University of Colorado Boulder

Lecture 6: Various!

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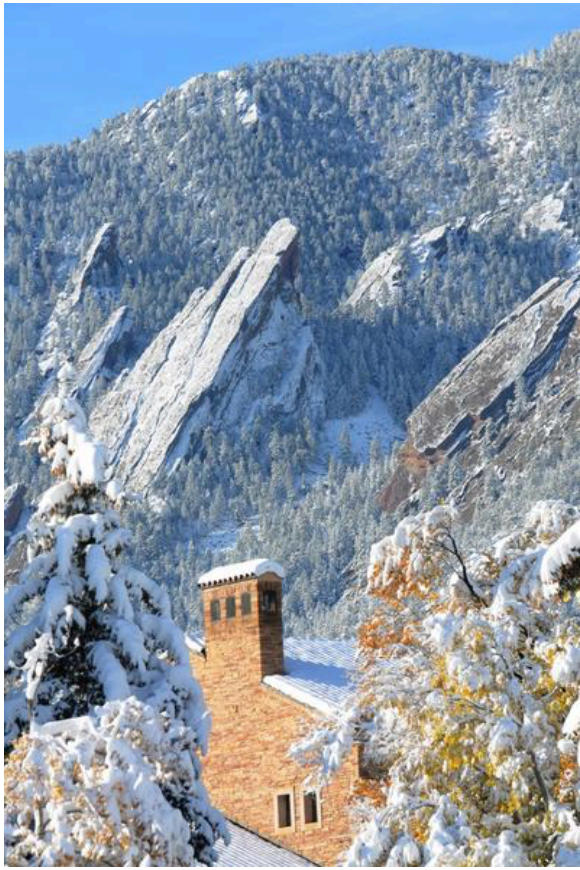
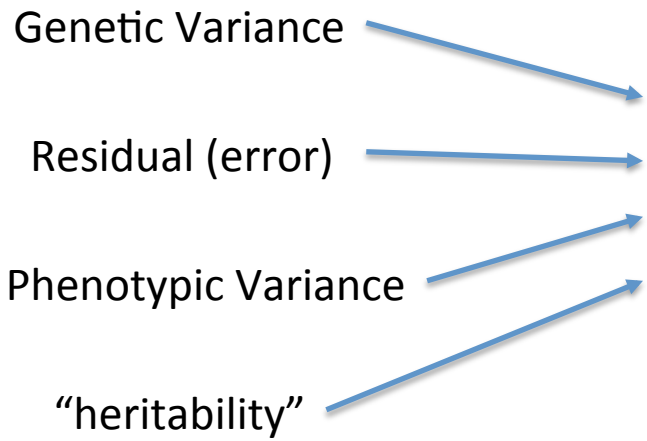


Wrap up topics

- Interpreting GCTA output
- Next Generation Sequencing
- Copy Number Variants
- Meta-Analysis

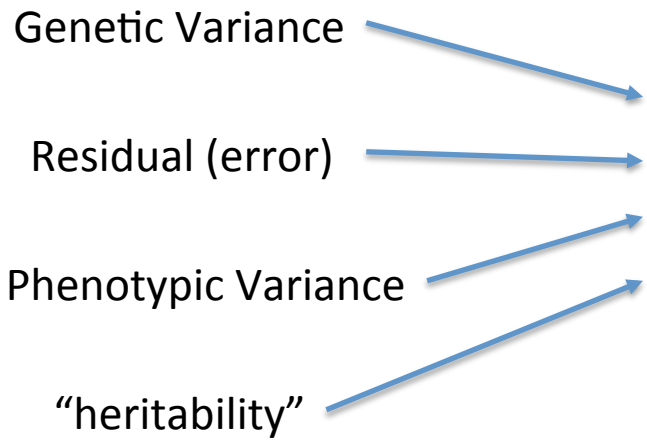
Interpreting GCTA Output

GCTA Output



Source	Variance	SE
V(1)	8.460930	5.852812
V(e)	9.985167	5.369622
Vp	18.446097	0.989077
V(1)/Vp	0.458684	0.304386
logL	−1791.054	
n	923	

GCTA Output



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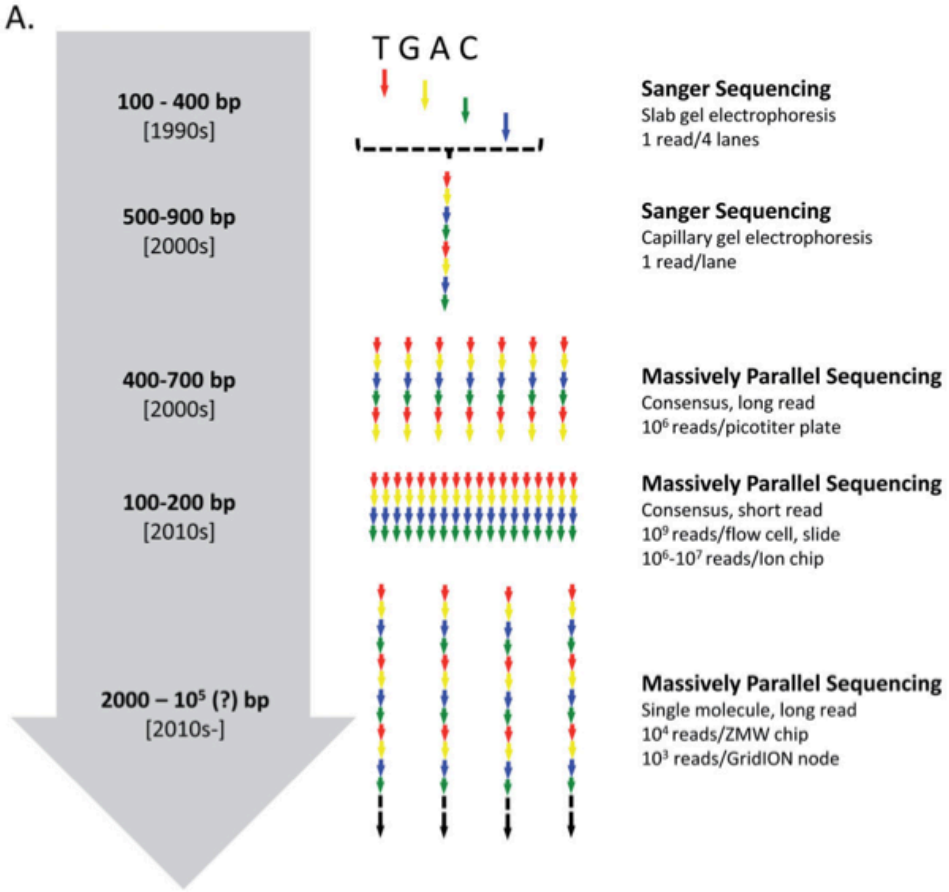
V(1)/Vp	**0.458684**	0.304386
logL	−1791.054	
n	923	

