Diagnostic Yield for Neurological and Neuromuscular Disorders Testing via High-Depth Multi-Gene Panel Analysis with Integrated Sequence and Copy Number Detection

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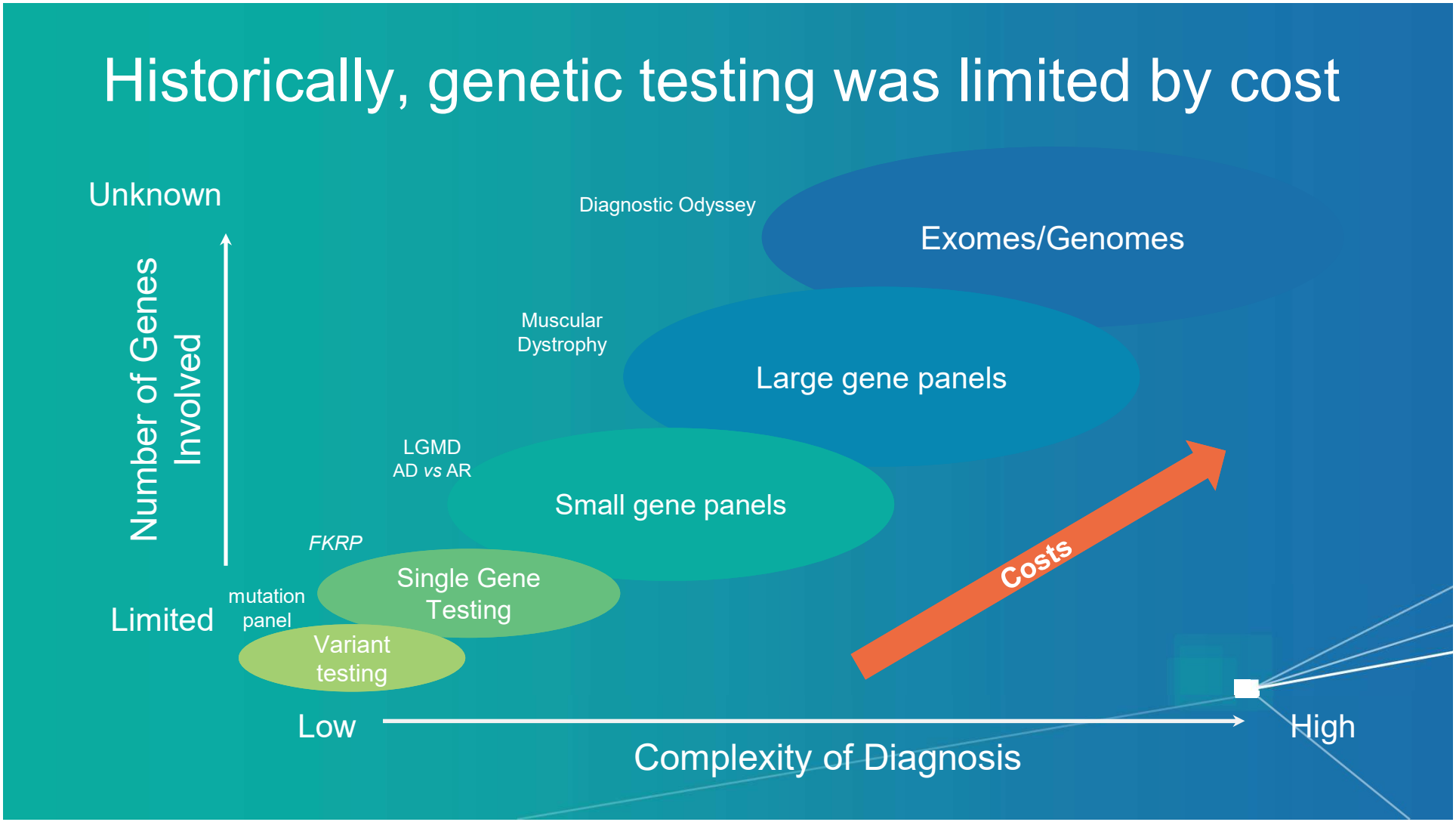
An Argument for Universal Del-Dup Testing for Neuromuscular Disorders

