Lecture 8: Introduction to Rare Variant Analysis and Collapsing Tests

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Lecture Overview

1. Limitations of GWAS
2. Sequencing and Rare Variants
3. Rationale for Rare Variant Analysis
4. Challenges
5. Collapsing/Burden Tests
6. Supervised Collapsing/Burden Tests

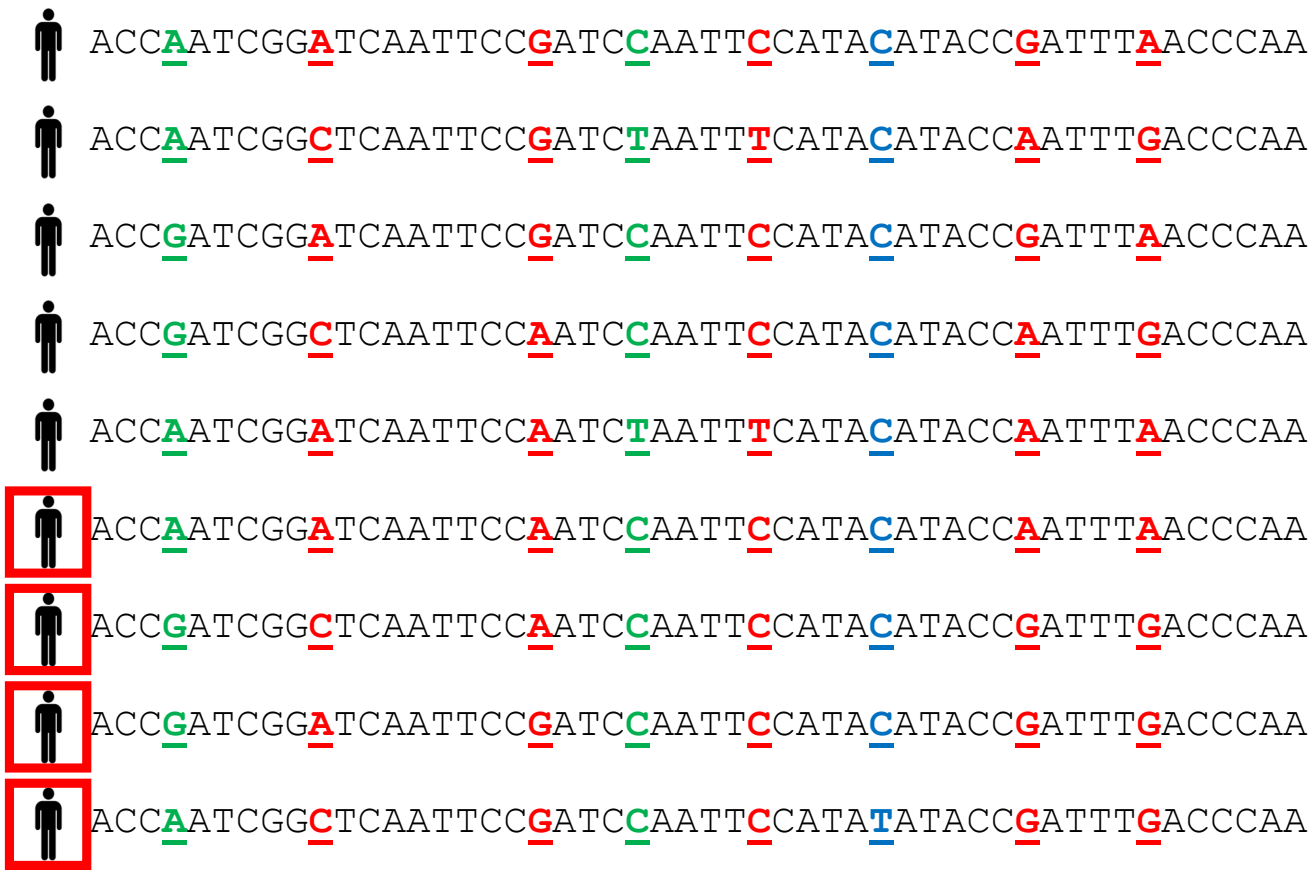
GWAS: Missing Heritability

- GWAS focus on common variants (MAF $\geq 5\%$) whose effects are small with RR$\approx$1.2-1.5.
- Missing heritability: Significant GWAS SNPs explain a small proportion of disease heritability.
- Possible reasons:
  - GxG and GxE interactions?
  - Many common causal variants: Each with a small effect?
  - Epigenetics?
  - Rare variants?