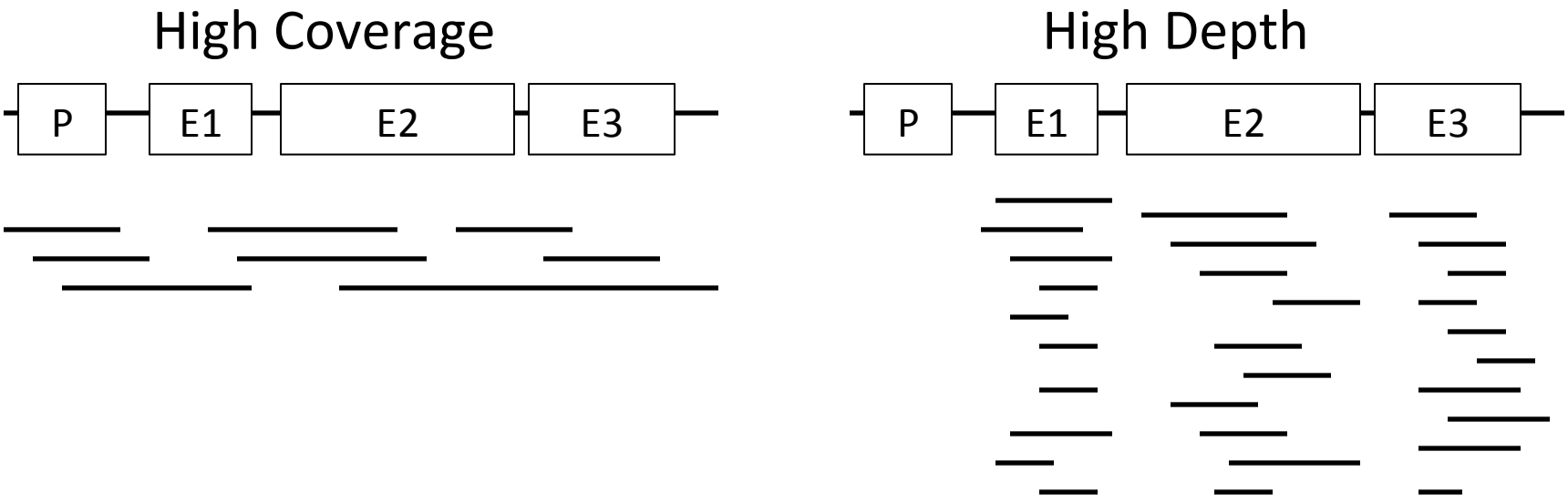
NOTE: This is the narrow sense heritability (additive effects)

Next Generation Sequencing

Sequencing


A.
100 - 400 bp
[1990s]
500-900 bp
[2000s]
400-700 bp
[2000s]
100-200 bp
[2010s]
2000 – 10⁵ (?) bp
[2010s-]
T G A C
Sanger Sequencing
Slab gel electrophoresis
1 read/4 lanes
Sanger Sequencing
Capillary gel electrophoresis
1 read/lane
Massively Parallel Sequencing
Consensus, long read
10⁶ reads/picotiter plate
Massively Parallel Sequencing
Consensus, short read
10⁹ reads/flow cell, slide
10⁶-10⁷ reads/Ion chip
Massively Parallel Sequencing
Single molecule, long read
10⁴ reads/ZMW chip
10³ reads/GridION node

Sequencing coverage vs depth



Next Generation Sequencing

- Moving fast
  - High depth, high coverage now possible
  - Prices falling

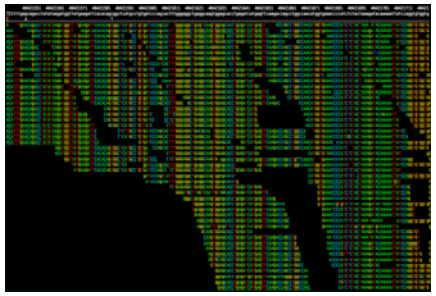
What are we expecting to find?

- Is this a looking under the lamp post issue?
  - More and more precise measurement
- Is there something new that we haven't seen?

Next Generation Sequencing

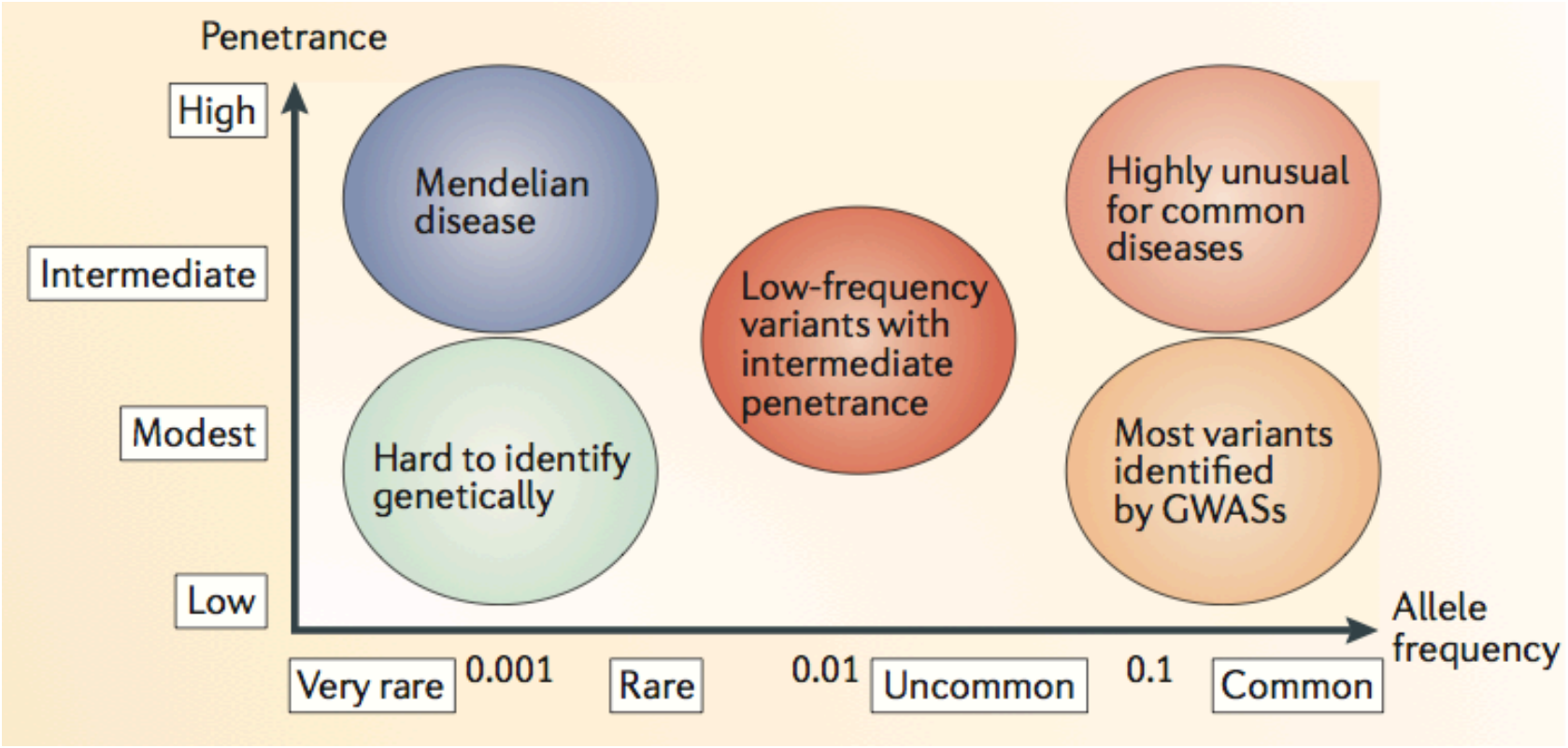
- Will this provide more answers than GWAS?