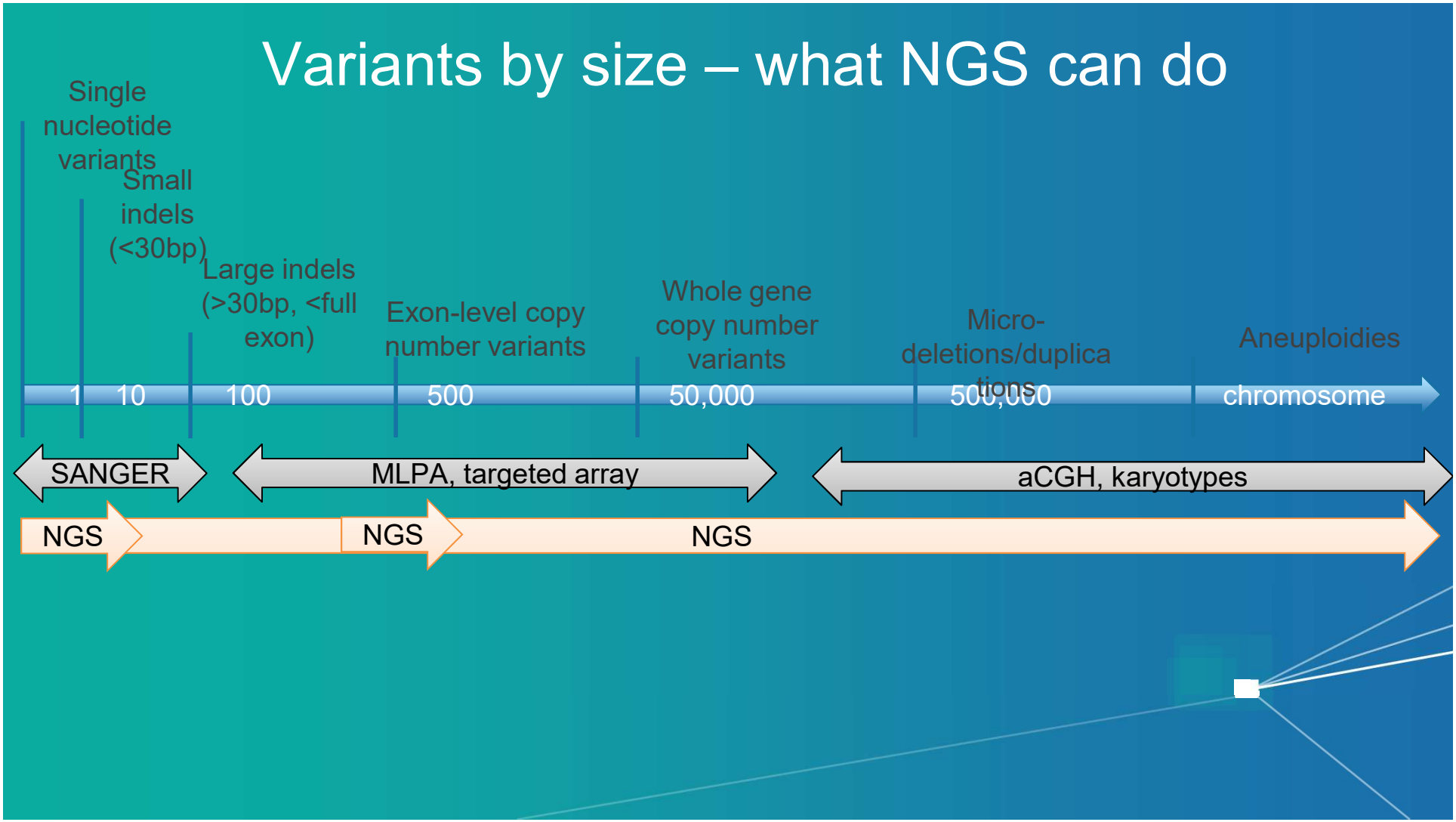
Disclosures

All authors are employees of
Invitae Corporation

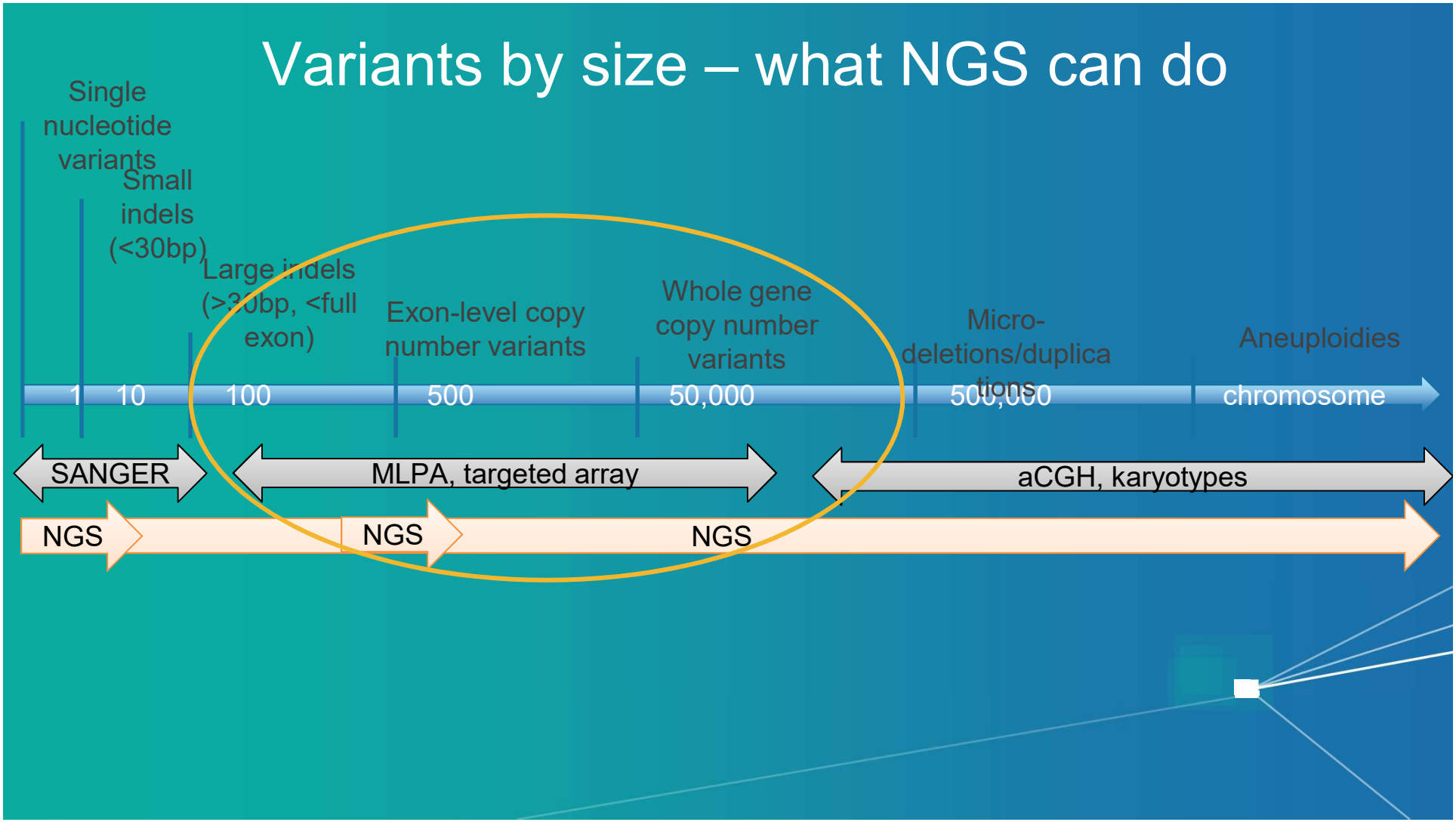
Historically, genetic testing was limited by cost



Variants by size – what NGS can do



Variants by size – what NGS can do



Why does CNV detection matter?

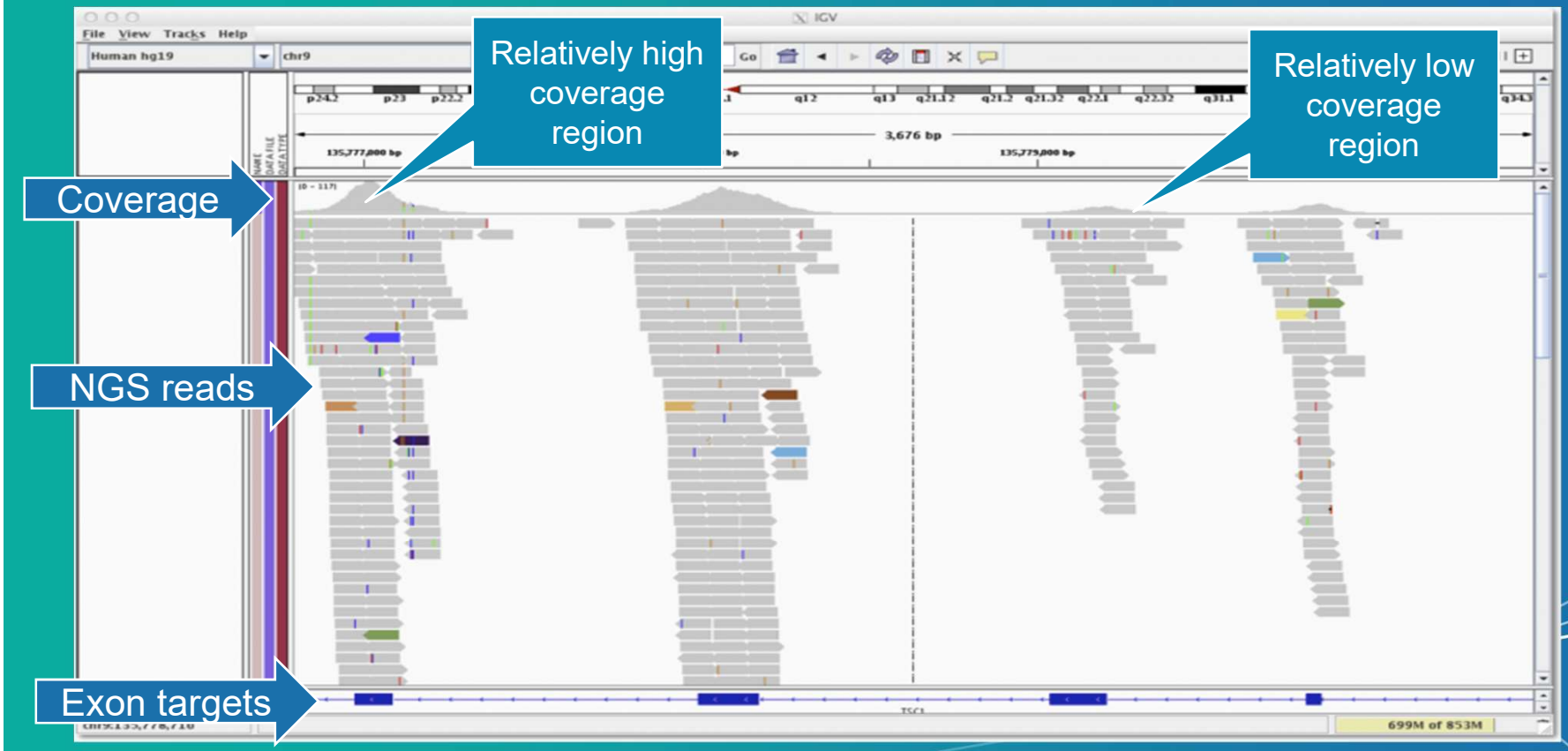
In >143,000 patients, del/dup events are seen:

- In 1.9% of all tested patients
- In 9.8% of patients with any pathogenic variant

➢ *A disproportionate fraction of del/dups are pathogenic*

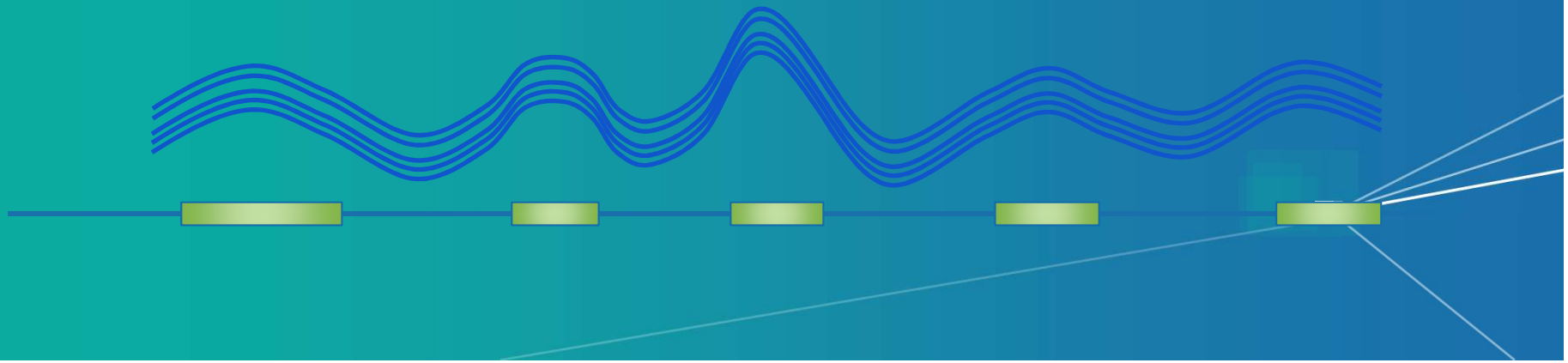
8.3% 4.8% 39% 7.7%

Read depth (a.k.a. coverage)

