Population Stratification & Genotype Imputation

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$ echo "Data Sciences Institute"
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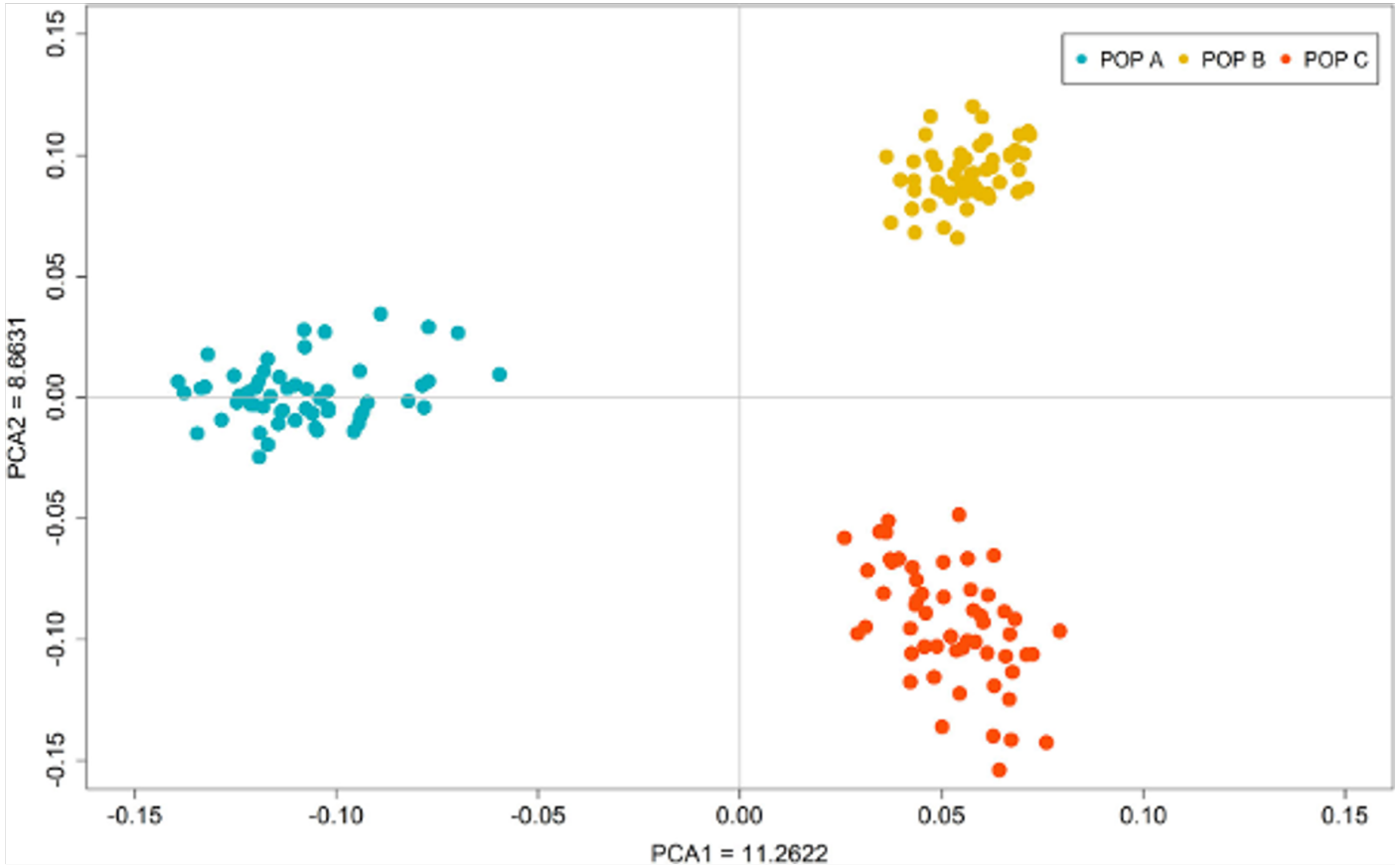
What You'll Learn Today

- **Why population structure matters:** Recognize how stratification and relatedness inflate association signals, learn core fixes (genomic control, PCA covariates, mixed models) as well as common pitfalls.
- **Use PCs and LMMs effectively:** Apply principal components for visualization/adjustment and deploy linear mixed models to obtain calibrated tests at scale.
- **Genotype imputation in practice:** Understand the reference-panel, LD-driven inference, quality metrics (e.g., INFO/r^2), and common pitfalls.