Sequencing



- Objective
  - Find **rare/common variants** associated with disease
- Design
  - Cohort, case-control, family-based
- Molecular information
  - 3B base-pair
- Desired outcome
  - Find genetic variation underlying disease

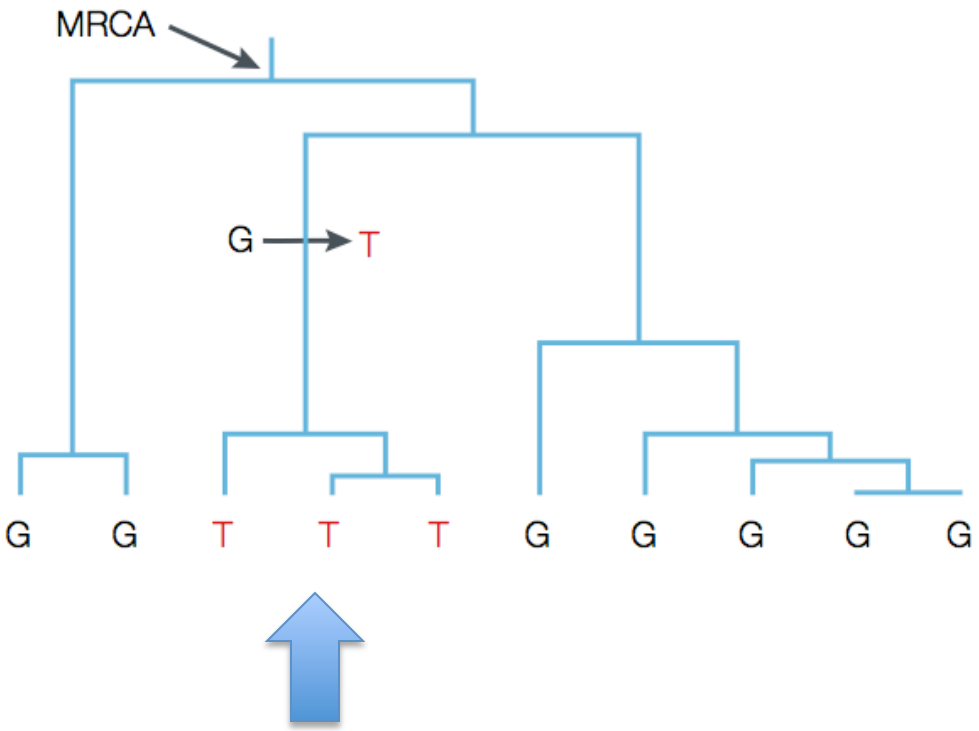
Disease and DNA Variation



Penetrance: P(D | G)