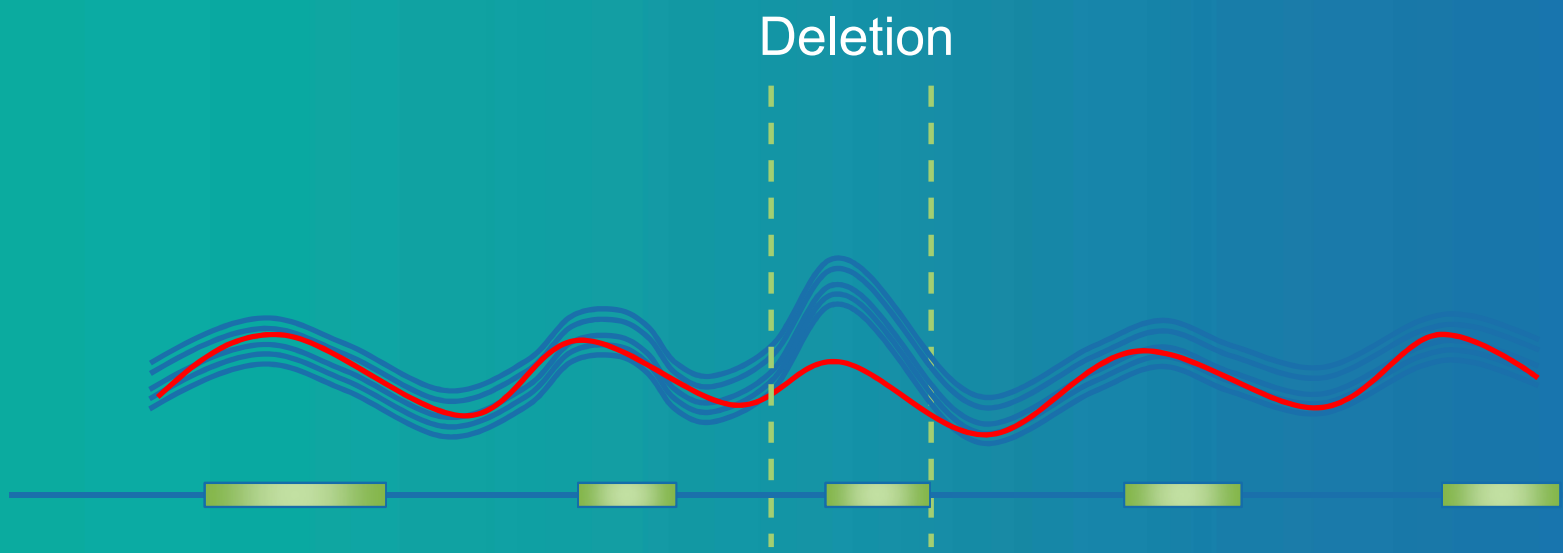


Copy number detection by NGS

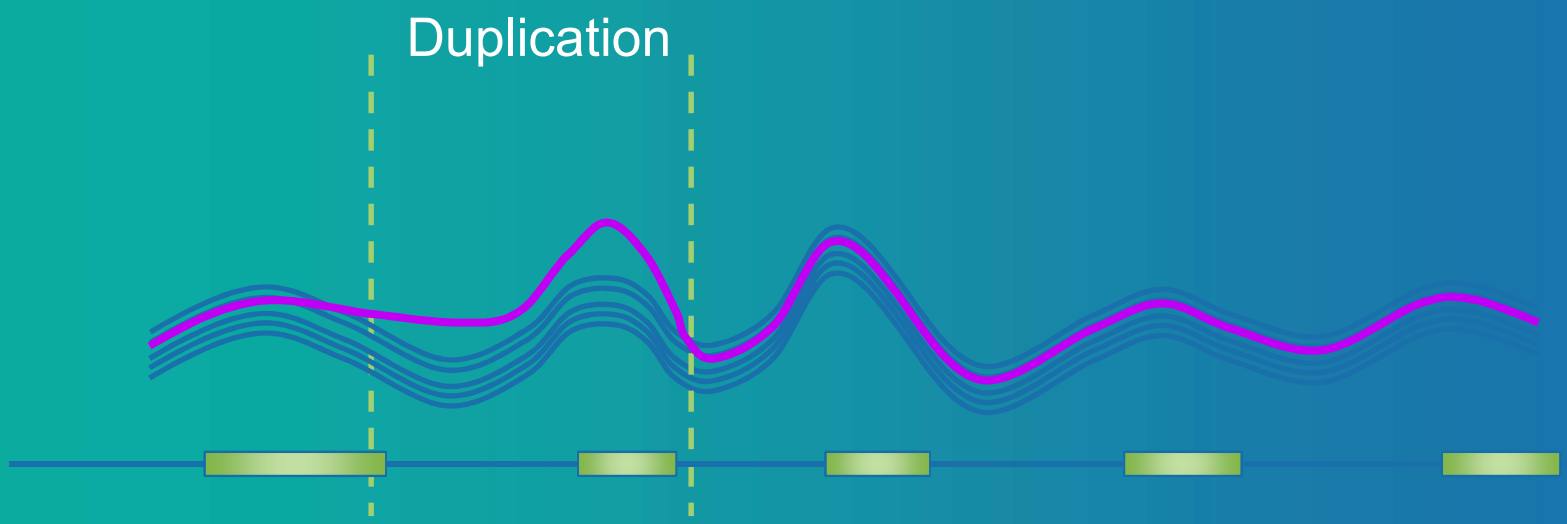
- Read depth varies between targets, due to both the target chemistry and NGS itself
- Depth profile *is non-uniform* but *reproducible*

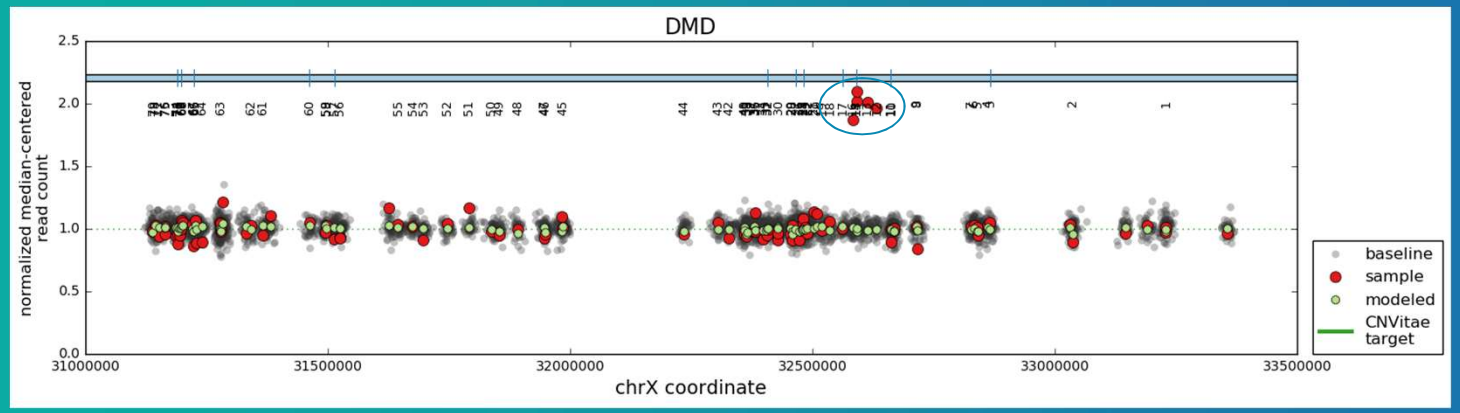


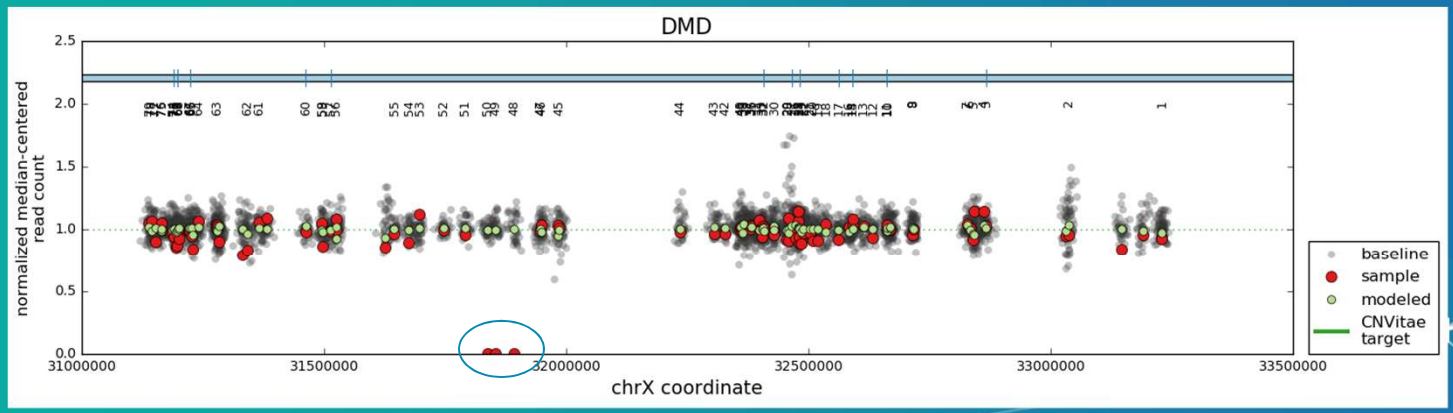
Deviations with respect to baseline samples

