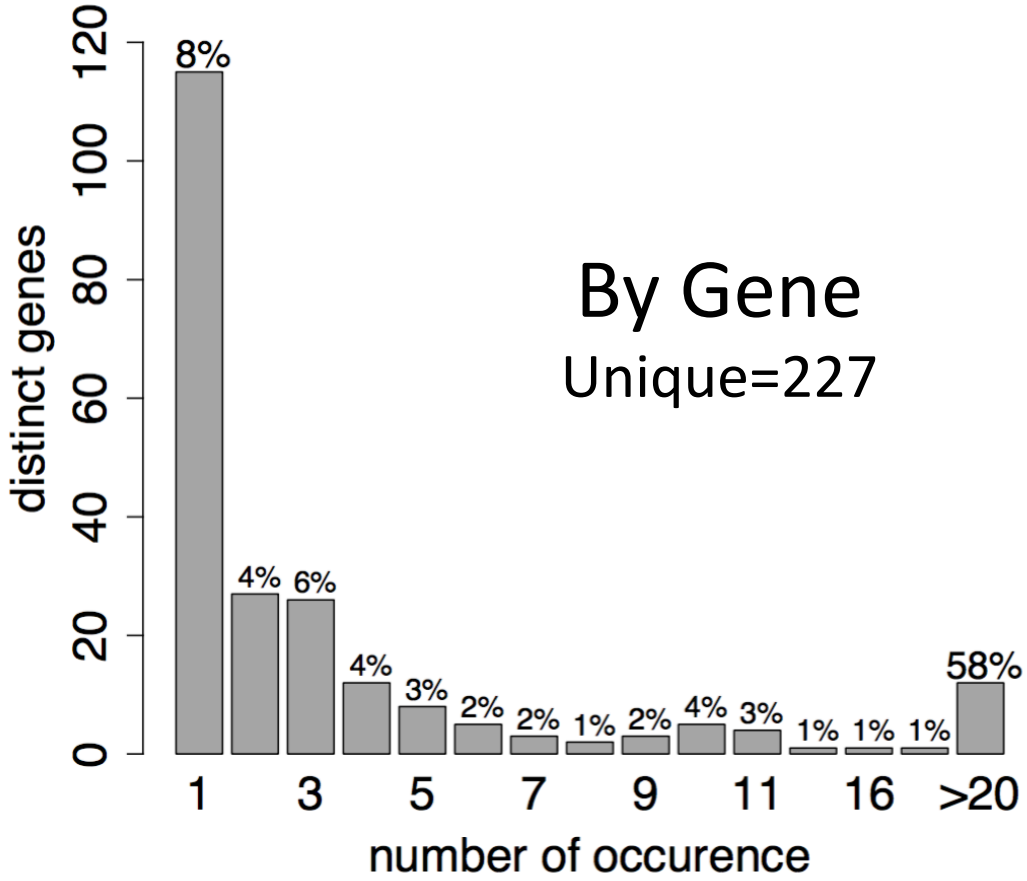
Some variants are common, many are unique



By Variant

N=1421

Unique = 745



By Gene

Unique=227

- ~65% of clinically reportable variants were rare in our cohort
  - Suggests that del/dup testing should go beyond just well known CNVs
- Most genes had very low del/dup prevalence, but these represent ~20% of events
  - Suggests that del/dup testing should go beyond genes with well known del/dup prevalence

Large variants affect genes across clinical areas

Clinical Area	Tests with CNVs in this cohort	Positive tests with CNV in this cohort
Hereditary Cancer	1.4%	9.2%
Metabolic Disorders and Newborn Screening	3.7%	10.6%
Pediatric Genetics	2.2%	7.8%
Cardiology	2.0%	5.7%
Neurology	11.0%	33.1%

Large variant prevalence is not uniform across disorders

Clinical Area	Positive tests with CNV	Disorder	Tests with CNVs	Positive tests with CNV
Pediatric Genetics	7.8%	neurofibromatosis	2.9%	12.2%
		epilepsy	3.4%	11.4%
		primary ciliary dyskinesia	3.5%	10.4%
		cystic fibrosis	0.7%	2.9%
		Noonan/RASopathies	1.3%	5.0%
Cardiology	5.7%	familial hypercholesterolemia	3.2%	10.9%
		aortopathies	1.9%	5.5%
		arrhythmias	1.8%	4.9%
		cardiomyopathies	1.3%	3.5%
Neurology	33.1%	neuropathies (including CMT)	14.4%	47.1%
		muscular dystrophy	19.9%	32.9%

Implications of CNV testing: recognizing complexity in molecular diagnoses

Case	**Patient with symptoms characteristic of Charcot-Marie-Tooth**
Standard testing result	*PMP22* del/dup negative, heterozygous Likely Pathogenic variant in *MFN2* Is this diagnostic? *MFN2* can be AD/AR
Comprehensive NGS result (small + large variants detected)	Also found likely pathogenic *GDAP1* partial Exon 3 deletion (1888 bp) Both variants should be pursued as potentially diagnostic and should be tested in family members
Another CMT example	A similar case had an apparently "homozygous" pathogenic SNV in *FGD4* which co-occurred with a pathogenic *FGD4* Exon 7-8 deletion
Clinical implications	**Comprehensive NGS immediately provided a complete result in these cases** **Copy number alterations exist in genes beyond *PMP22* in CMT patients**

Implications of CNV testing: availability of complete molecular data can impact clinical care

Cases	**Children with disorders that have treatment implications need quick answers (epilepsy, biochemical disorders)**
Standard testing result	Single pathogenic small variant found in recessive gene (ACADM, PCCA)
Comprehensive NGS result (small + large variants detected)	Found pathogenic exonic deletion in the same gene as compound heterozygous event
Additional examples	Intragenic exonic CNVs were found in recently discovered epilepsy genes: SLC35A2, DEPDC5, WWOX, KCTD7, PRRT2
Clinical implications	- **No reflex testing necessary: no additional sample or testing expense** **- Quick return of results to aid clinicians with clinical management** **- Comprehensive testing of all implicated genes increases diagnostic yield**

Conclusions

- Novel NGS methods now allow us to capture a broad spectrum of variants
- The prevalence of large variants, including intragenic CNVs, is higher than currently appreciated
  - ~10% of all positive molecular diagnoses in our cohort include large variants
- Many patients in our cohort had common CNVs, but the majority (62%) had rare events and 20% had events in genes with a low prevalence of large variants in this cohort
- Identifying CNVs alongside SNVs speeds up the time to diagnosis and can impact care in treatable hereditary disorders

Acknowledgements

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