Population Stratification

- Different subgroups present within your population.



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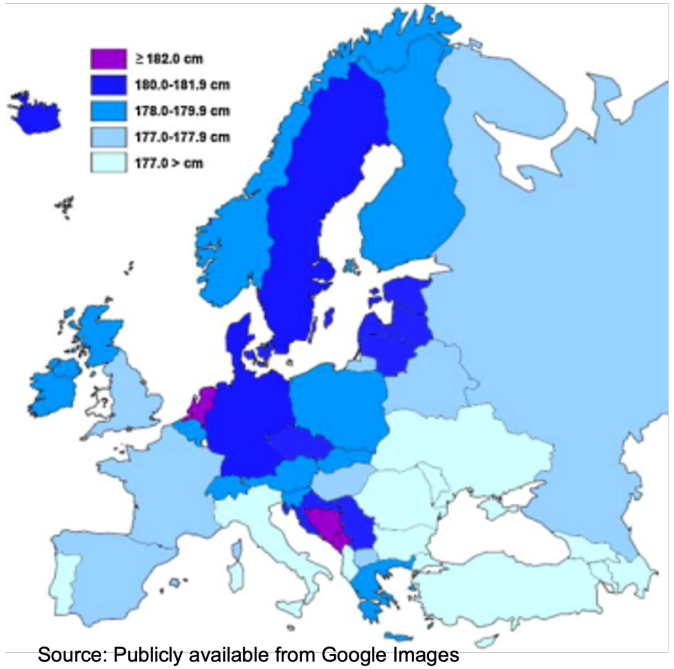
Population Stratification

- Population structure leads to differences in both traits and allele frequencies across regions.

Height varies across countries



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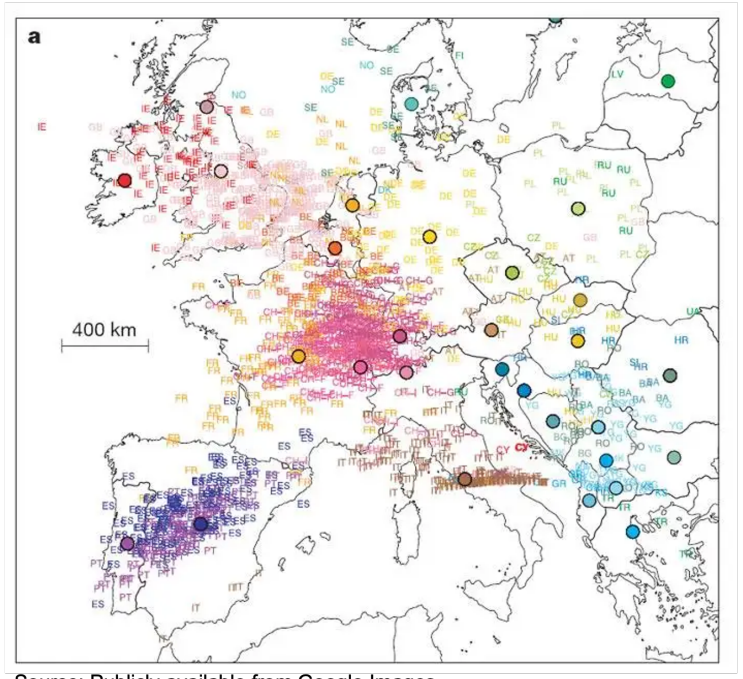
Allele frequencies vary as well