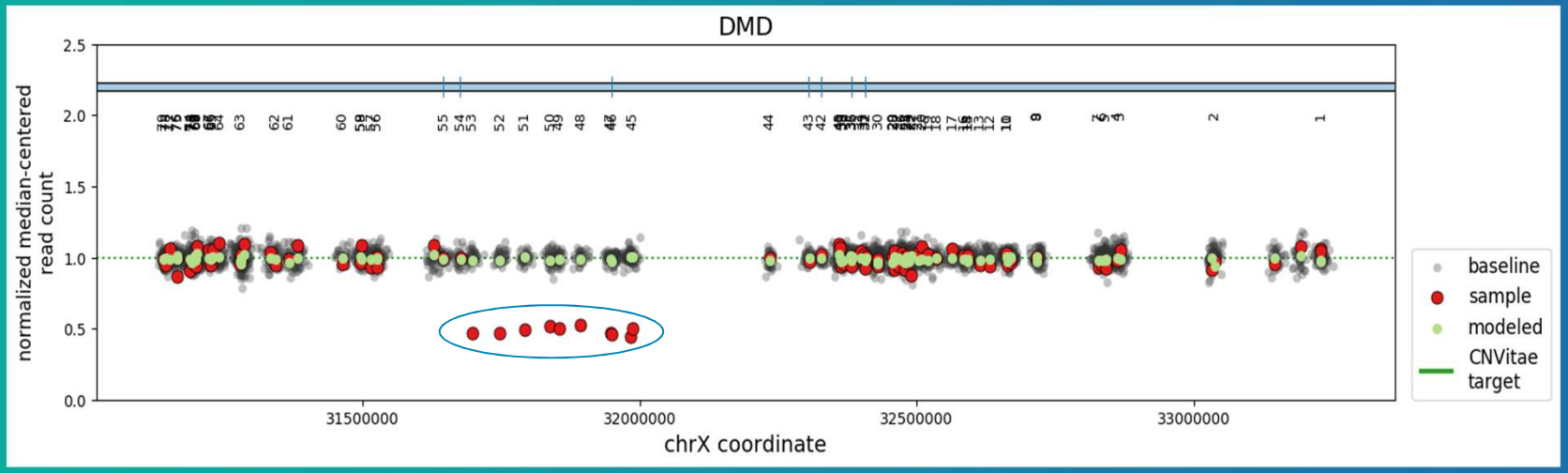


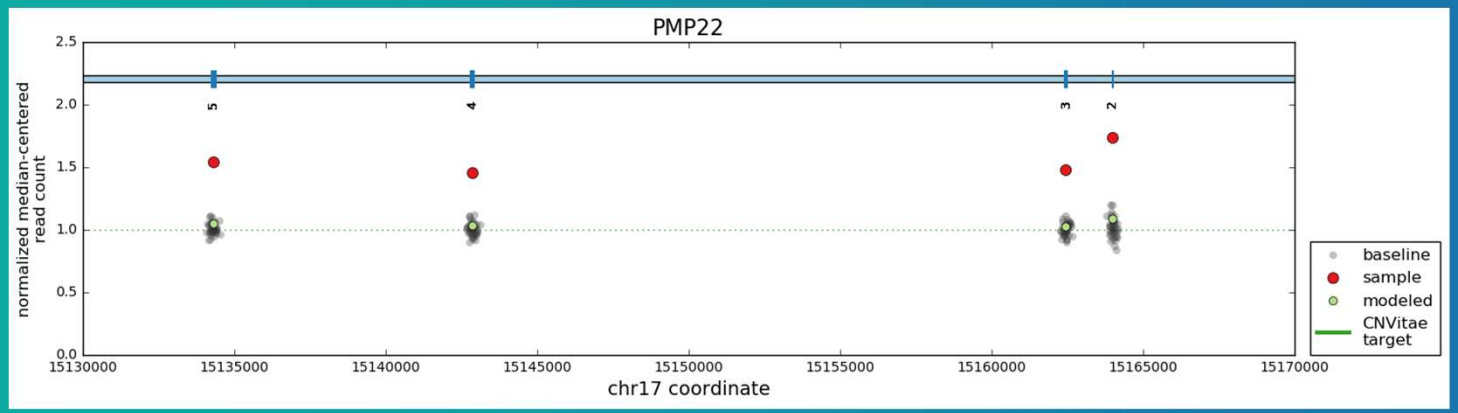
Deviations with respect to baseline samples

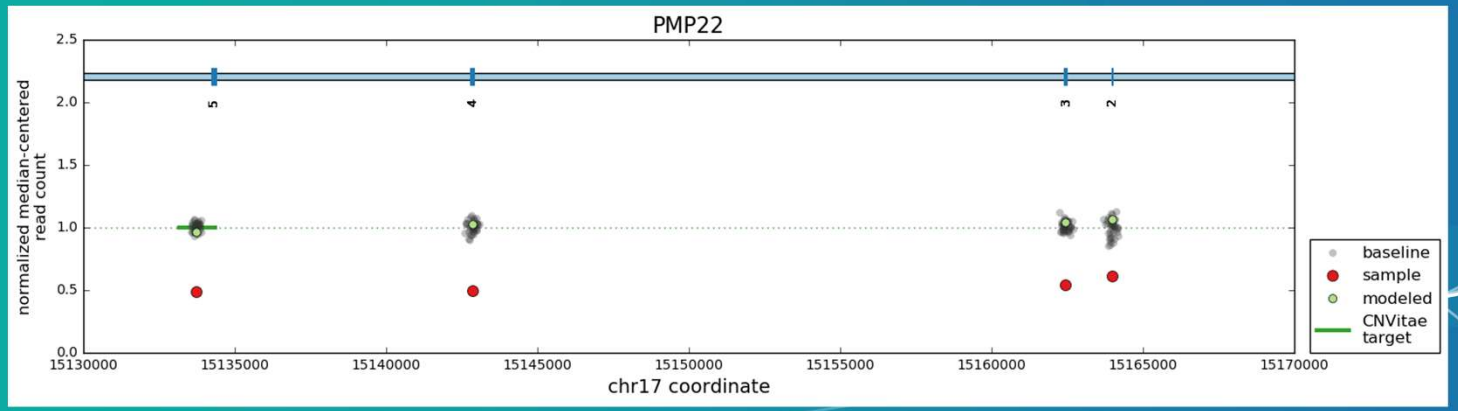


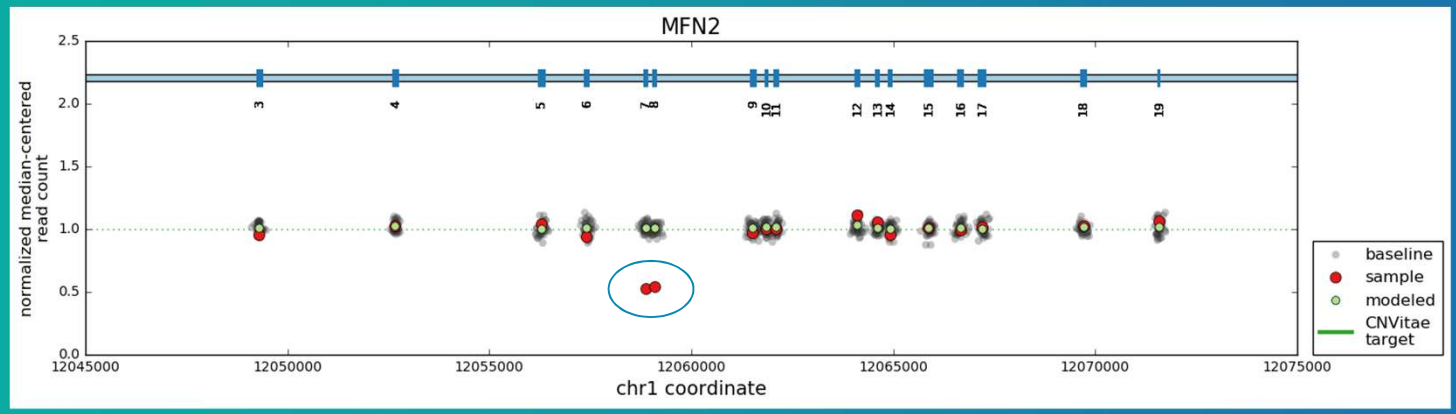

DMD
normalized median-centered read count
chrX coordinate
baseline
sample
modeled
CNVitae target