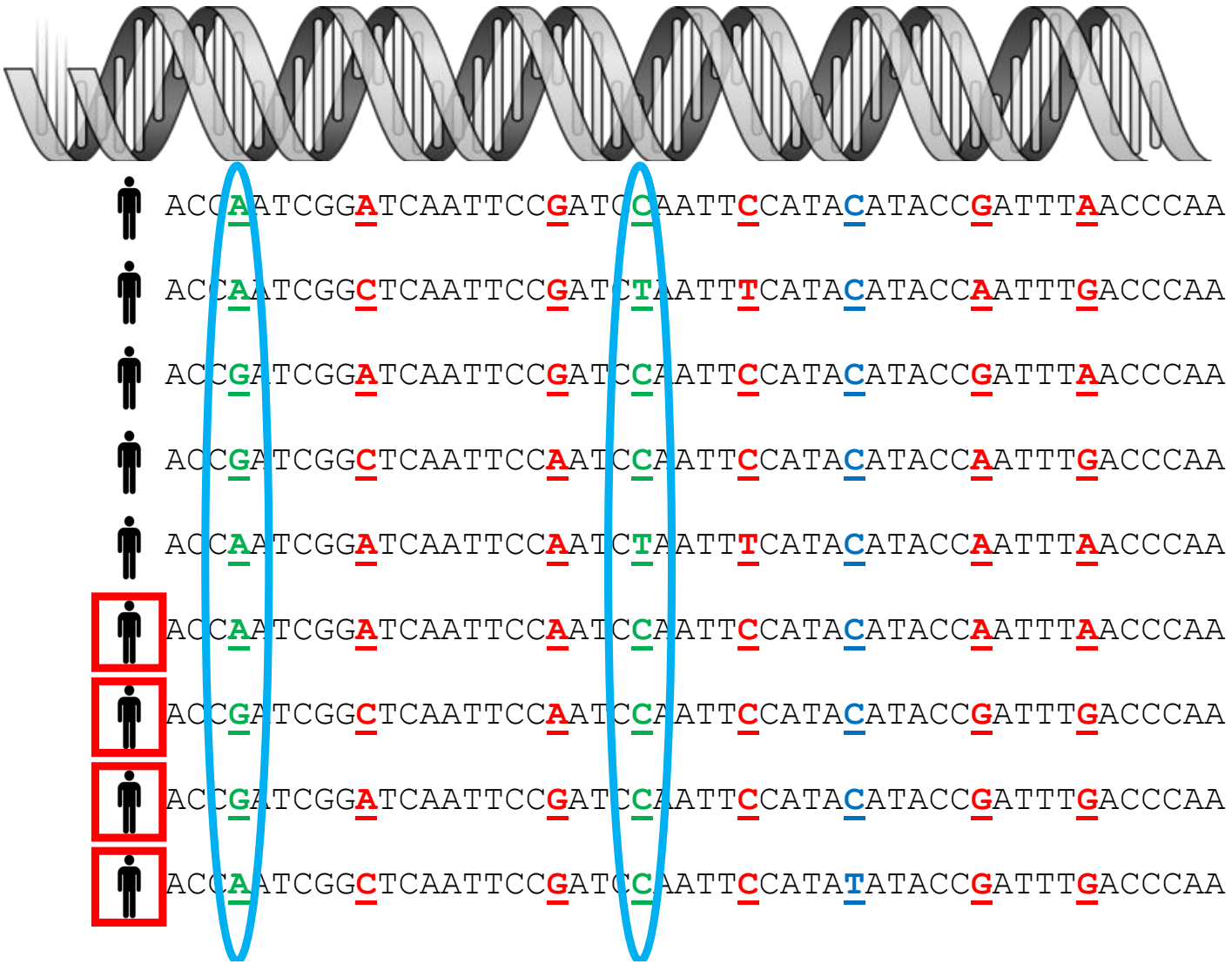
Next Generation Sequencing (NGS)

- Genotype all basepairs (bps) in a gene, the whole exome, or the whole genome (3 billion bps).
- Allow to identify all SNPs or other types of variants. No need to rely on LD to tag untyped causal SNPs.