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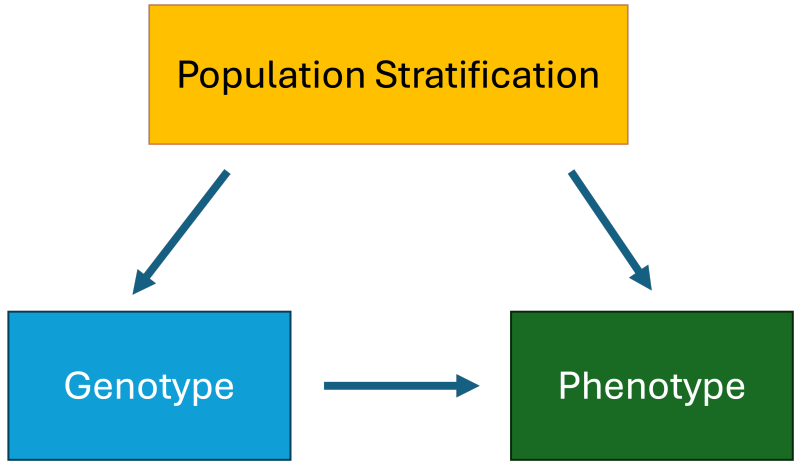
Population Structure Acts as a Confounder



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- Failing to account for population stratification results in incorrect variance estimates for the association test statistic $\rightarrow$ **false positive associations**.

How to Address Population Stratification?

1. **Hardy-Weinberg failure**
    - Not powerful, provides no adjustment.
2. **Modern approaches**
    - Use large sets of *null markers*.
    - Stratification causes global inflation of test statistics at null markers (not disease-associated).
    - By examining these markers, we can estimate the extent of stratification.
    - Enabled by GWAS: millions of markers available, most are null.

Approaches to Adjust for Population Stratification

- Genomic Control
- Clustering approaches
- Principal component analysis (PCA)
- Mixed effect models

Genomic Control (GC)

- WE define GC lambda as

$$\lambda = \frac{\text{median}\left(\chi_1^2, \ldots, \chi_L^2\right)}{0.4549}$$

- If $\lambda > 1.05 \rightarrow$ population stratification likely
- Corrected test statistic:

$$\chi^2_{l,GC} = \frac{\chi^2_l}{\lambda}, \quad \forall l = 1, \ldots, L.$$

Key Principles of Genomic Control

- Most SNPs are expected not associated with the trait.
- If many SNPs show inflated test statistics $\rightarrow$ evidence of stratification.
- Compare **observed median $\mathbf{X}^2$** to 0.4549 (expected under the null).
- Ratio measures inflation due to stratification.
- Stratification should affect all loci equally $\rightarrow$ similar inflation across SNPs.
- Adjust each statistic by rescaling with GC λ.
- Genomic control uses **a constant adjustment factor for all SNPs**.
- Can lead to overadjustment at some SNPs and underadjustment at other SNPs.