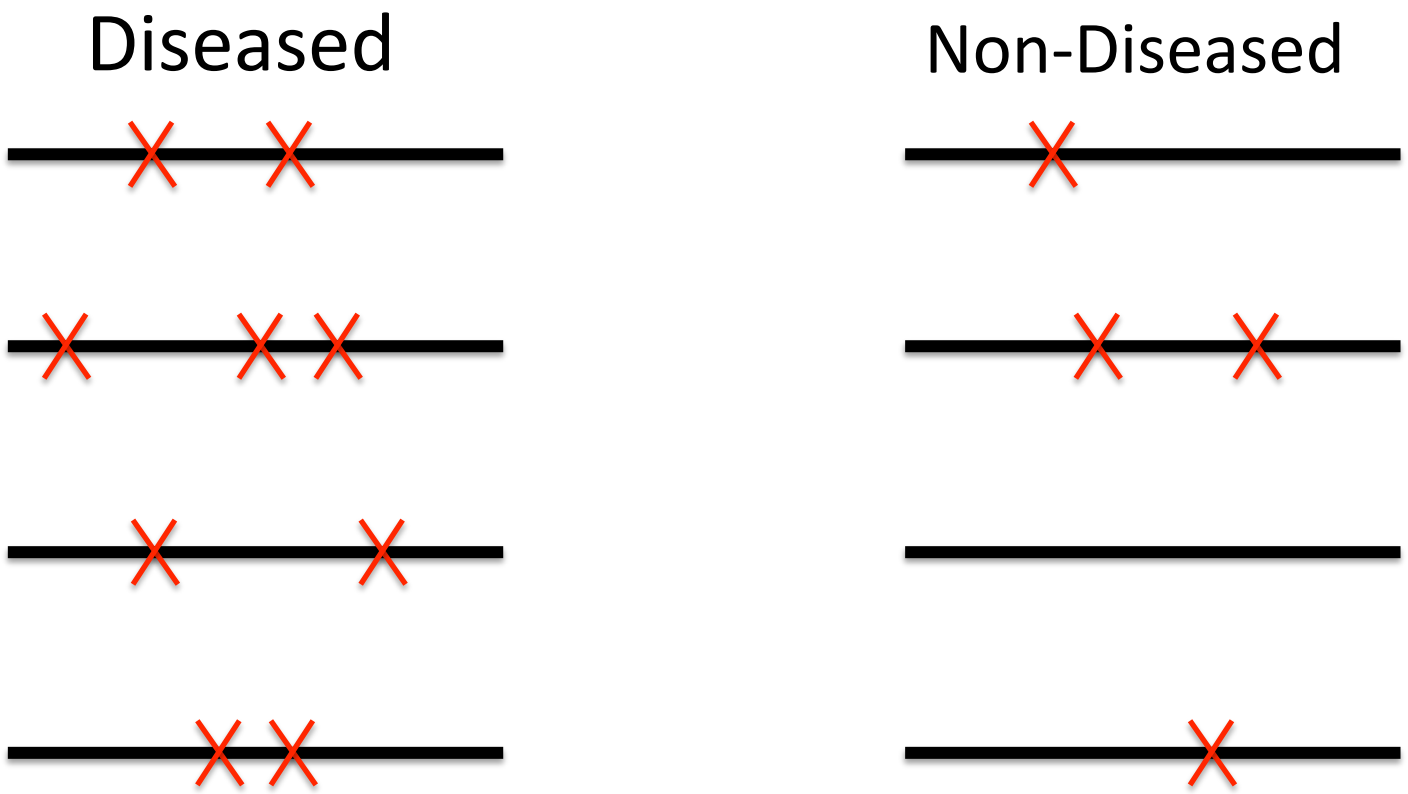
2012 Nature Reviews | Genetics

GWAS: Common Disease / Common Variant



Higher disease prevalence associated with T allele

Sequencing: Rare Variant Hypothesis

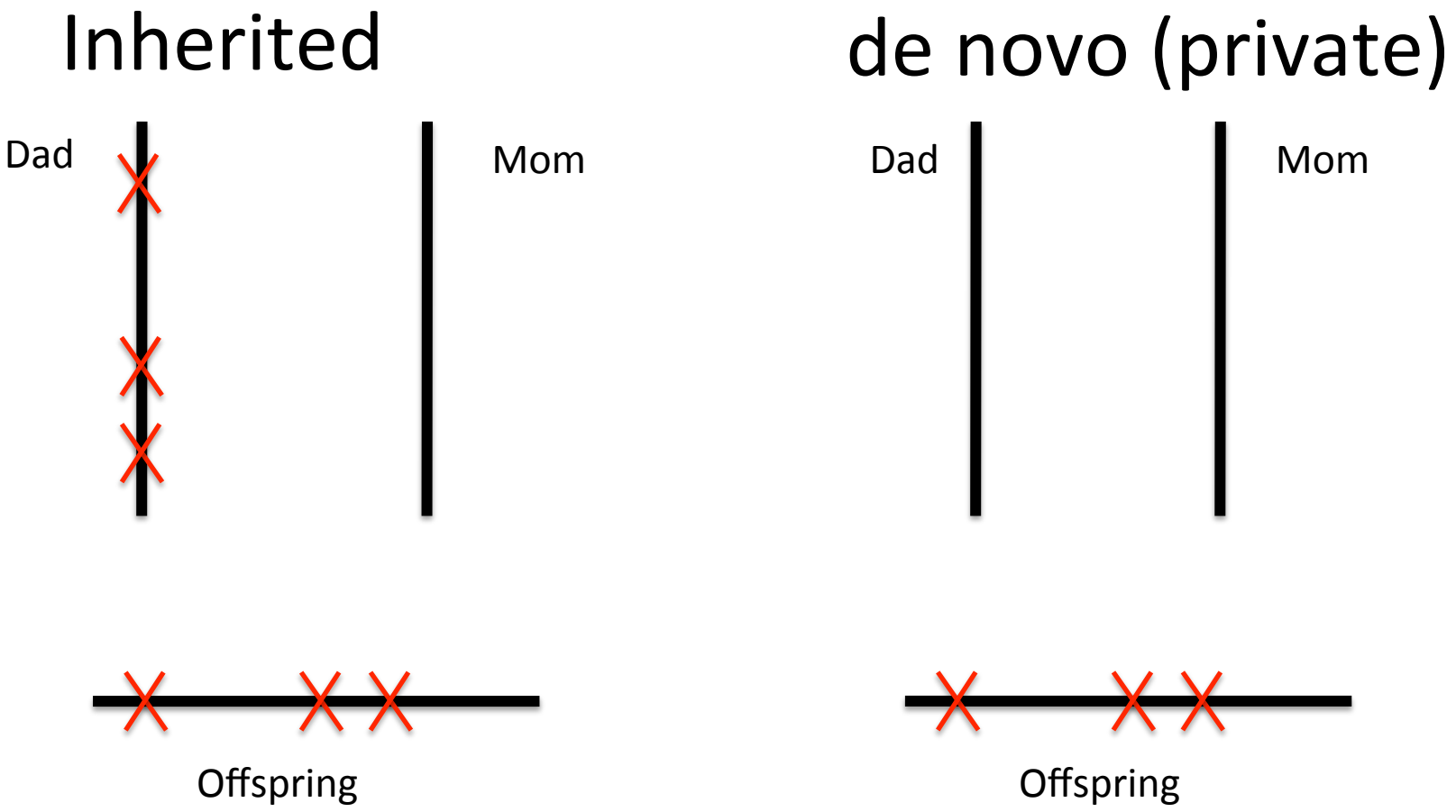


Inherited vs de novo mutation

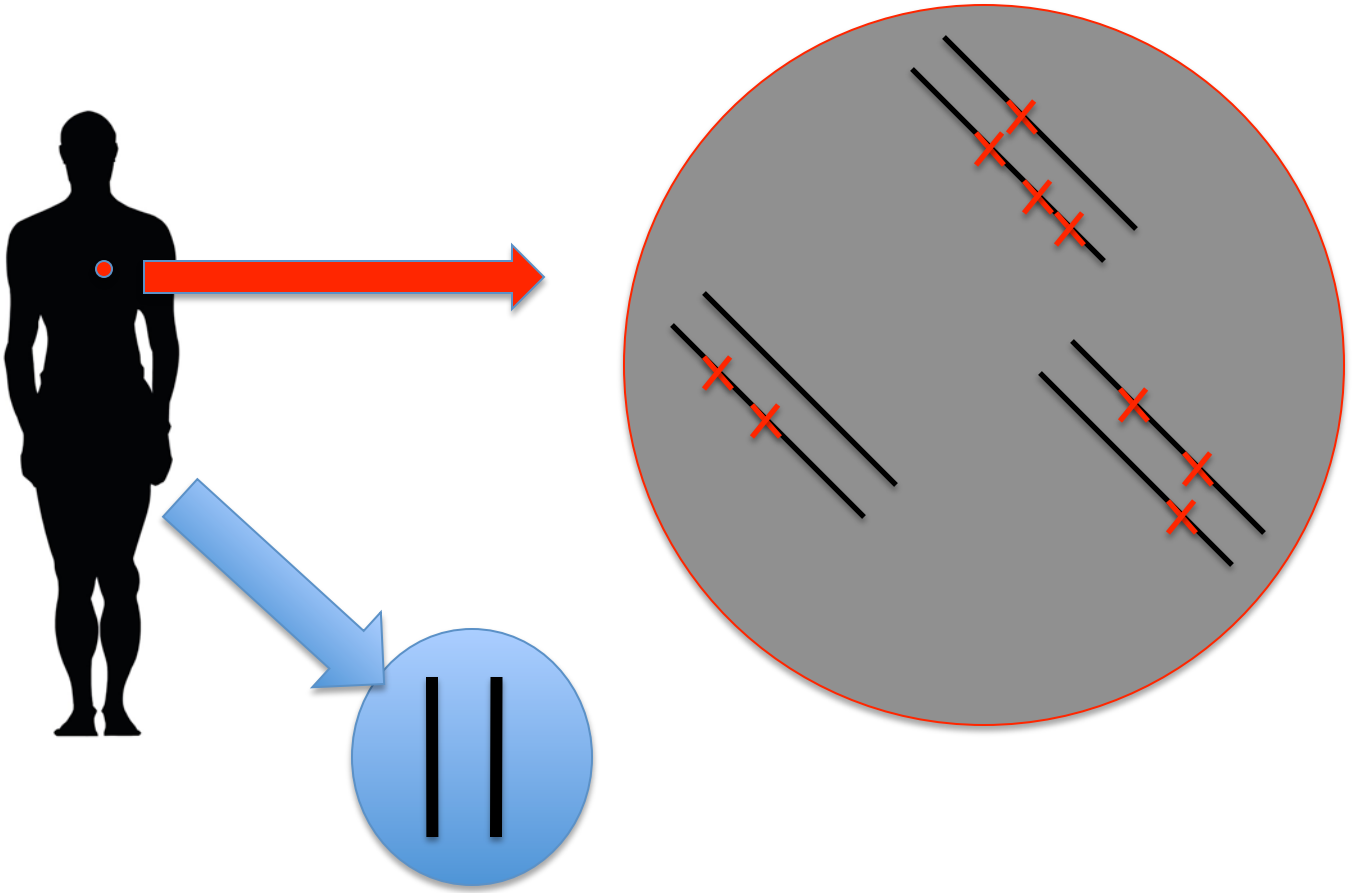
Offspring

Offspring

Inherited vs de novo mutation

