# Lecture 7: 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 (First Major Category of Test)

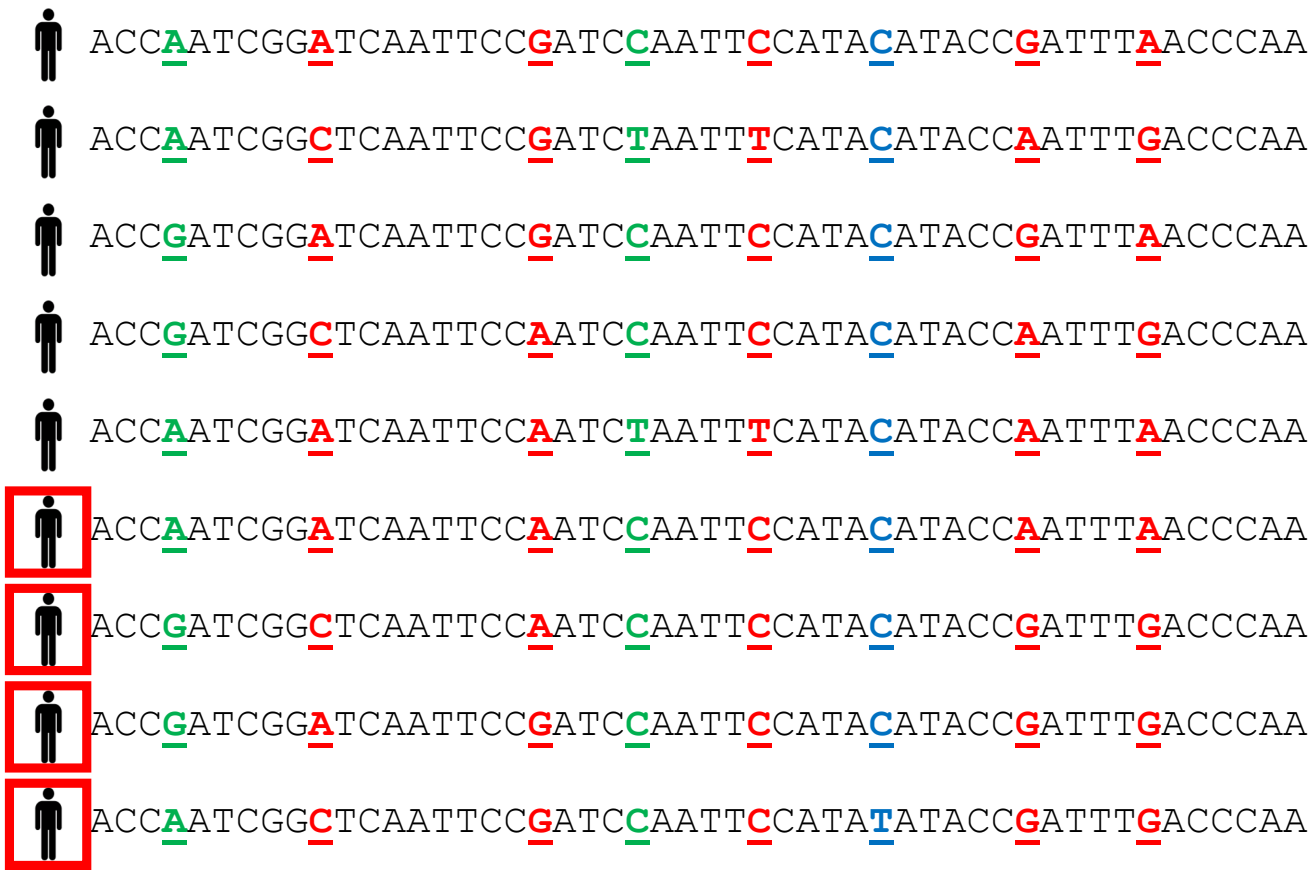
# GWAS: Missing Heritability

- 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?