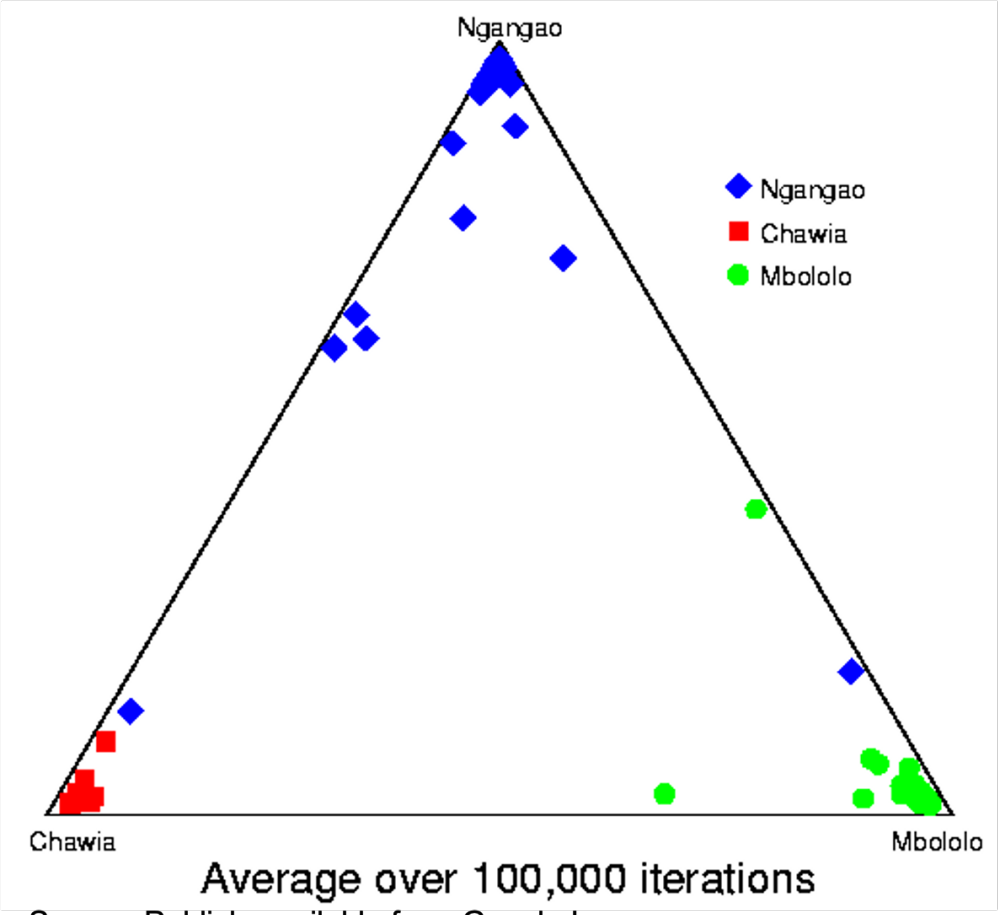
STRUCTURE

- Bayesian clustering model (Pritchard, Stephens, Donnelly, Genetics 2000) **infer the latent population structure** by a stratified analysis.
- Assigns individuals to K source populations; under admixture, each individual can have **fractional ancestry** across populations.
- Works best with **strong population structure** (a few distinct ancestries) and **many variants / ancestry-informative markers (AIMs)**.
- A practical issue is choosing K in advance.
    - Larger K can overfit, so model-selection/diagnostic procedures are needed.
    - Often used exploratorily by comparing results across several values of K.

STRUCTURE

Convergence of Structure: Taita thrush data (K=3).

- The ternary plot shows each individual's proportional ancestry.
- Points near a vertex indicate near-pure membership in one cluster; interior points indicate admixture.