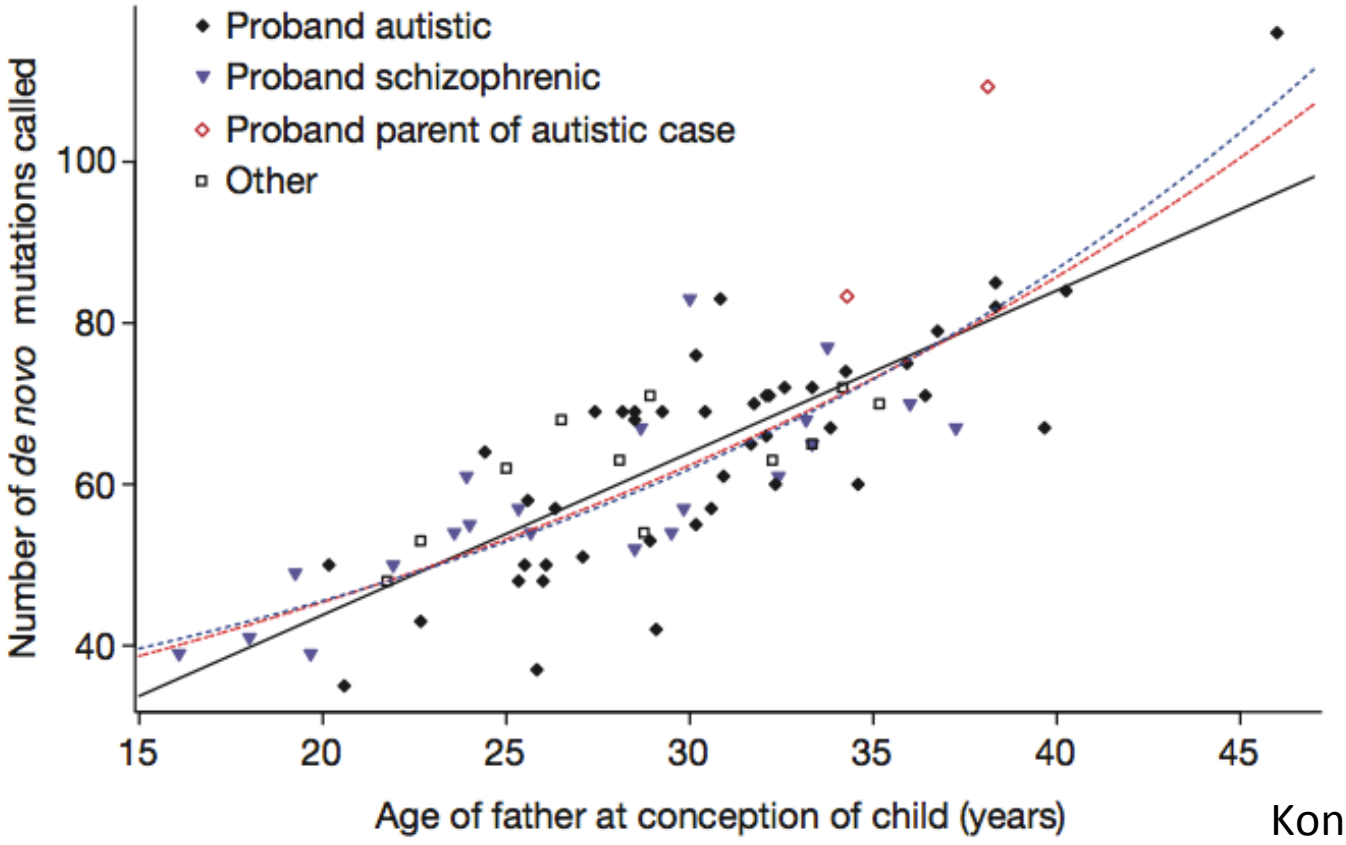


Tumor genomes



Gerlinger et al (2012) | NEJM

Paternal Age, Autism and Mutations



Kong et al., 2012

Disease characteristic vs prediction

- Mutations and genetic variation may be part of the disease process
- However, can we use our DNA to predict future disease?
  - Using “clones” (monozygotic twins) might help us answer the question…