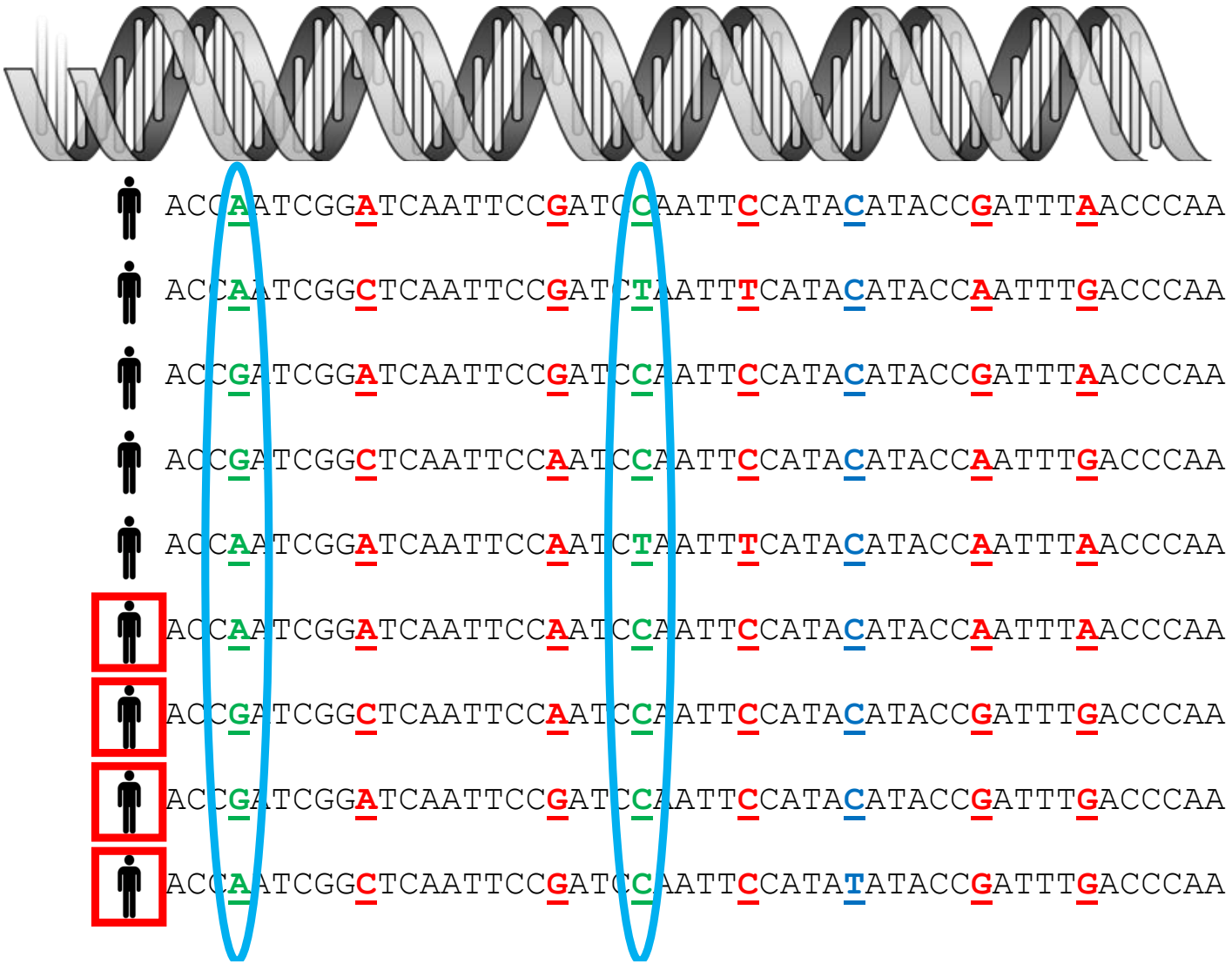
# Next Generation Sequencing (NGS)

- 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.