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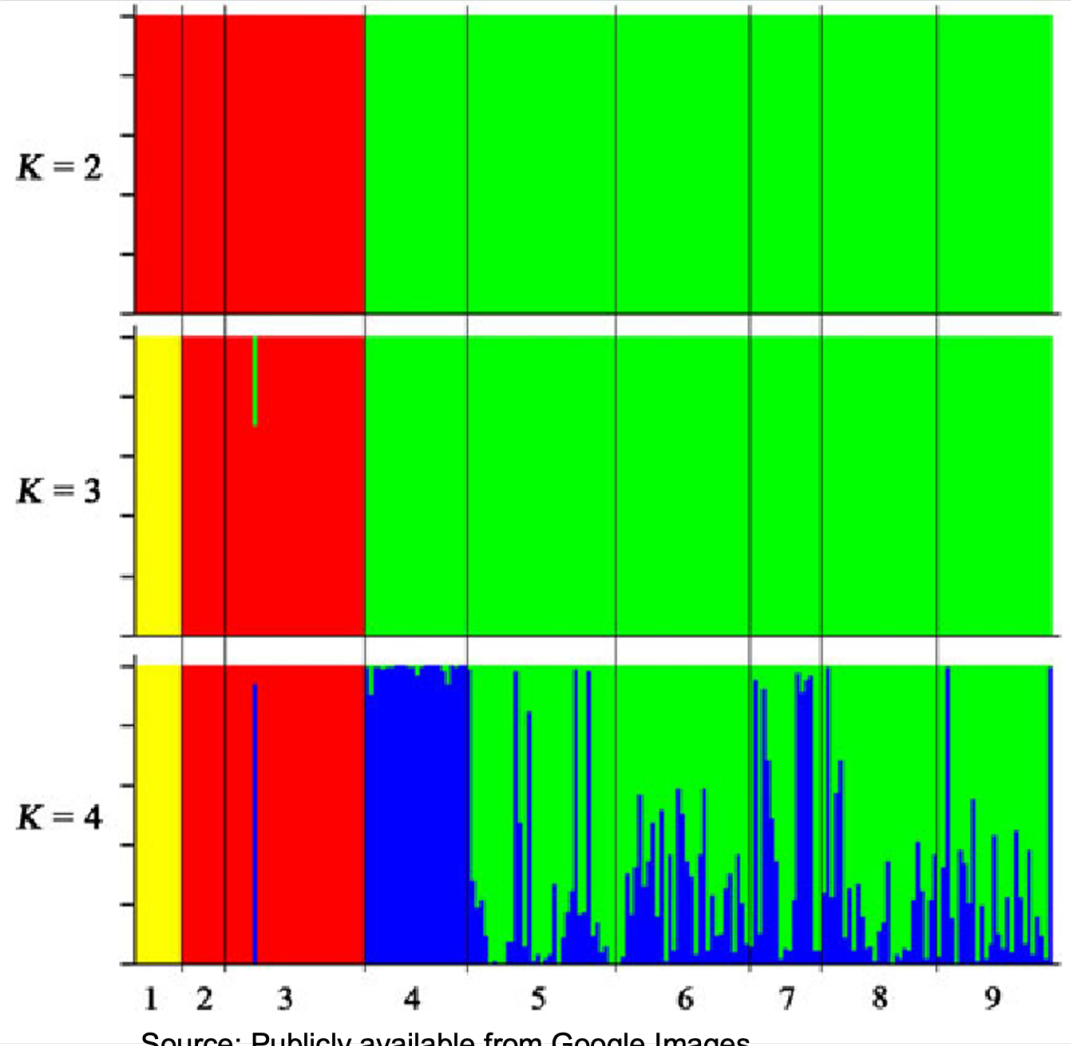
STRUCTURE Bar Plots

- Each vertical bar = one individual; the color fractions are the estimated membership coefficients.
- Individuals with same color patterns are inferred to belong to the same cluster.



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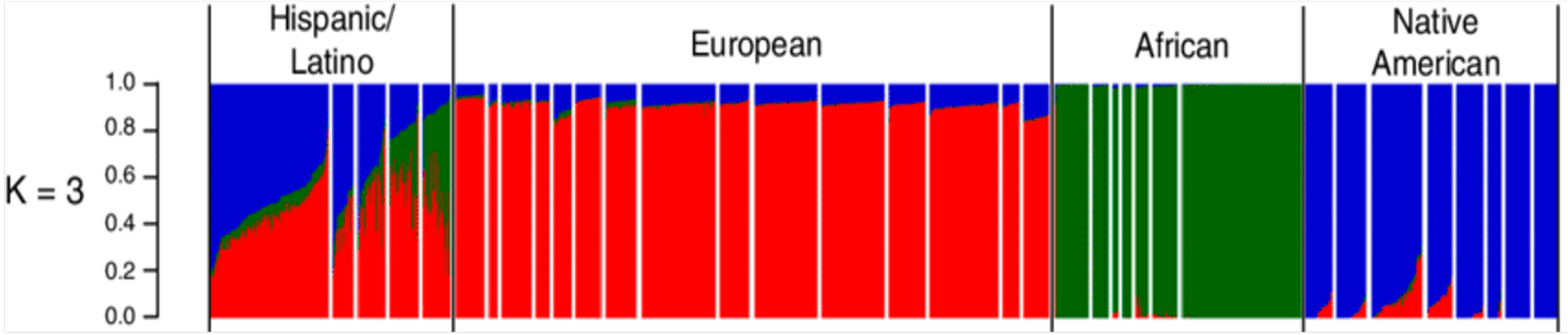
Applying STRUCTURE to Admixed Populations