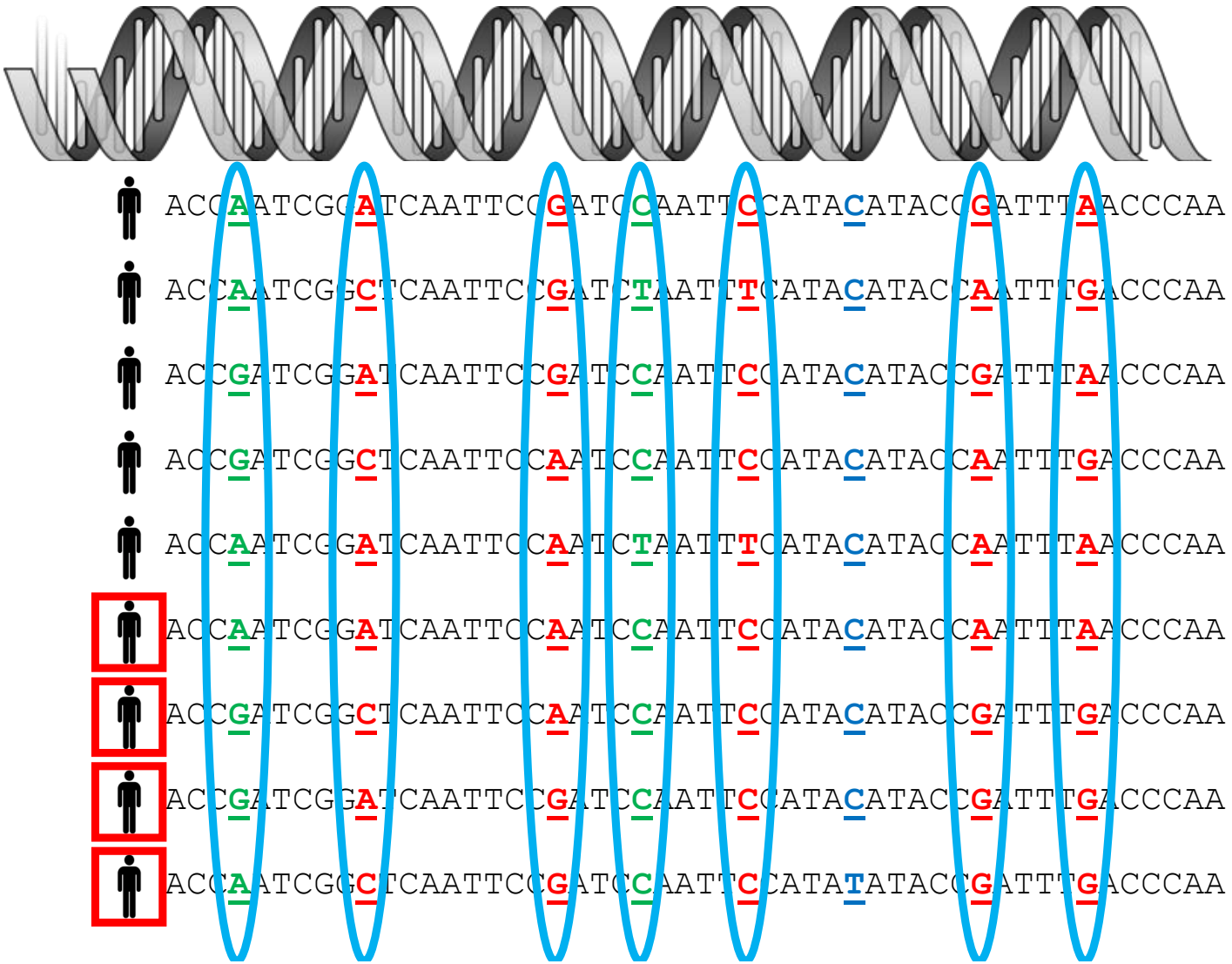
Genetic Association Studies



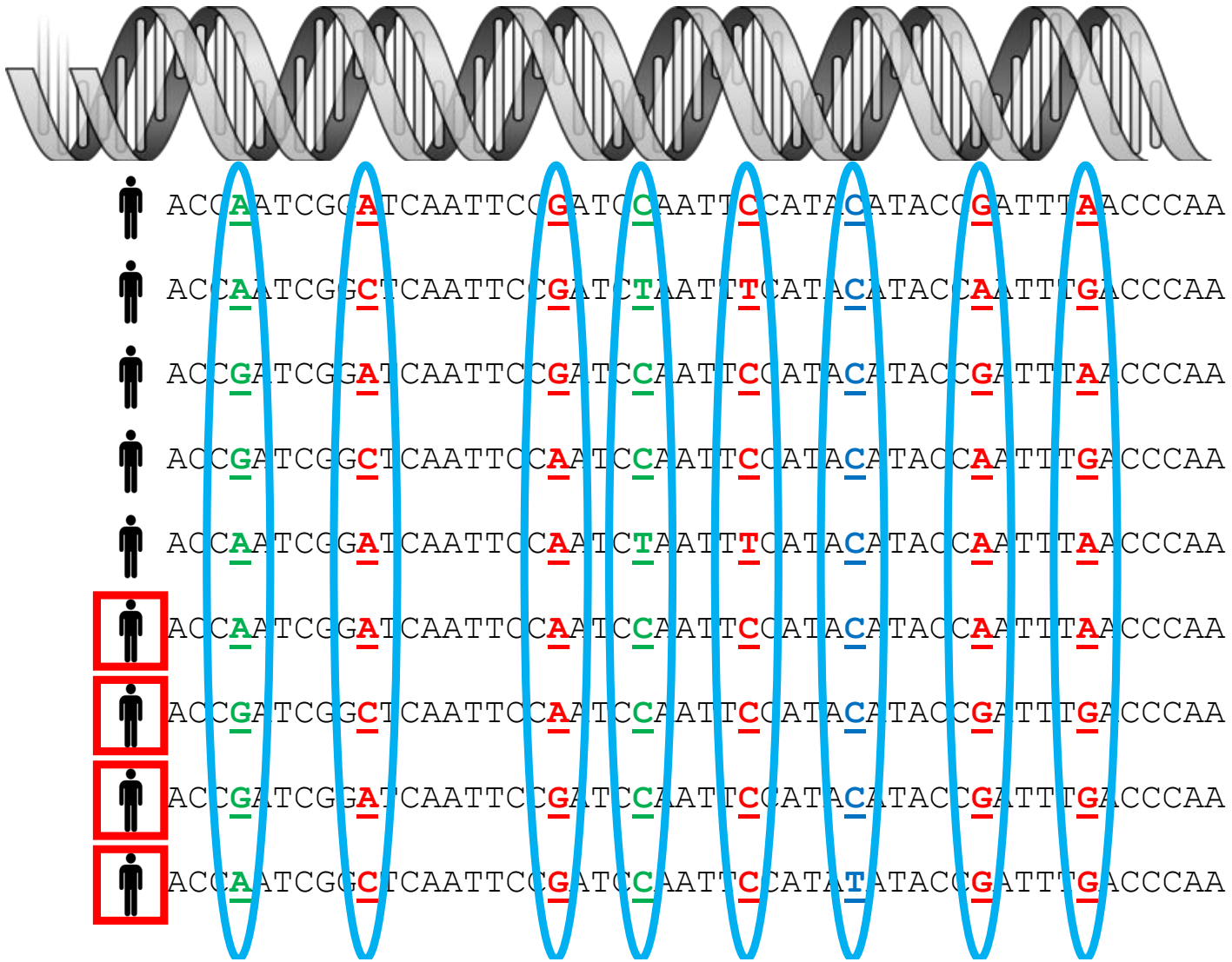
GWAS: A few years ago



GWAS: current (+ imputation)