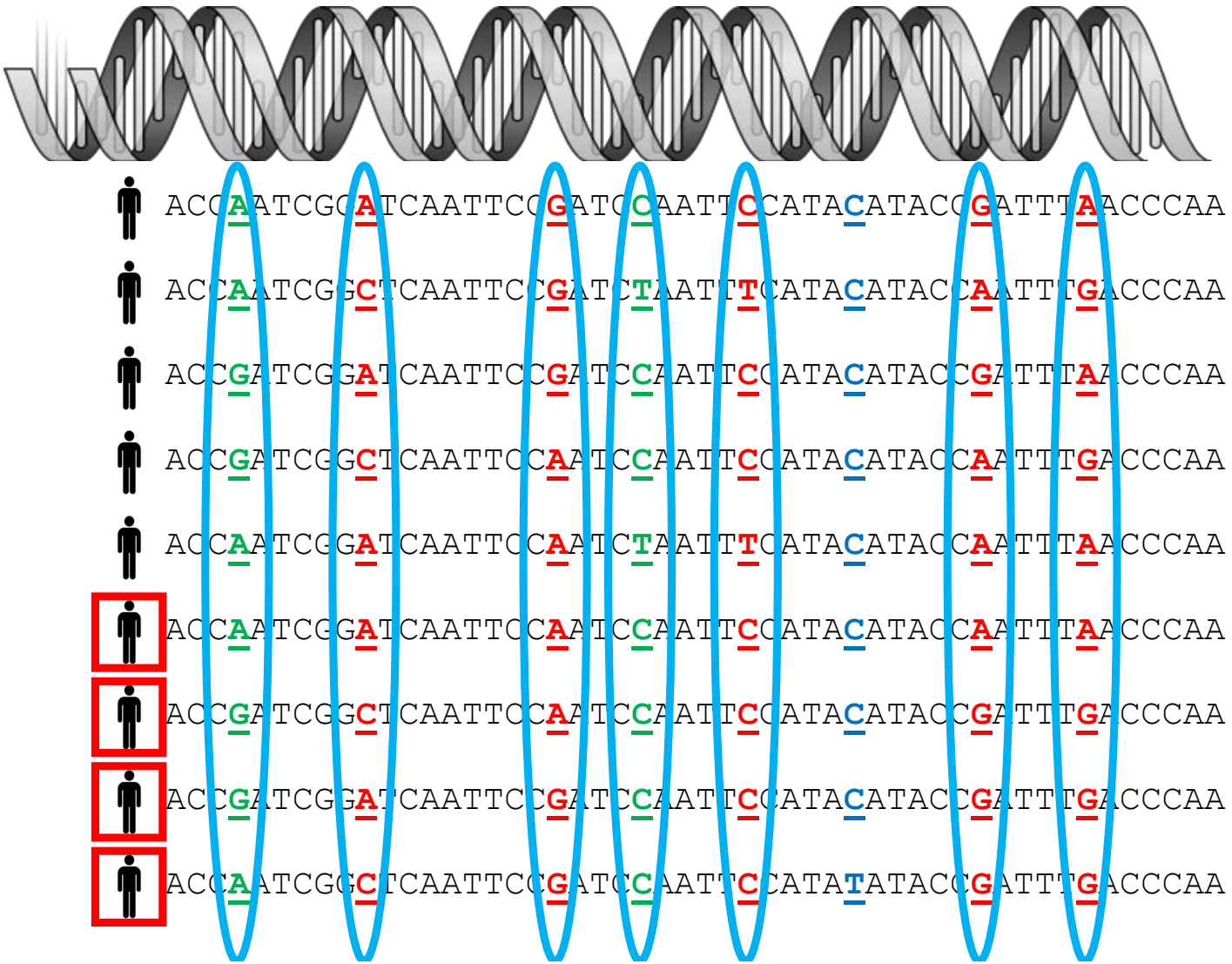
# Genetic Association Studies



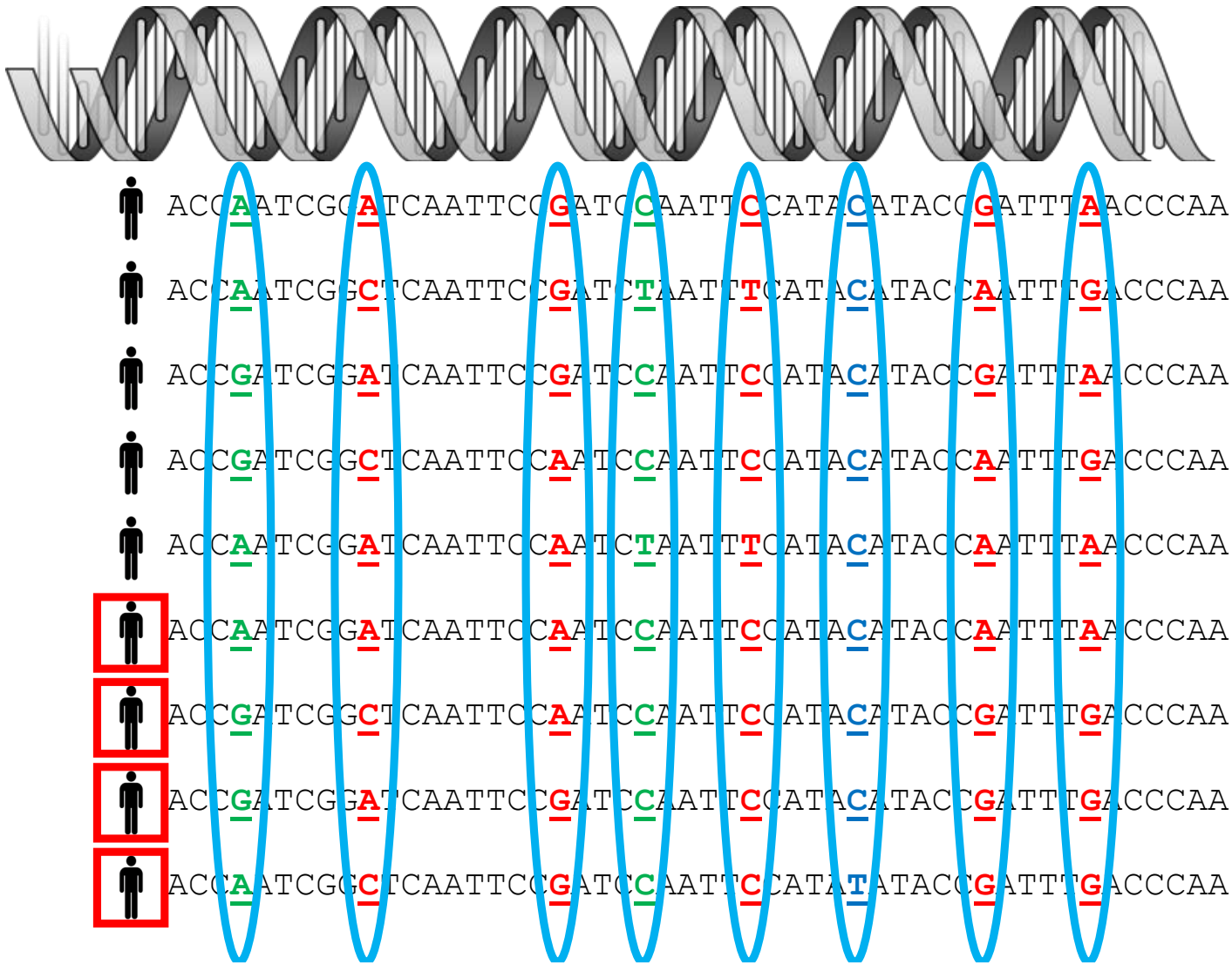
# GWAS: A few years ago



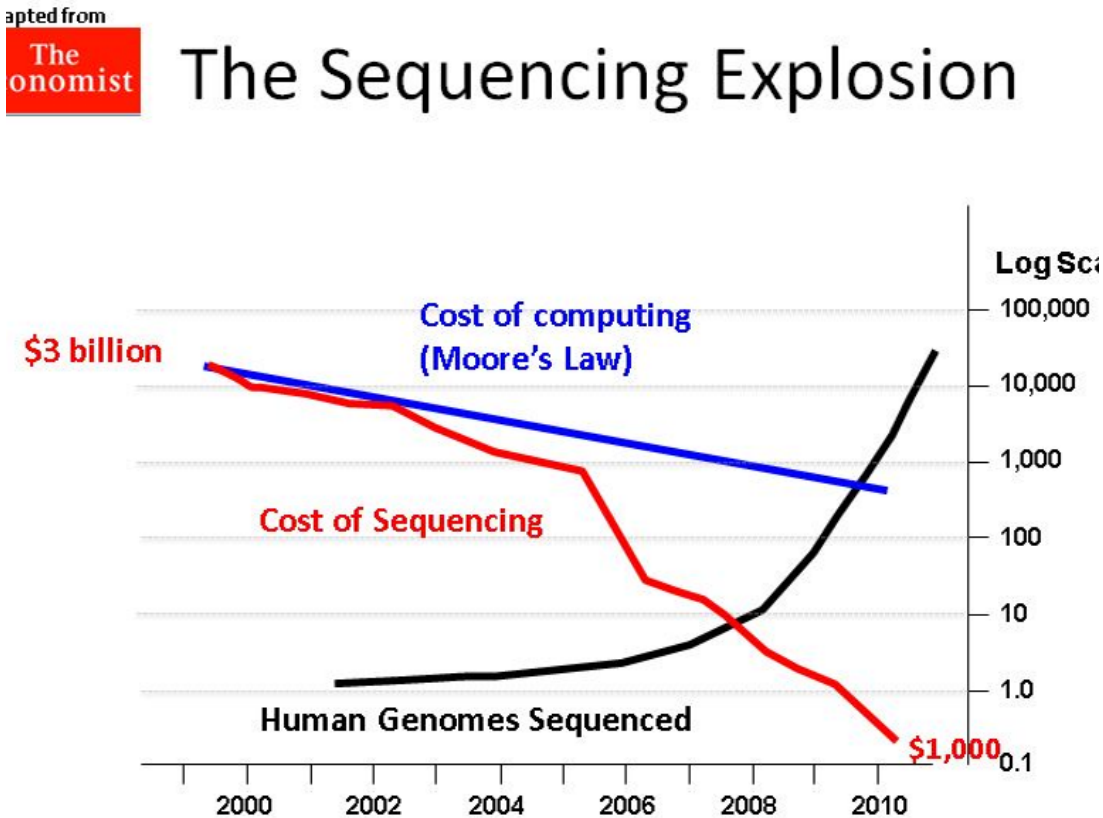
# GWAS: current (+ imputation)



# Sequencing


ACCAATCGGATCAATTCCGATCCAATTCCATACATACCGATTTAACCCAA
ACCAATCGGCTCAATTCCGATCTAATTTCATACATACCAATTTGACCCAA
ACCGATCGGATCAATTCCGATCCAATTCCATACATACCGATTTAACCCAA
ACCGATCGGCTCAATTCCAATCCAATTCCATACATACCAATTTGACCCAA
ACCAATCGGATCAATTCCAATCTAATTTCATACATACCAATTTAACCCAA
ACCAATCGGATCAATTCCAATCCAATTCCATACATACCAATTTAACCCAA
ACCGATCGGCTCAATTCCAATCCAATTCCATACATACCGATTTGACCCAA
ACCGATCGGATCAATTCCGATCCAATTCCATACATACCGATTTGACCCAA
ACCAATCGGCTCAATTCCGATCCAATTCCATATATACCGATTTGACCCAA