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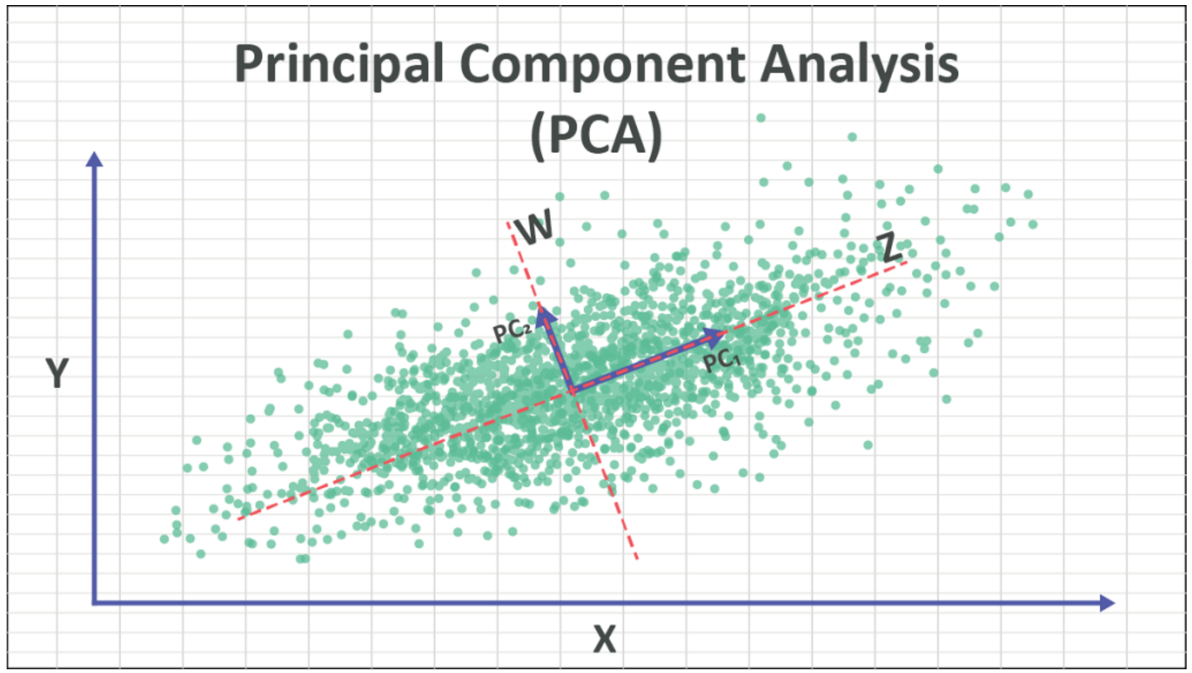
Principal Component Analysis

(**Eigenstrat**, Nature Genetics 2006)

- Widely used approach to control for population structure in genetic association studies.
- Intuition: ancestry/geography leaves correlated patterns in genotypes that can be summarized by a few axes.
- Start with a genotype matrix $X \in \mathbb{R}^{n \times m}$ (n individuals, m SNPs); aim for a low-dimensional summary with $k \ll m$.

What PCA Does

- Principal component analysis (PCA) is a statistical procedure that allows us to perform **dimension reduction**.
    - Finds orthogonal directions ("principal components") that capture the **largest possible variance** in the data.