Disease/Condition	Sex	Number of MZ Twin Pairs	Number MZ Disease Concordant Pairs	Number MZ Disease Discordant Pairs	Disease Prevalence in Cohort (*CR*)
Bladder Cancer	Male & Female	15668	5	189	0.6%
Breast Cancer	Female	8437	42	505	3.5%
Colorectal Cancer	Male & Female	15668	30	416	1.5%
Leukemia	Male & Female	15668	2	103	0.3%
Lung Cancer	Male & Female	15668	18	296	1.1%
Ovarian Cancer	Female	8437	3	125	0.8%
Pancreatic Cancer	Male & Female	15668	3	123	0.4%
Prostate Cancer	Male	7231	40	299	2.6%
Stomach Cancer	Male & Female	15668	11	223	0.8%
Thyroid Autoimmunity	Male & Female	284	7	17	5.5%
Type 1 Diabetes	Male & Female	4307	3	20	0.3%
Gallstone Disease	Male & Female	11073	112	956	5.3%
Type 2 Diabetes	Male & Female	4307	29	113	2.0%
Alzheimer's Disease	Male & Female	398	2	8	1.5%
Dementia	Male & Female	398	3	16	2.8%
Parkinson Disease	Male & Female	3477	7	60	1.1%
Chronic Fatigue	Female	1803	133	526	22.0%
Chronic Fatigue	Male	1426	48	266	12.7%
Gastro Esophageal Reflux Disorder (GERD)	Female	1260	63	284	16.3%
Gastro Esophageal Reflux Disorder (GERD)	Male	918	32	185	13.6%
Irritable Bowel Syndrome	Male & Female	1252	14	97	5.0%
Coronary heart disease (CHD) Death	Female	2004	97	424	15.4%
Coronary heart disease (CHD) Death	Male	1640	153	451	23.1%
Stroke-related Death	Male & Female	3852	35	316	5.0%
General Dystocia	Female	928	40	173	13.6%
Pelvic Organ Prolapse	Female	3376	34	157	3.3%
Stress Urinary Incontinence	Female	3376	13	87	1.7%

MZ: Monozygotic. Disease prevalence in cohort (cohort risk, *CR*) was determined as described in the Materials and Methods.

Roberts et al., 2012

NGS Analytic Considerations

- Common variation
  - GWAS pipeline applies
- Rare variation
  - Might require new methods/thinking

Analysis of rare variants

- Effectively count data
  - Number of mutations/variants
- Accumulation of rare variants
  - Genome-wide
  - Genic region
  - Pathway/system

Analysis of rare variants

- Counts follow a Poisson distribution
  - "rate" of mutational load
- Weight variants
  - Prior biological information
  - Up-weight specific variants

Hecht *et al. BMC Genomics* 2015, **16**(Suppl 8):S1
http://www.biomedcentral.com/1471-2164/16/S8/S1

BMC Genomics

RESEARCH Open Access

Better prediction of functional effects for sequence variants

Maximilian Hecht[1*], Yana Bromberg[2,3,4], Burkhard Rost[1,4]

From VarI-SIG 2014: Identification and annotation of genetic variants in and disease
Boston, MA, USA. 12 July 2014

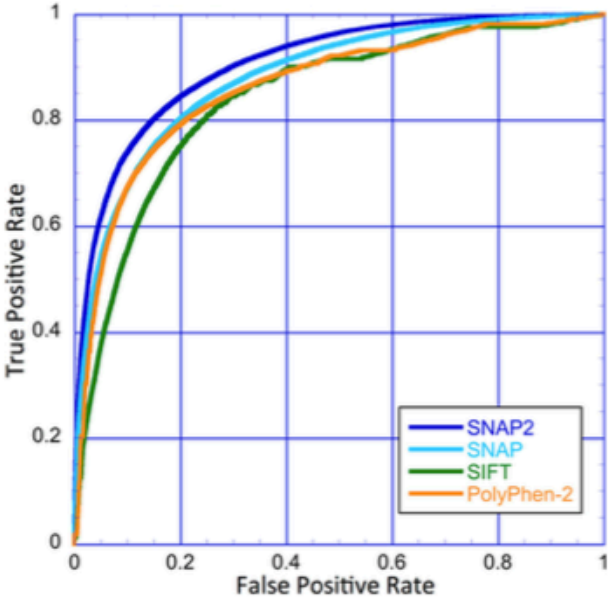


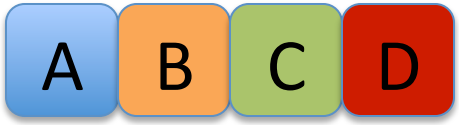
Figure 1 SNAP2 performs best for the ALL data set. This figure shows performance estimates for the *ALL* data set. Our new method SNAP2 (dark blue, AUC = 0.905) outperforms its predecessor SNAP (light blue, AUC = 0.880), PolyPhen-2 (orange, AUC = 0.853) and SIFT (green, AUC = 0.838) over the entire spectrum of the Receiver Operating Characteristic (ROC) curve. Curves are significantly different from each other at a significance level of P < 10-4 as measured by the DeLong method [59]. All SNAP2 results were computed on the test sets not used in training after a rigorous split into training, cross-training and testing. Results for PolyPhen-2 and our original SNAP included some of those proteins in their training, suggesting over-estimated performance.