Sequencing


ACCAATCGGATCAATTCCGATCCAATTCCATACATACCGATTTAACCCAA
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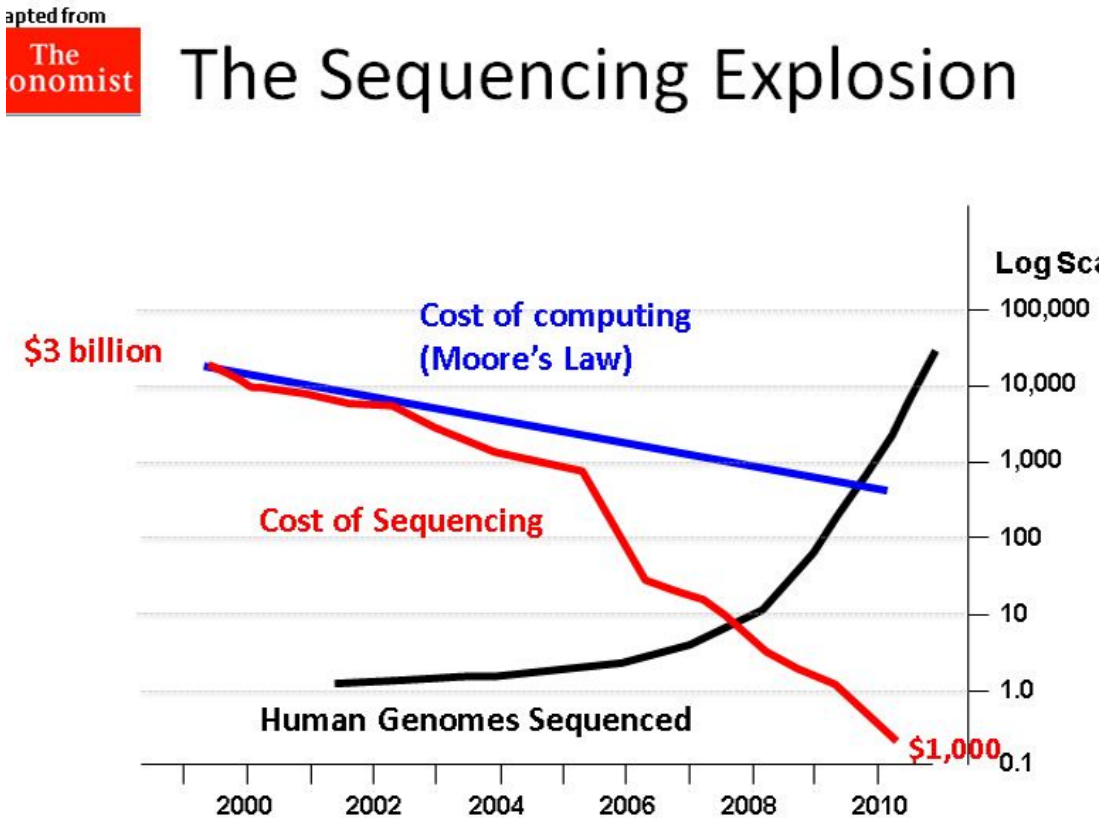
ACCAATCGGATCAATTCCAATCTAATTTCATACATACCAATTTAACCCAA
ACCAATCGGATCAATTCCAATCCAATTCCATACATACCAATTTAACCCAA
ACCGATCGGCTCAATTCCAATCCAATTCCATACATACCGATTTGACCCAA
ACCGATCGGATCAATTCCGATCCAATTCCATACATACCGATTTGACCCAA
ACCAATCGGCTCAATTCCGATCCAATTCCATATATACCGATTTGACCCAA