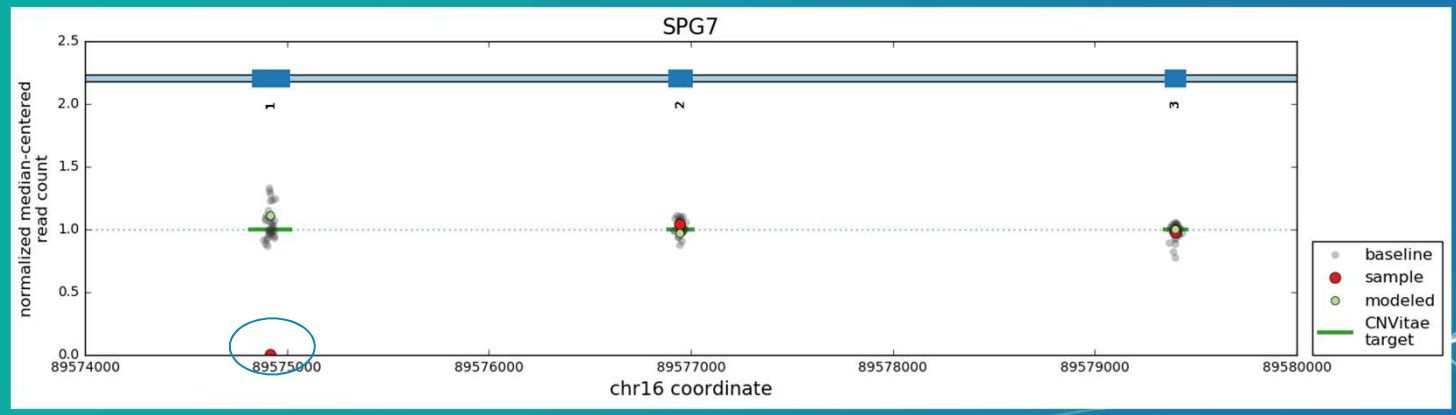

DMD
normalized median-centered read count
chrX coordinate
baseline
sample
modeled
CNVitae target


DMD
normalized median-centered read count
2.5
2.0
1.5
1.0
0.5
0.0
31500000
32000000
32500000
33000000
chrX coordinate
baseline
sample
modeled
CNVitae target


PMP22
normalized median-centered read count
2.5
2.0
1.5
1.0
0.5
0.0
5
4
3
2
15130000
15135000
15140000
15145000
15150000
15155000
15160000
15165000
15170000
chr17 coordinate
baseline
sample
modeled
CNVitae target


PMP22
normalized median-centered read count
2.5
2.0
1.5
1.0
0.5
0.0
5
4
3
2
15130000
15135000
15140000
15145000
15150000
15155000
15160000
15165000
15170000
chr17 coordinate
baseline
sample
modeled
CNVitae target


MFN2
normalized median-centered read count
2.5
2.0
1.5
1.0
0.5
0.0
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
12045000
12050000
12055000
12060000
12065000
12070000
12075000
chr1 coordinate
baseline
sample
modeled
CNVitae target


SPG7
normalized median-centered read count
2.5
2.0
1.5
1.0
0.5
0.0
1
2
3
89574000
89575000
89576000
89577000
89578000
89579000
89580000
chr16 coordinate
baseline
sample
modeled
CNVitae target

Validity of method: retrospective cohorts

- Kerkof *et al. J Mol Diag* 2017.
  - 43 CNVs among 391 patients
    - 100% concordance using NGS methods
- Lincoln *et al*. *J Mol Diag* 2015.
  - 29 CNVs among 1,105 patients
    - 100% concordance using NGS methods

Prevalence of CNVs in neuromuscular disorders

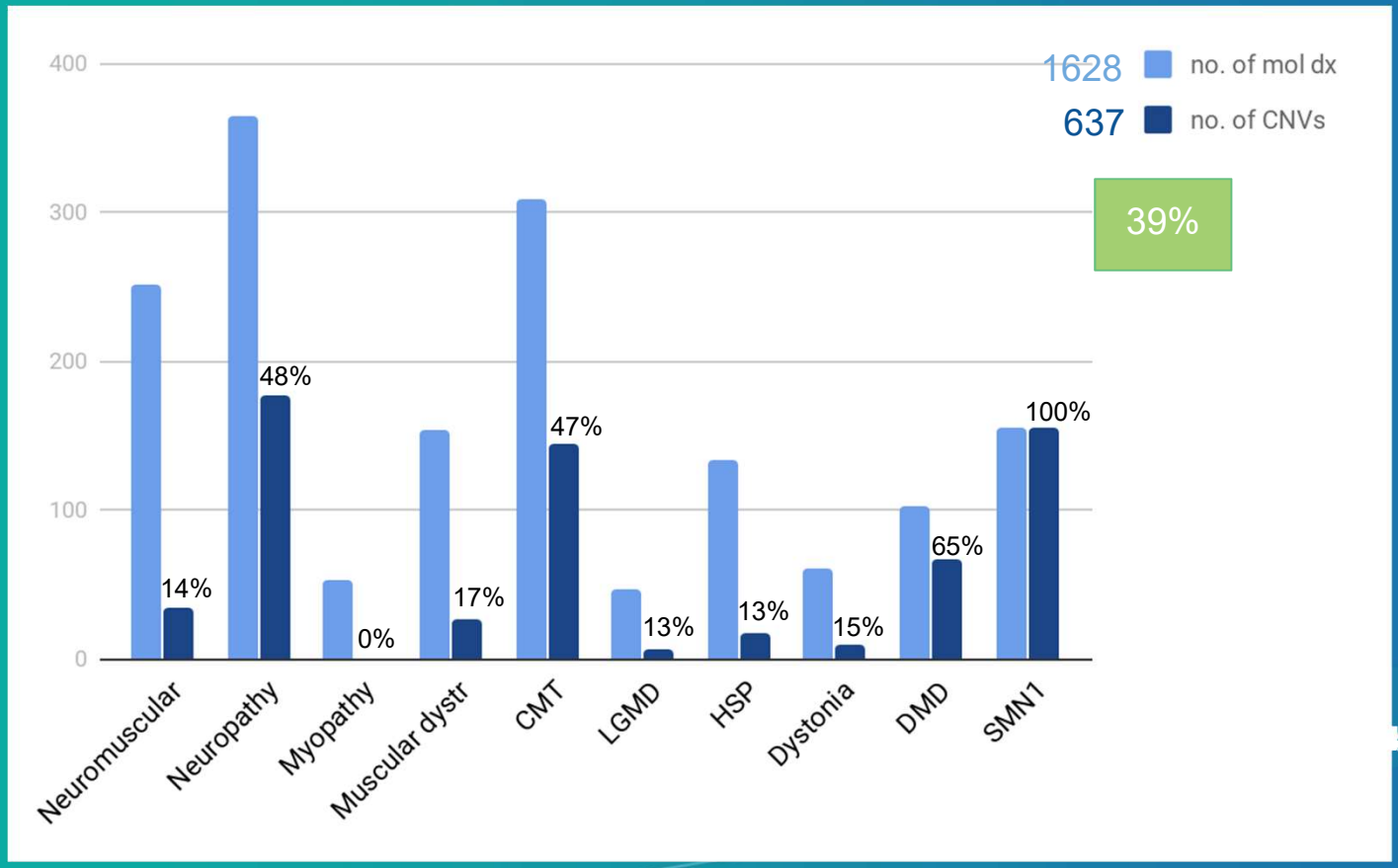
- Referrals to a commercial lab
- Sole criterion: *physician ordered*
- Diagnostic (not carrier)
- Typically limited clinical/lab data