# Sequencing Cost Has Dropped Dramatically



# Massively parallel sequencing

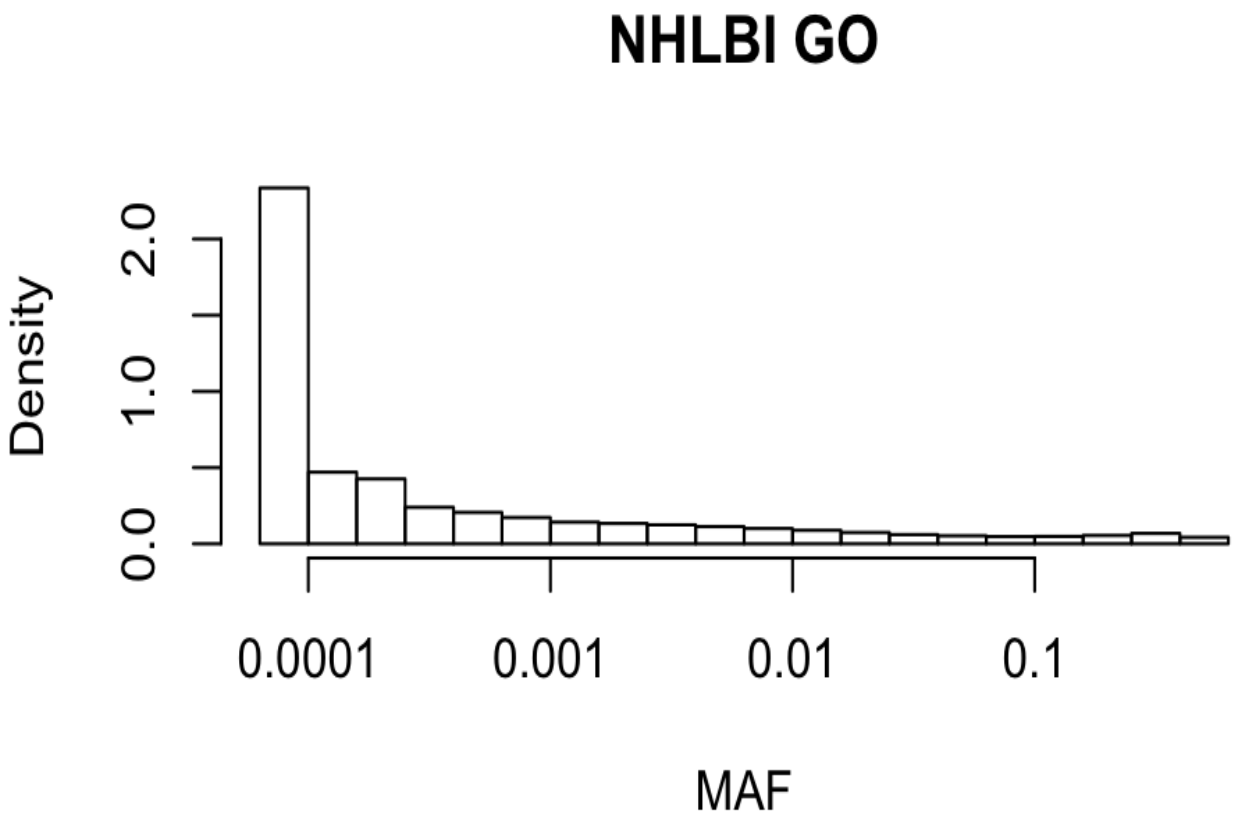
- Illumina can achieve $\$1,000$ per whole genome.

# 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.

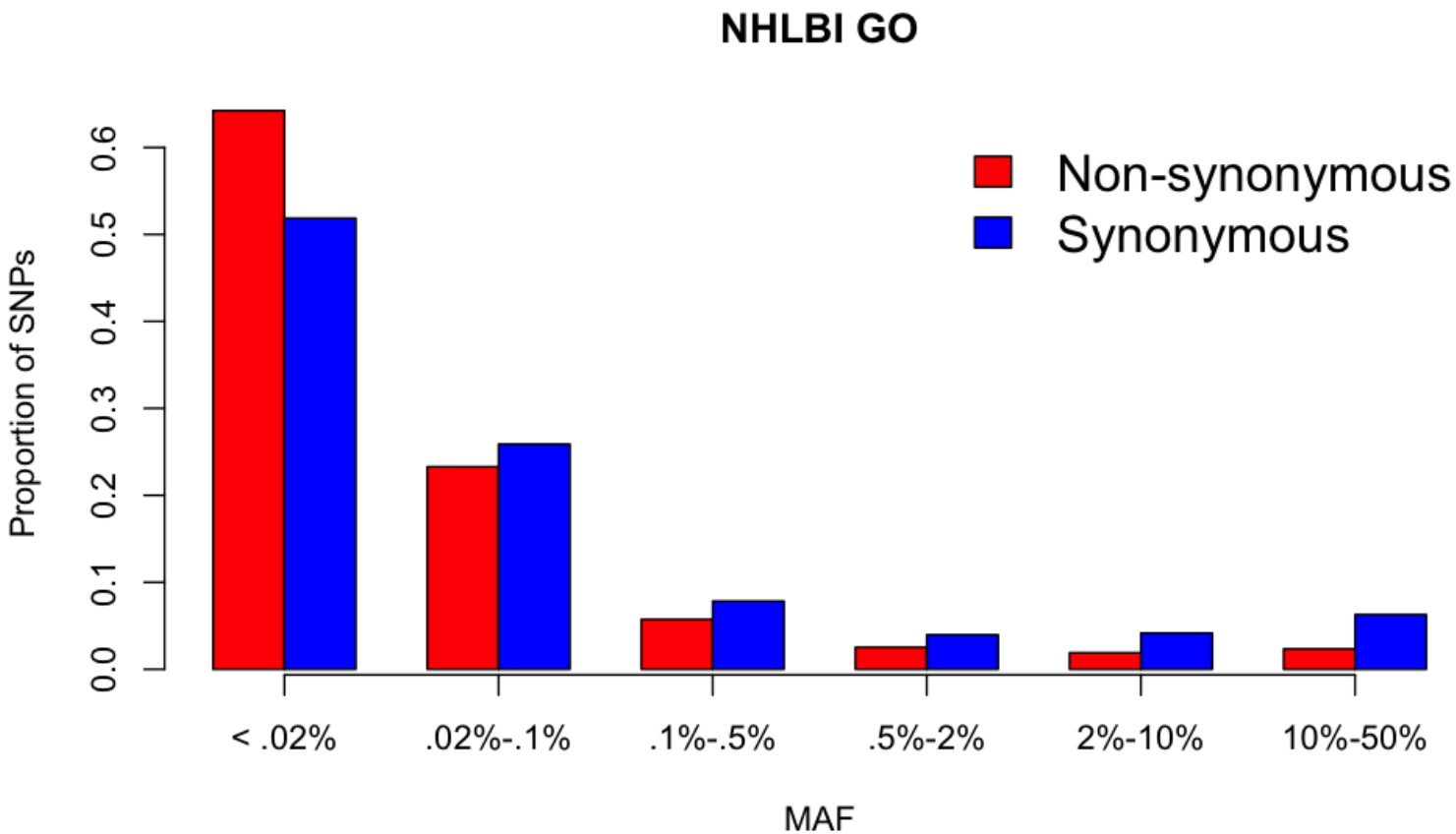
# Why rare variants?