Sequencing Cost Has Dropped Dramatically



Massively parallel sequencing

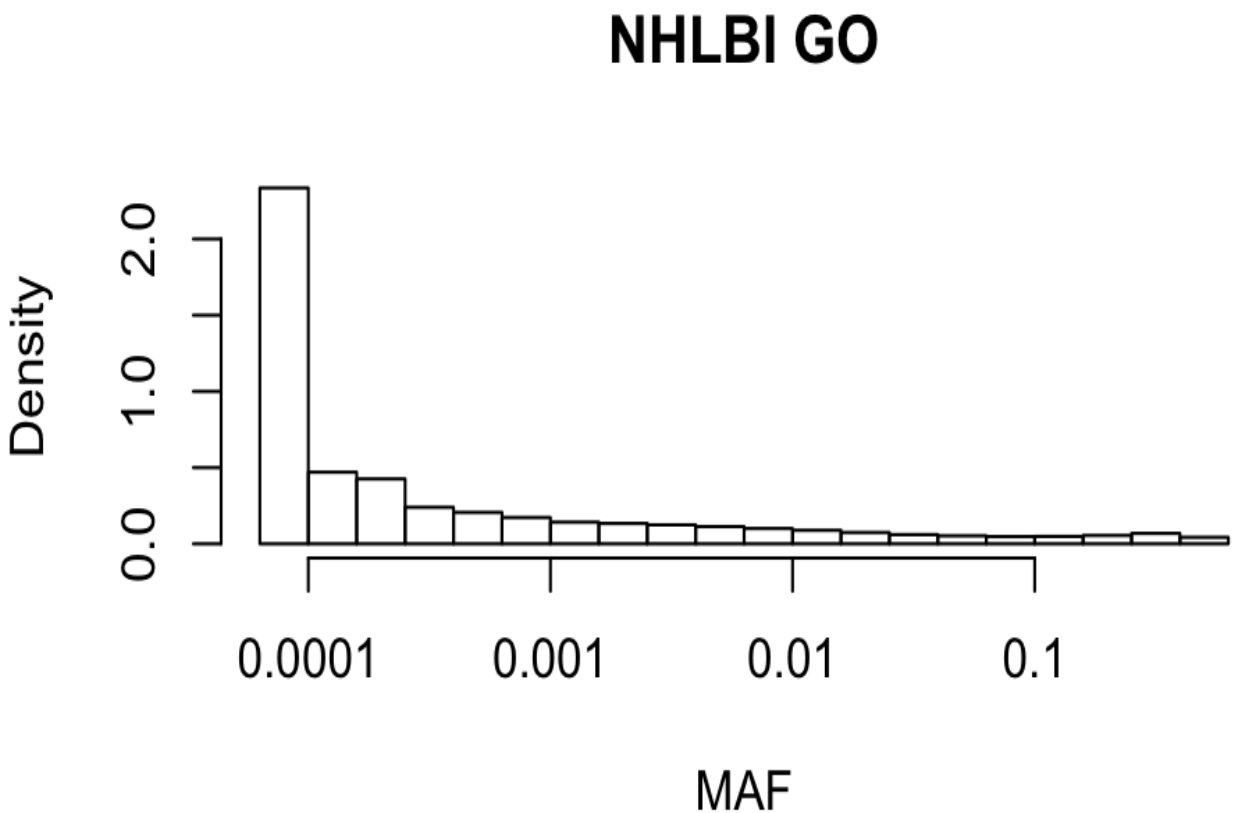
- Illumina can achieve $\$1,000$ per whole genome.

Common vs Rare variants

- Common Variants (Common SNPs):
  - MAF $> 0.01 \sim 0.05$.
  - Often high correlation with adjacent SNPs (Strong Linkage Disequilibrium(LD)).
- Rare Variants (Rare SNPs):
  - MAF $\leq 0.01 \sim 0.05$.
  - Relatively new mutations.
  - Often weak correlation with other SNPs.

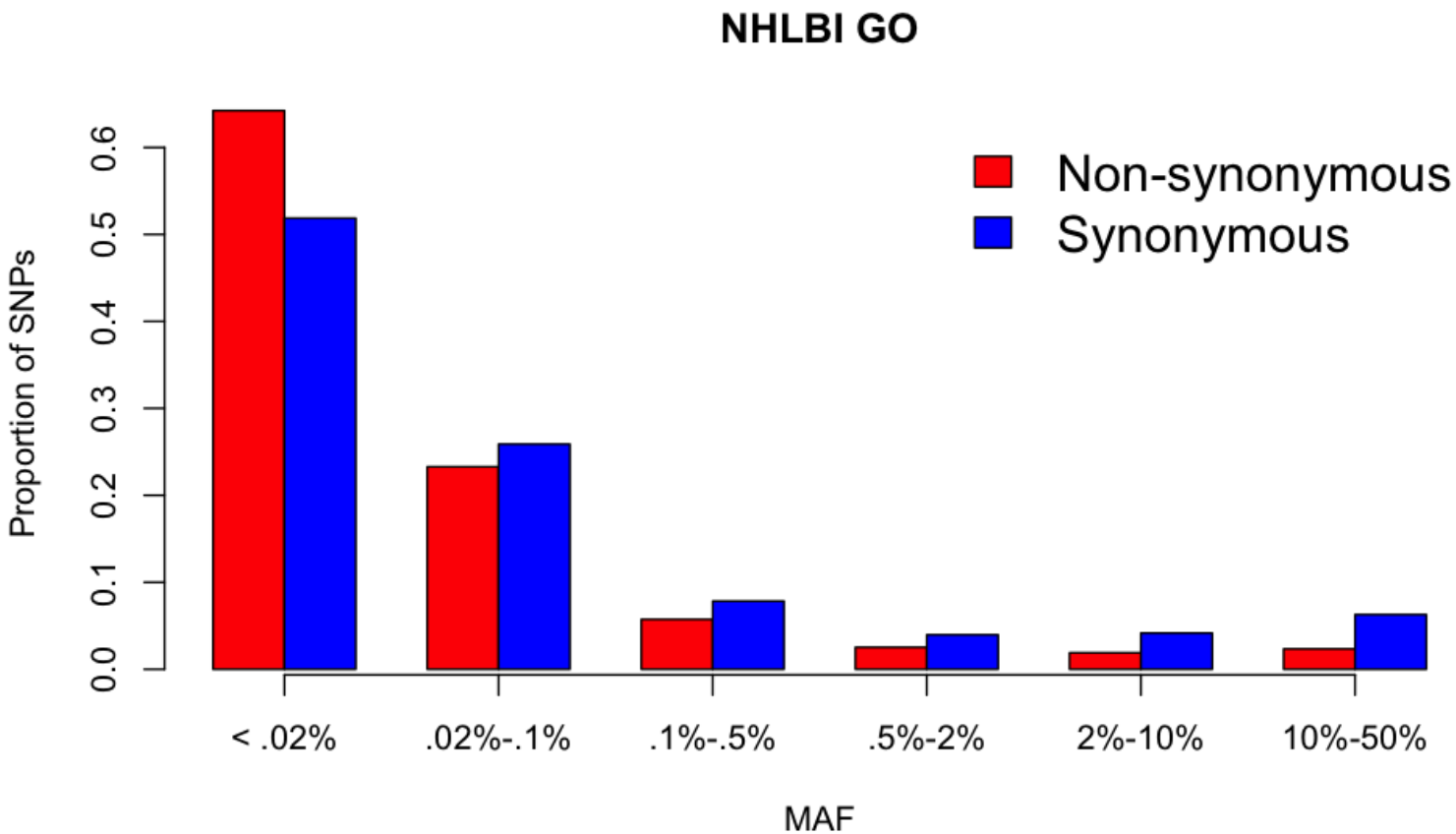
Why rare variants?