CNVs account for 39% of positive results

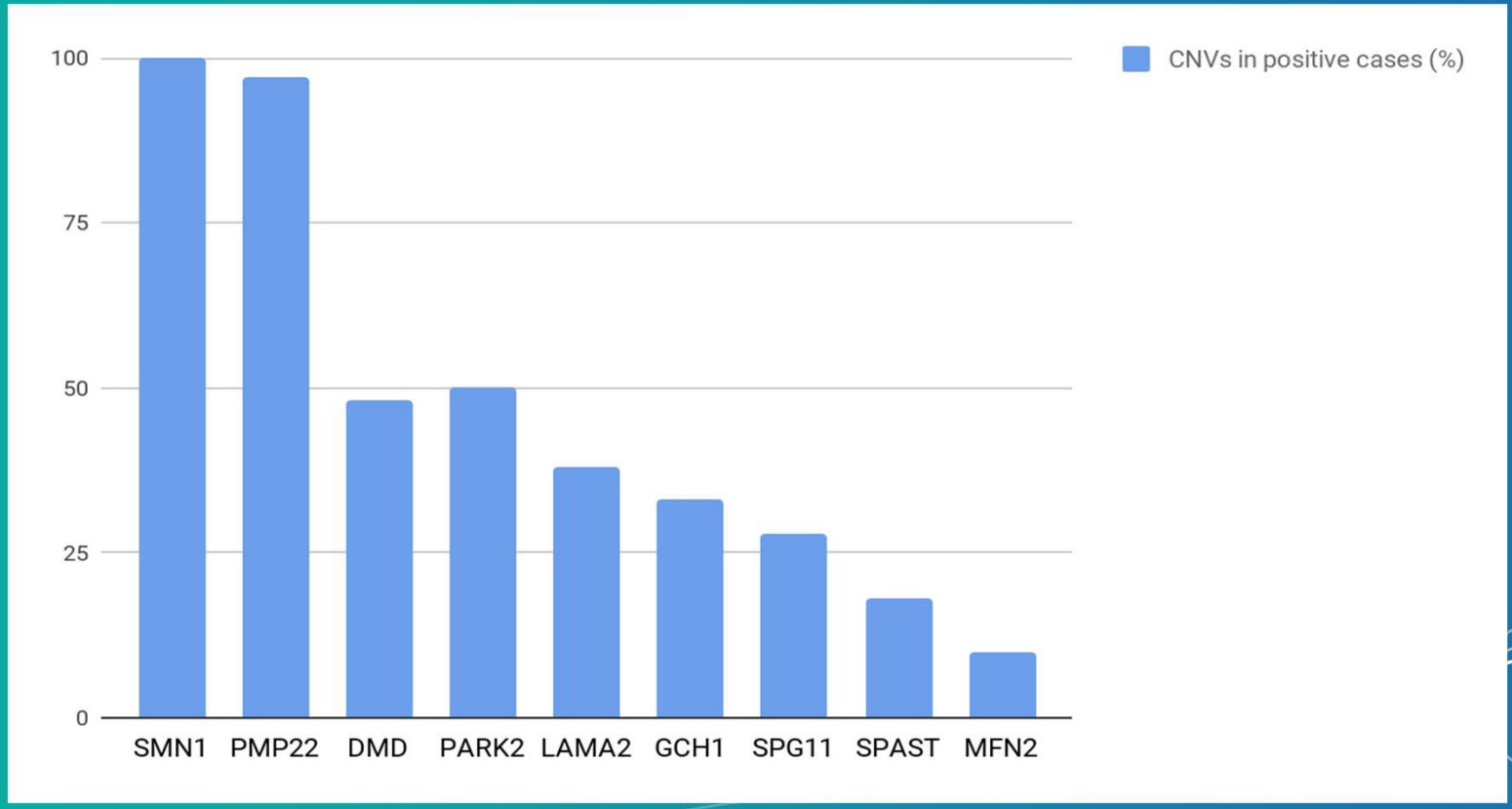
7,092 unrelated individuals tested

test panel	no. of genes	no. of requests	no. of mol dx	(%)
Neuromuscular disorders	104	1073	251	(23)
Neuropathy	70	2741	364	(13)
Myopathy	50	352	53	(15)
Muscular dystrophy	46	399	154	(39)
Charcot-Marie Tooth	42	1040	308	(30)
LGMD	30	174	46	(26)
HSP	43	843	133	(16)
Dystonia	16	535	61	(11)
DMD	1	187	103	(55)
SMN1	1	246	155	(63)
	Total	7590	1628	(23)

CNV rates by panel



CNV rate by gene



Case for universal CNV analysis

for DMD, how many 'CNV-positive' cases occur among multi-gene panel tests?