- 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 $\theta$ chance

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

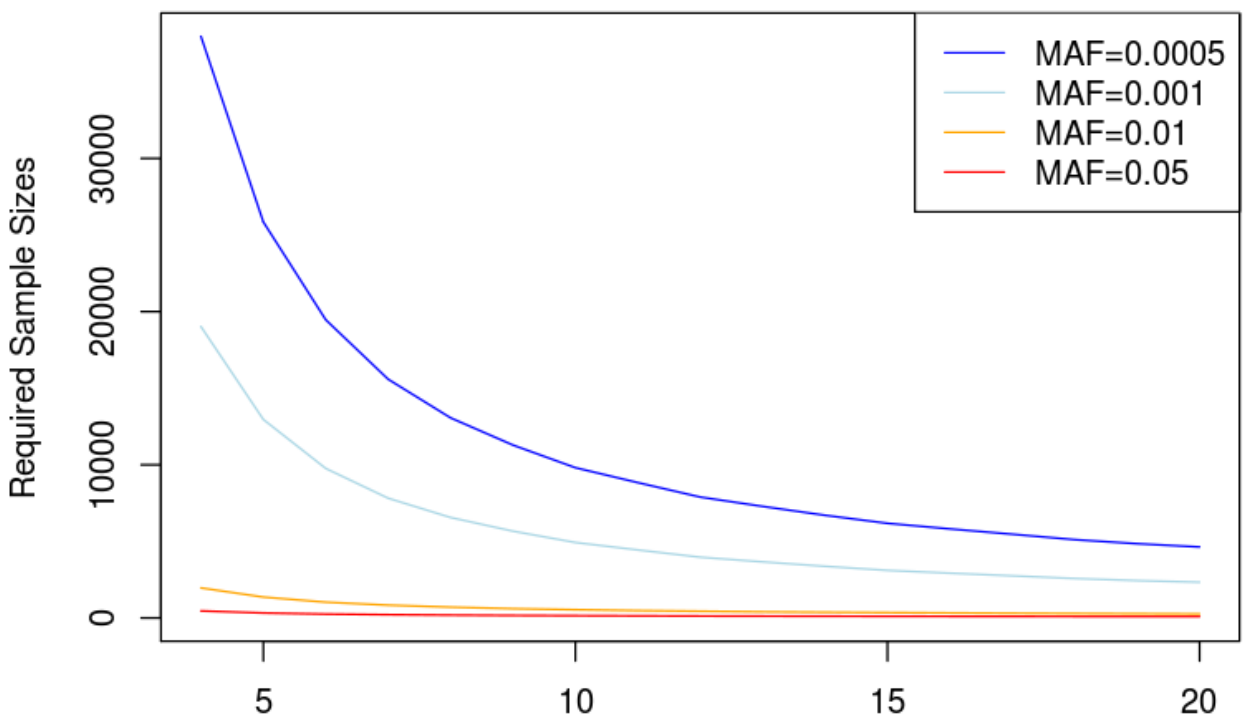
- For $\theta = 99.9\%$, the required minimum sample size is