Watch this space

- Methods are changing fast

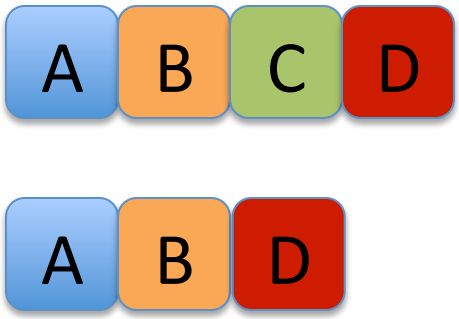
Copy Number Variation

CNV



Reference

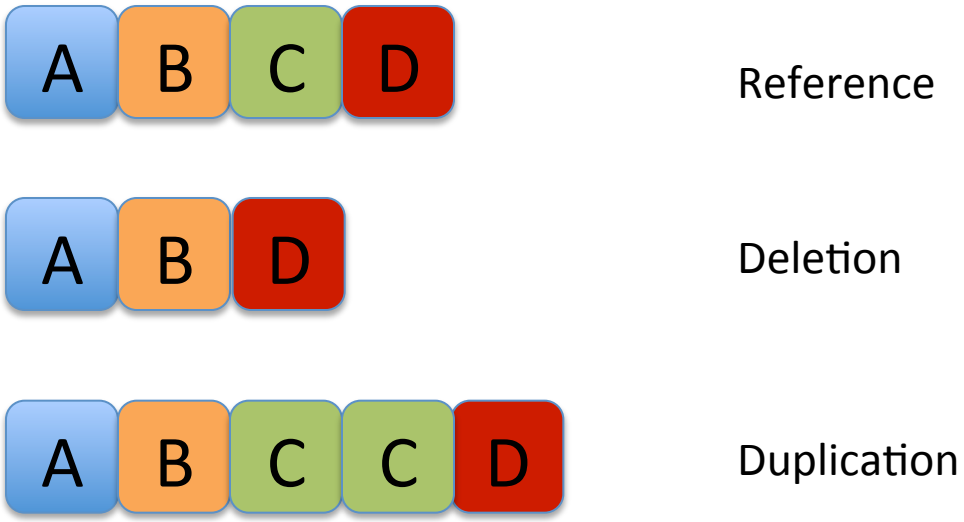
CNV



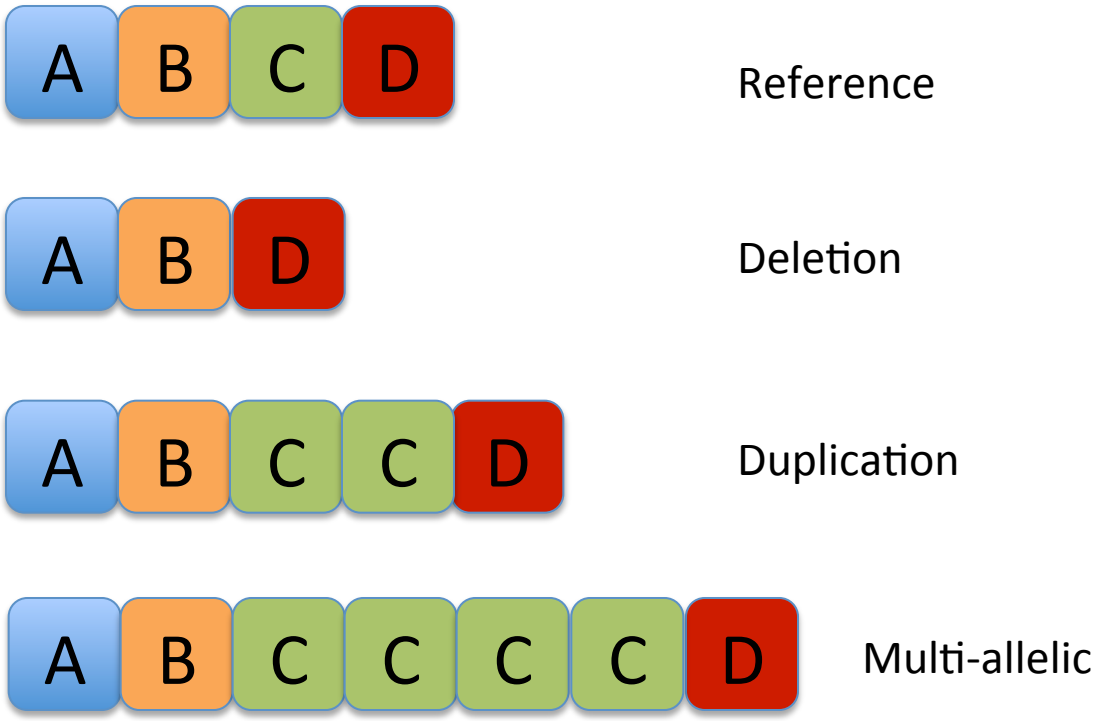
Reference

Deletion

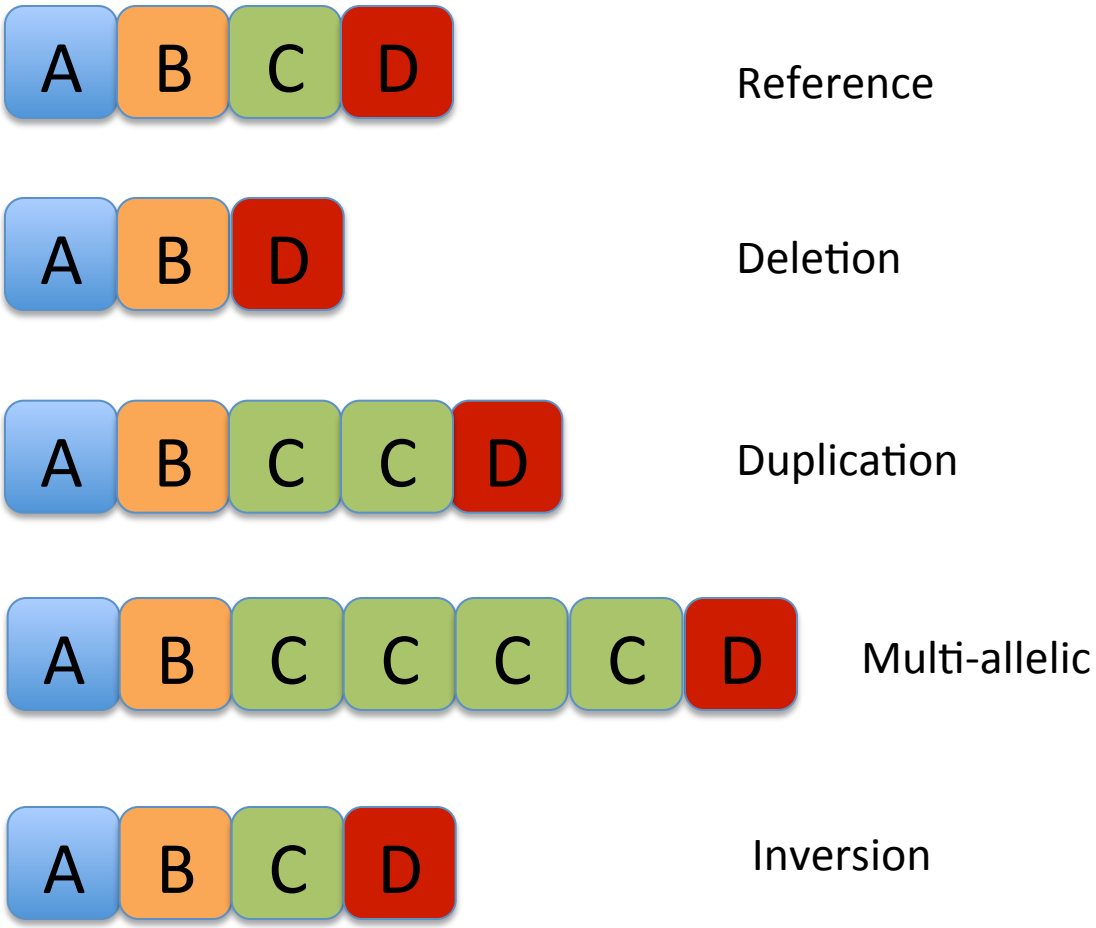
CNV



CNV


A B C D
Reference
A B D
Deletion
A B C C D
Duplication
A B C C C C D
Multi-allelic

CNV


A B C D
Reference
A B D
Deletion
A B C C D
Duplication
A B C C C C D
Multi-allelic
A B C D
Inversion

How do we measure CNVs?

- GWAS platforms
- RT PCR and dPCR methods
- Next Gen Sequencing

GWAS Platform

- PennCNV is a common tool designed to harness Illumina and Affy data
  - Reliable and well-documented

CNV Analysis

Analysis of copy number variations at 15 schizophrenia-associated loci

Elliott Rees, James T. R. Walters, Lyudmila Georgieva, Anthony R. Isles, Kimberly D. Chambert, Alexander L. Richards, Gerwyn Mahoney-Davies, Sophie E. Legge, Jennifer L. Moran, Steven A. McCarroll, Michael C. O'Donovan, Michael J. Owen and George Kirov

CNV Analysis

Table 1 Findings from our data-set for previously implicated copy number variation (CNV) loci in schizophrenia[a]

Locus	Position in Mb	Case group ($n = 6882$)		Control group ($n = 6316$)		OR (95% CI)	P
		CNVs, n	Frequency, %	CNVs, n	Frequency, %		
1q21.1 del	chr1:146,57-147,39	12	0.17	1	0.016	11.03 (1.43–84.86)	**0.0027**
1q21.1 dup	chr1:146,57-147,39	8	0.12	5	0.079	1.47 (0.48–4.49)	0.35
NRXN1 del	chr2:50,15-51,26	11	0.16	0	0.00	NA (1.25–∞)	**7.7×10^{-4}**
3q29 del	chr3:195,73-197,34	4	0.058	0	0.00	NA (0.44–∞)	0.074
WBS dup	chr7:72,74-74,14	3	0.044	1	0.016	2.75 (0.29–26.48)	0.35
VIPR2 dup	chr7:158,82-158,94	1	0.015	6	0.095	0.15 (0.02–1.27)	0.99
15q11.2 del	chr15:22,80-23,09	44	0.64	26	0.41	1.56 (0.96–2.53)	**0.046**
AS/PWS dup	chr15:24,82-28,43	8	0.12	0	0.00	NA (0.90–∞)	**0.0055**
15q13.3 del	chr15:31,13-32,48	4	0.058	2	0.032	1.84 (0.34–10.03)	0.38
16p13.11 dup	chr16:15,51-16,30	24	0.35	12	0.19	1.84 (0.92–3.68)	0.056
16p11.2 distal del	chr16:28,82-29,05	0	0.00	2	0.032	NA (0–3.82)	1
16p11.2 dup	chr16:29,64-30,20	27	0.39	0	0.00	NA (3.09–∞)	**2.3×10^{-8}**