- Most of human variants are rare



Why rare variants?

- Functional variants tend to be rare.



Challenges in Association Studies for Rare Variants

- Compared to common variant studies, individual SNP analysis in rare variant studies is seriously underpowered.

How many subjects are needed to observed a rare variant?

- Sample size required to observe a variant with MAF$=p$ with at least θ chance

$$n > \frac{ln(1-\theta)}{2ln(1-p)}$$

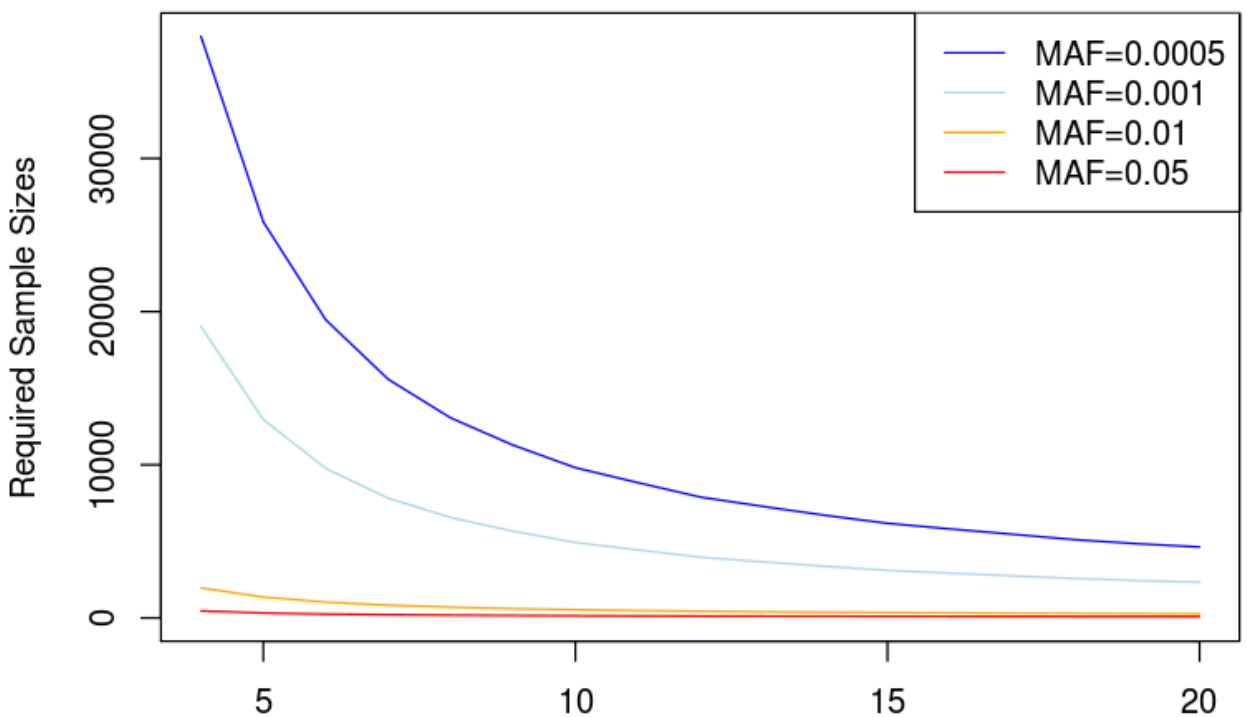
- For $\theta = 99.9\%$, the required minimum sample size is

MAF	0.1	0.01	0.001	0.0001
Minimum n	33	344	3453	34537

- Large samples are required to observe rare variants.