| MAF | 0.1 | 0.01 | 0.001 | 0.0001 |
|---|---|---|---|---|
| Minimum $n$ | 33 | 344 | 3453 | 34537 |

- Large samples are required to observe rare variants.

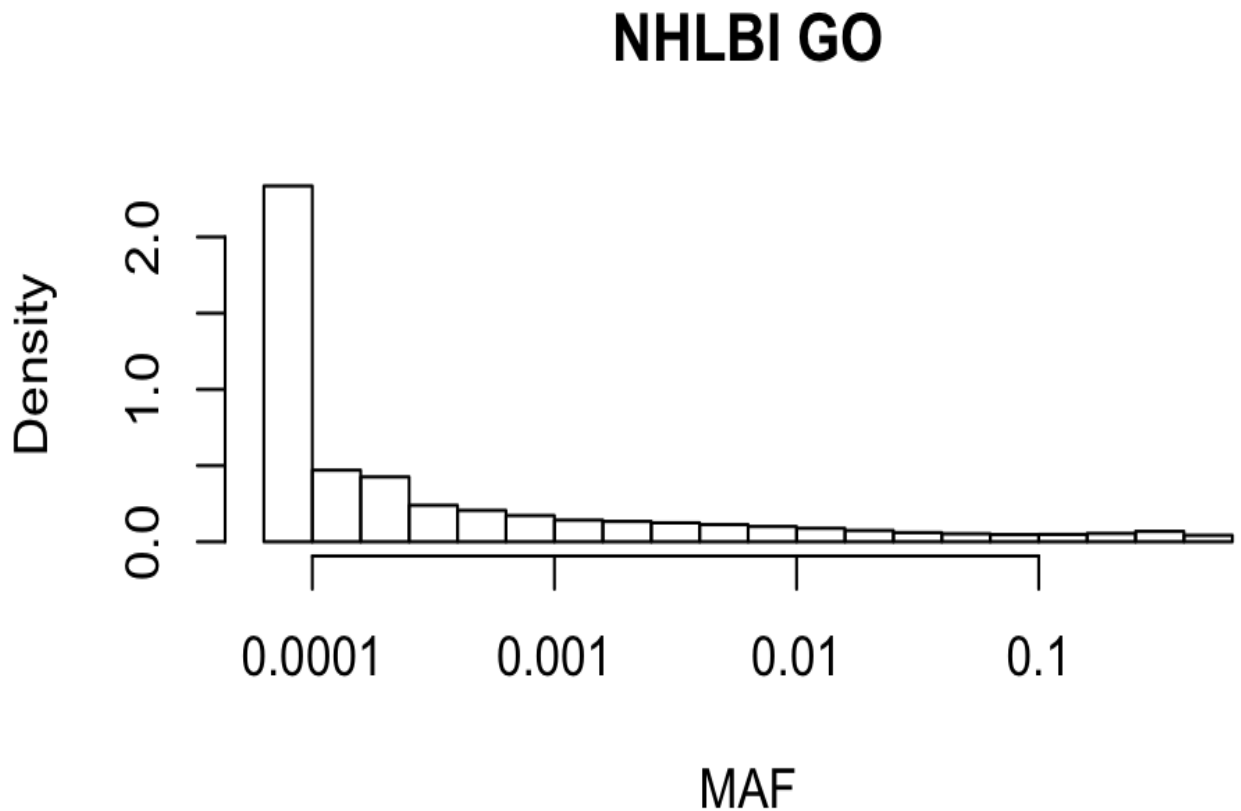
# How many subjects are needed to achieve 80% of power ($\alpha = 10^{-6}$) by single variant test?

- 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.