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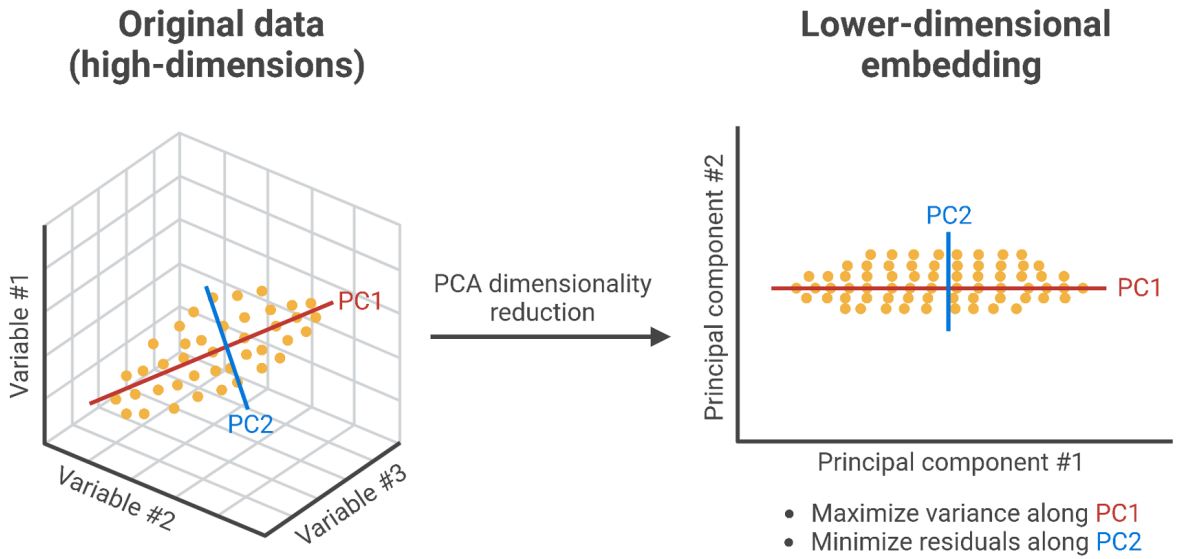
Geometric Picture

- Identify a pair of orthogonal vectors (red) that define a lower-dimensional plane (gray) and **maximize the variance of the projection**.

Principal Component Analysis (PCA) Transformation



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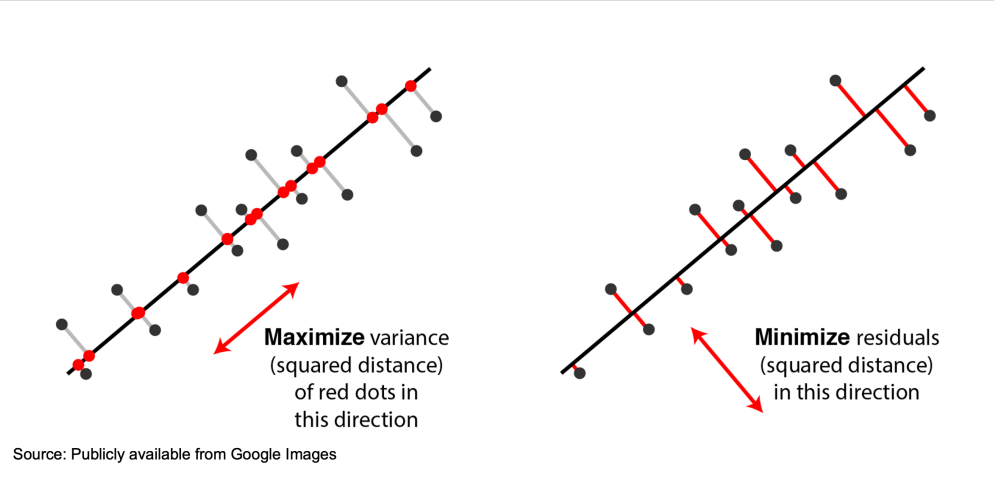
Formal Objective

- Given $X = [x_1, \ldots, x_n]^\top$, find a k-dimensional subspace minimizing the mean squared reconstruction error (equivalently, maximizing captured variance).
- Two equivalent views:
  - Maximize variance along chosen directions.
  - Minimize residuals from projecting onto the subspace.