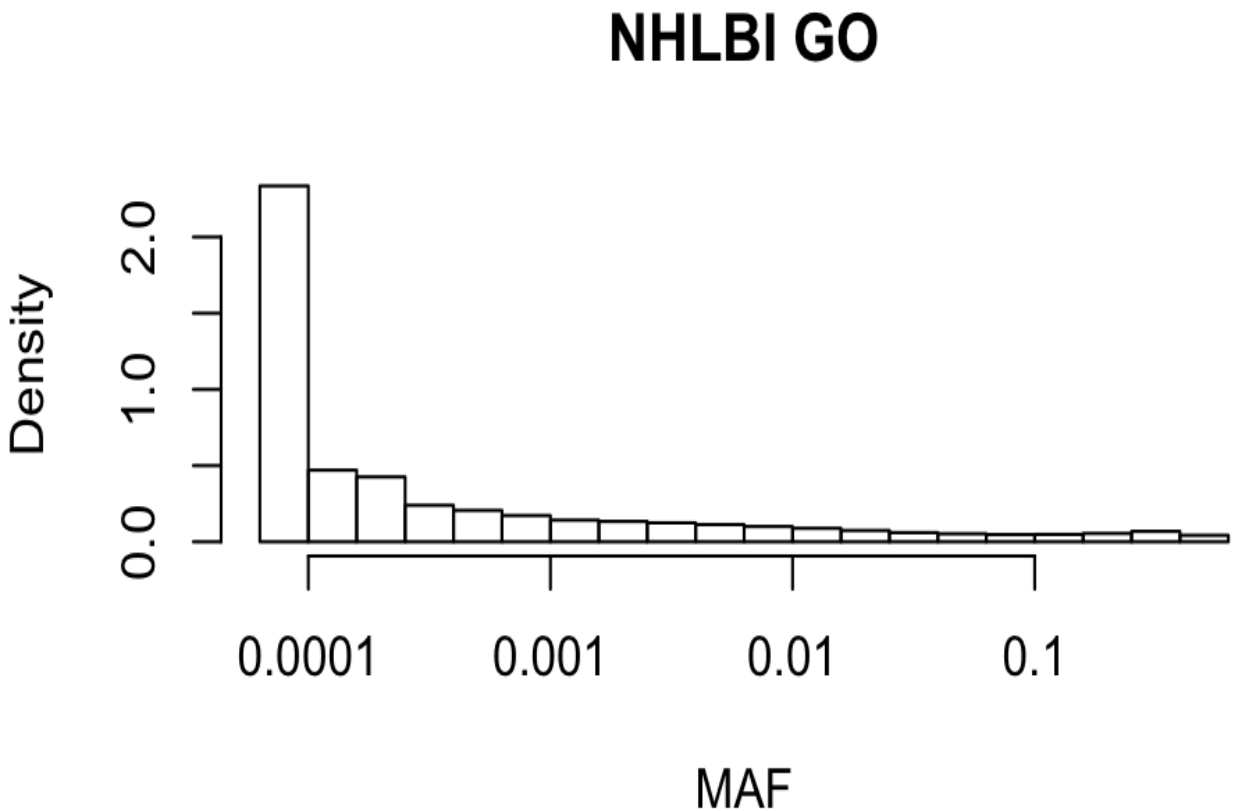
How many subjects are needed to achieve 80% of power ($\alpha = 10^{-6}$) by single variant test?

- Single variant test is not powerful to identify rare variant effects.



Multiple Testing:

- A lot more rare variants than common variants → larger multiple testing burden



Challenges in Association Studies for Rare Variants

- Individual rare variant tests are underpowered
- Need cost-effective study designs to genotype a large number of individuals
- Need powerful statistical methods and strategies to test for associations
  - Region based analysis: genes, moving windows, networks/pathways
  - Integrate with bioinformatics: Incorporate functional information

Region Based Analysis of Rare Variants

- Single variant test is not powerful to identify rare variant associations
- Gene (or Region) based tests
- Strategy:
  - Identify all observed variants within a sequenced (sub)-region.
  - Regions: gene, regulatory region, ...
  - Test the joint effect of rare/common variants while adjusting for covariates.

Regression Models

- p variants in a certain region.
- SNPs in a region $\mathbf{G}_i = (g_{i1}, g_{i2}, \ldots, g_{ip})'$, $(g_{ij} = 0, 1, 2)$
- Covariates $\mathbf{X}_i$: age, gender, PC scores (for population stratification).
- Continuous/binary traits:

$$\mu_i / logit(\mu_i) = \alpha_0 + \mathbf{X}_i'\boldsymbol{\alpha} + \mathbf{G}_i'\boldsymbol{\beta}$$

- Test of no genetic region effect:

$$H_0 : \boldsymbol{\beta} = (\beta_1, \ldots, \beta_p) = 0$$

Major Classes of Tests

- Burden/Collapsing tests
- Supervised/Adaptive Burden/Collapsing tests
- Variance component (similarity) based tests
- Omnibus tests: hedge against difference scenarios

Note: "Burden" tests sometimes refers to collapsing tests or to any region based test — inconsistent notation.

Burden Tests

- Aggregate rare variant information in a region into a summary dose variable
  - Binary Collapsing: CAST
  - CMC
  - Count Collapsing: MZ (GRANVIL)
  - Weighted Sum Test
- Most powerful if all rare variants are causal variants with the same effect sizes (and association directions).

Burden Tests- Principle

- If p is large, multivariate test $\boldsymbol{\beta} = 0$ is not powerful.
- Collapsing: Suppose $\beta_1 = \cdots = \beta_{\boldsymbol{p}} = \beta$

$$\mu_i / logit(\mu_i) = \alpha_0 + \mathbf{X}_i^T \alpha + C_i \beta$$

- $C_i = g_{i1} + \cdots + g_{ip}$: genetic burden/score
- Test $H_0 : \beta = 0$ (df=1)

Burden Tests

- Collapse rare variants

Y	G_1	G_2	G_3	G_4
1	1	0	0	0
1	0	1	0	0
1	0	0	1	1
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
0	0	0	0	0
0	0	0	0	0
0	0	0	0	0

Burden Tests

- Collapse rare variants

Y	G_1	G_2	G_3	G_4		C
1	1	0	0	0		1
1	0	1	0	0		1
1	0	0	1	1	→	2
.	.	.	.	.		.
.	.	.	.	.		.
.	.	.	.	.		.
0	0	0	0	0		0
0	0	0	0	0		0
0	0	0	0	0		0

Burden Tests

- Many different types of tests exist based on different C_i
- Existence of any rare variants can cause loss of function of a region (ex. CAST)

$$C_i = \begin{cases} 1 & \text{if} \quad \sum_{j=1}^{p} g_{ij} > 0 \\ 0 & \text{if} \quad \sum_{j=1}^{p} g_{ij} = 0 \end{cases}$$

- Dominant genetic model (ex. MZ-test)

$$C_i = \sum_{j=1}^{p} I(g_{ij} > 0)$$

Weighted Burden

- Assume that rarer variants have larger effects
- Suppose $\beta_j = w(MAF_j)\beta$.
  - Ex: $w(MAF_j) = 1/\sqrt{MAF_j(1 - MAF_j)}$ (Madsen and Browning).
- $C_i = w_1 g_{i1} + \cdots + w_p g_{ip}$
  - Weighted count of rare variants, where $w_j = w(MAF_j)$.