17p12 del	chr17:14,16-15,43	4	0.058	3	0.047	1.22 (0.27–5.47)	0.55
17q12 del	chr17:34,81-36,20	1	0.015	0	0.00	NA (0.11–∞)	0.52
22q11.2 del	chr22:19,02-20,26	20	0.29	0	0.00	NA (2.28–∞)	**2.2×10^{-6}**
Totals		171	2.48	58	0.92		**1.4×10^{-12}**

del, deletion; dup, duplications, NA, not applicable; WBS, Williams–Beuren syndrome; AS/PWS, Angelman/Prader–Willi syndrome.
a. Copy number variation positions are in UCSC Build 37. Significant results are in bold (using Fisher exact test, 1-tailed).

Meta-Analysis

Aggregating the evidence

- Often, we are interested in combining evidence across independent studies
- There are a variety of ways to do this

Differing approaches…

- Mega-Analysis
- Combining Significance
- Meta-Analysis
- Weighted Hypothesis Testing

Mega-Analysis

- Combine two or more samples
- Requires access to raw data
- Many consortia utilize this approach

Mega-Analysis

- Strengths
  - Unprecented statistical power
- Weaknesses
  - Combining across heterogeneous samples
  - Ignore variation between studies

Combining significance

- Rather than combine raw data, you combine test statistics and/or p-values
- Simplest approach
  - Fisher's Method

$$X_{2k}^2 \sim -2\sum_{i=1}^{k} \ln(p_i)$$

Fisher's Method

- Strengths
  - Simple approach
  - Does not require raw data
- Weaknesses
  - Assumptions
    - Independent tests
    - Uniform distribution of p-values
  - Lack of effect size (only p-values)

Meta-Analysis

- Combining effect size estimates across studies
  - Odds ratios, risk ratios, etc.
- Important distinction
  - Random vs Fixed Effects

Fixed vs Random Effects

- Fixed Effects Meta-Analysis
  - Ignores between-study variance
- Random Effects Meta-Analysis
  - Incorporates between-study variance
  - More conservative (wider confidence intervals)

Conducting a meta-analysis

- Requirements
  - Proper extensive literature search
  - Parameter estimate (i.e. odds ratio)
  - Standard error
- Various tools to conduct a meta-analysis
  - R packages
    - Metafor is a good option
    - Provides graphics

Examples

- See alzgene.org