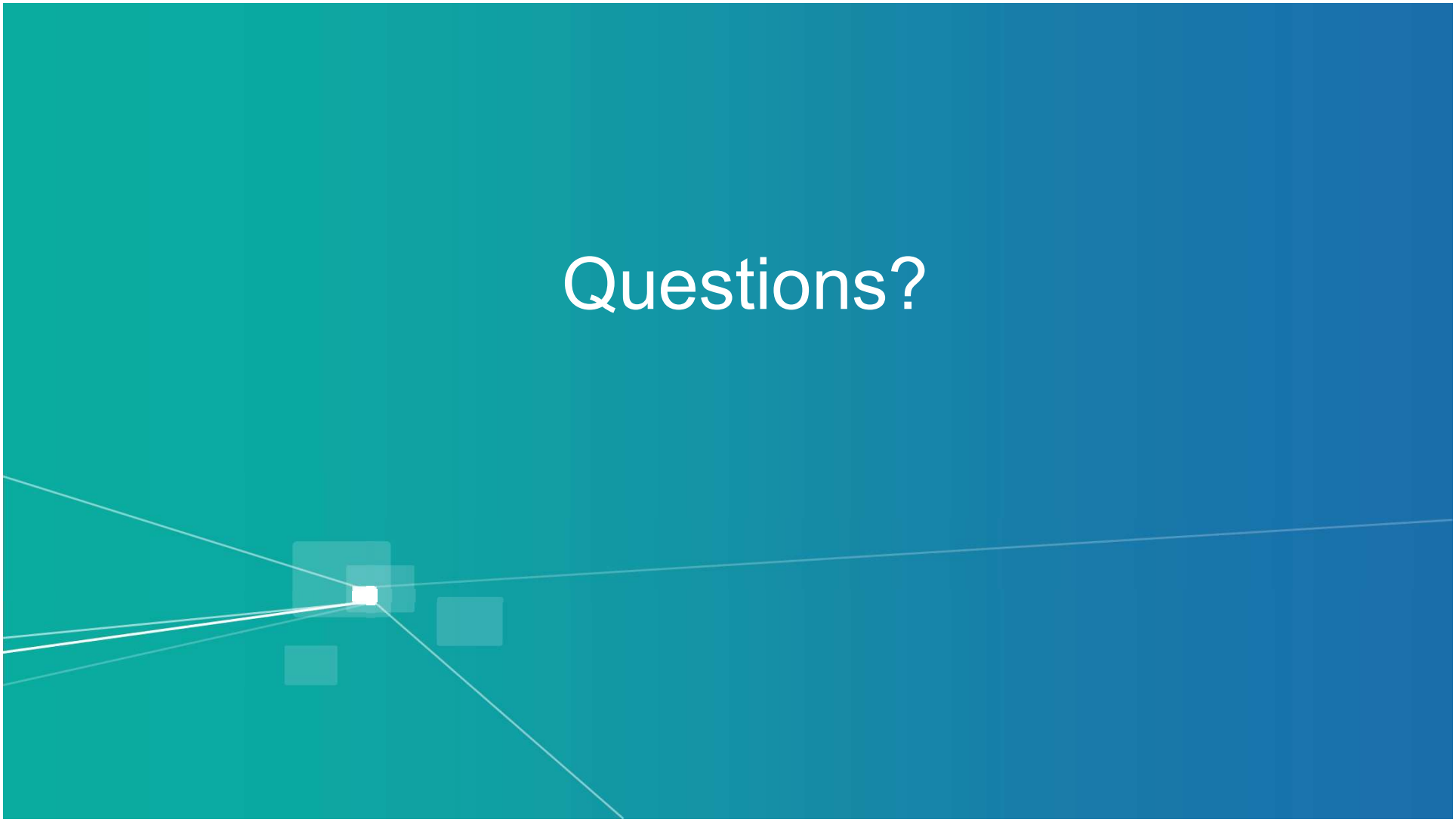
p a n e l

Mutation type	LGMD	MD	NMD	Total (%)
CNV	5/46 (11)	24/154 (16)	13/251 (5)	42/451 (9)

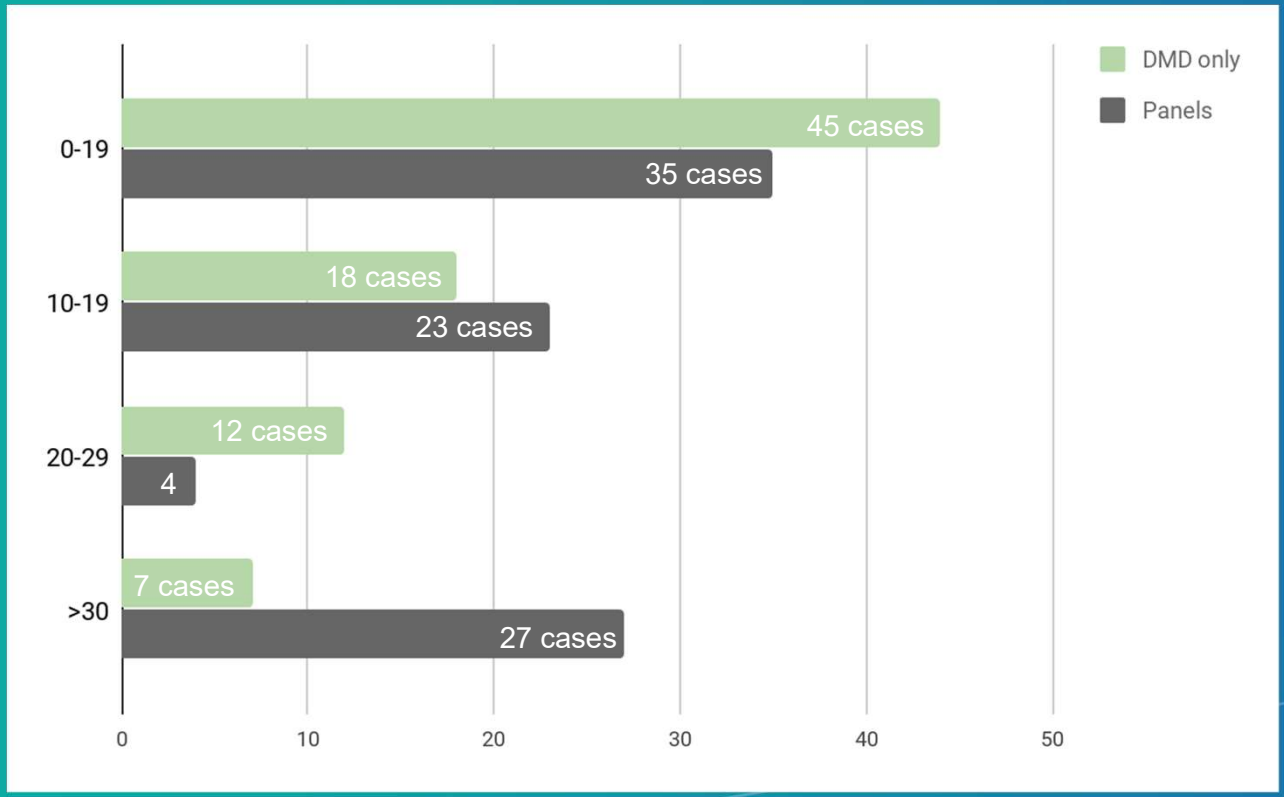
Summary

- NGS data can be used for highly sensitive del/dup analysis
- CNVs are prevalent in neuromuscular disorders
- Universal CNV detection may increase diagnostic rate

Questions?



Age at diagnosis



Validity of method: prospective cohorts

- Kerkof *et al. J Mol Diag* 2017.
  - 130 CNVs among 2375 patients
    - 106 confirmed by alternate methods (FP=1%)
- Invitae
  - 4,028 CNVs among 227,022 patients
    - 3,910 confirmed by alternate methods (FP=0.05%)

What do real data look like?