Burden test - CMC test

Li and Leal (2008) *AJHG*

- There exists many variations of burden tests.
- CMC test
  - Group variants based on their MAFs
  - Collapse each group using CAST approach
  - Conduct Hoteling's T-test

Burden tests - Original Weighted Sum

Madsen and Browning (2009) *Plos Genetics*

- Assume binary trait without covariates
- Control only MAFs and rank sum test
  - Weight: $w_j = 1/\sqrt{q_j^u(1-q_j^u)}$, where q_j^u is the estimated MAF from control samples.
  - Test statistic:

$$T_{wst} = \sum_{i \in case} rank(C_i), \quad C_i = \sum w_j g_{ij}$$

  - P-values from normal approximation:

$$Z = (T_{wst} - \widehat{\mu})/\widehat{\sigma}$$

Power of Burden Tests

- Power of burden tests depends on
  - Number of associated variants
  - Number of non-associated variants
  - Direction of the effects.
- Powerful if most variants are causal and have effects in the same direction.

Burden vs. Single Variant Test

	Single Variant Test	Combined Test
10 variants / all have risk 2 / All have frequency .005	.05	.86
10 variants / all have risk 2 / Unequal Frequencies	.20	.85
10 variants / average risk is 2, but varies / frequency .005	.11	.97

[Li and Leal (2008) AJHG]

- Power from simulated data
- Combining variants can greatly increase the power.

Burden vs. Single Variant Test

	Single Variant Test	Combined Test
10 disease associated variants	.05	.86
10 disease associated variants + 5 null variants	.04	.70
10 disease associated variants + 10 null variants	.03	.55
10 disease associated variants + 20 null variants	.03	.33

[Li and Leal (2008) AJHG]

- Null variants reduce the power.
- Existence of variants whose effects are in different directions can reduce power more substantially

Burden Test: Mixed effect directions

- Lose power if variants have positive and negative effects.

Y	G_1	G_2	G_3	G_4
1	1	0	0	0
1	0	1	0	0
1	0	0	0	0
.	.	.	.	.
.	.	.	.	.
.	.	.	.	.
0	0	0	0	0
0	0	0	1	0
0	0	0	0	1

Burden Test: Mixed effect directions

- Lose power if variants have positive and negative effects.

Y	G_1	G_2	G_3	G_4		C
1	1	0	0	0		1
1	0	1	0	0		1
1	0	0	0	0	→	0
.	.	.	.	.		.
.	.	.	.	.		.
.	.	.	.	.		.
0	0	0	0	0		0
0	0	0	1	0		1
0	0	0	0	1		1

Burden Test: Mixed effect directions

- Several methods have been developed to estimate association directions and incorporate them in the burden test framework.
  - Adaptive Sum Test
  - Estimated regression coefficient (EREC) test

Adaptive sum test

Han F and Pan W. (2010) *Hum Hered*

- Model:

$$C_i = \sum_{j=1}^{p} w_j g_{ij}$$

$$logit(Pr(Y = 1)) = \alpha_0 + C_i \beta$$

- Fit individual SNP models

$$logit(Pr(Y = 1)) = \alpha_0 + g_j \beta_j$$

  - Assign $w_j = -1$ if $\widehat{\beta}_j < 0$ and the p-value is small
  - $w_j = 1$ otherwise.

Adaptive sum test

- Compute p-values using permutation.
- Step-up procedure assign $w_j = 0$ if g_j is unlikely associated with the trait (Hoffmann *et al.*Plos One, 2010)

Estimated regression coefficient (EREC) test

Lin DY. and Tang Z. (2011) *AJHG*

- Estimate regression coefficient β and use it as a weight.

$$C_i = \sum_{j=1}^{p} w_j g_{ij}, \quad w_j = \widehat{\beta}_j$$

  - Motivation: True β_j is the optimal weight
  - Estimate $\widehat{\beta}_j$ by fitting individual SNP regression models
  - Use $w_j = \widehat{\beta}_j + \delta$ when the sample size is small (n$<$ 2000)

Estimated regression coefficient (EREC) test

- Calculate

$$C_i = \sum_{j=1}^{p} w_j g_{ij}, \quad w_j = \widehat{\beta}_j$$

- Test statistic:

$$T_{EREC} = \sum_{i=1}^{n} C_i (y_i - \widehat{\mu}_{0,i}).$$

  - Use score test statistics
  - P-values from the parametric bootstrap.

Estimated regression coefficient (EREC) test

- Cons:
  - Individual SNP regression models are difficult to fit for very rare variants.
  - The constant δ is arbitrary.

Adaptive burden test

- Adaptive burden tests have robust power.
- Compute p-values through permutation or bootstrap
  - Computationally intensive