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Optimization Formulation

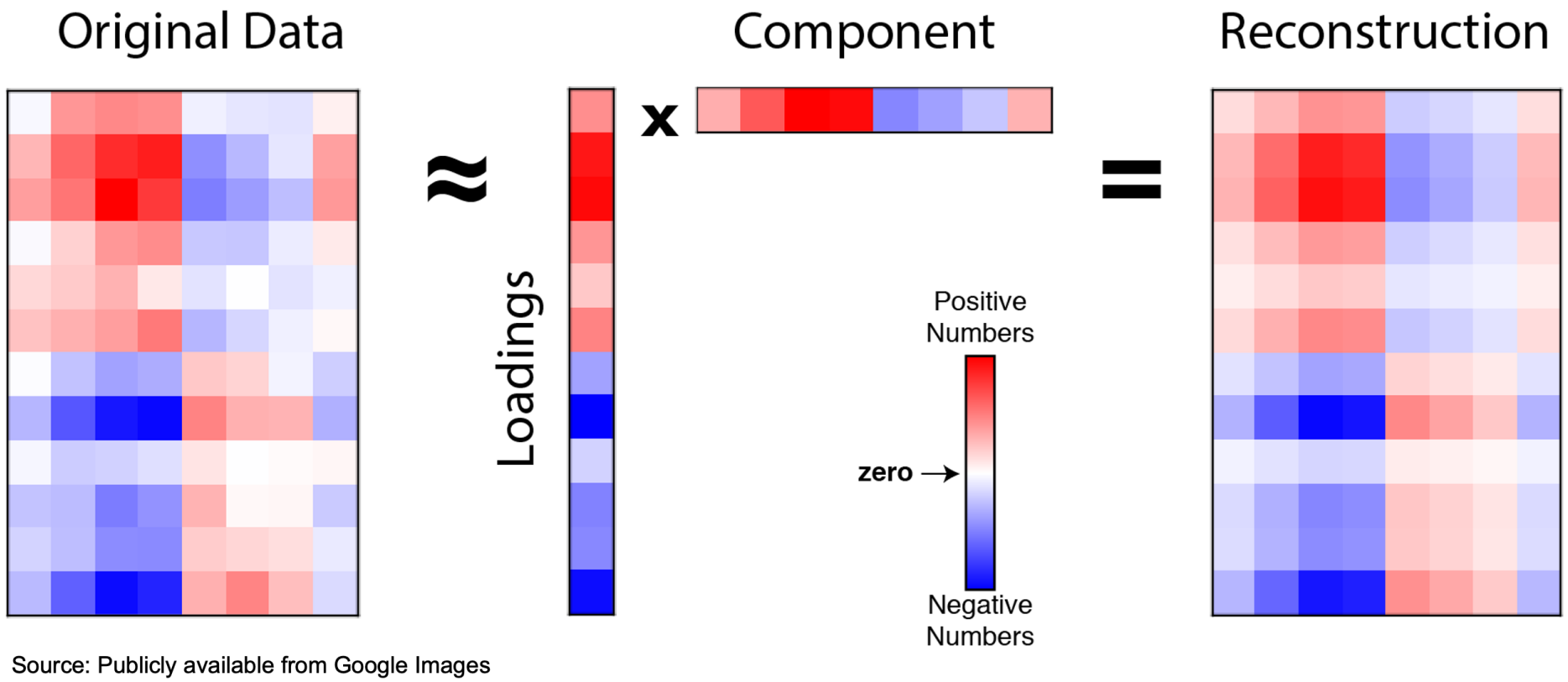
$$\min_{W,C} \left\| X - WC^\top \right\|_F \quad \text{s.t. } C^\top C = I_k$$

- Shapes: $W \in \mathbb{R}^{n \times k}$ (scores), $C \in \mathbb{R}^{m \times k}$ (loadings).
- Many solvers exist; a standard route is via **eigendecomposition/SVD** of a covariance matrix.

Practical Algorithm

1. Center the data (i.e., each SNP): column means of X are set to zero.
2. Compute the covariance matrix of the data: $S = X^\top X$.
3. Take the top k eigenvectors of the covariance matrix that have the largest eigenvalues
   $\rightarrow$ the top k principal component.
4. Low-rank reconstruction: $X \approx WC^\top$.

Lower-Dimensional Representation



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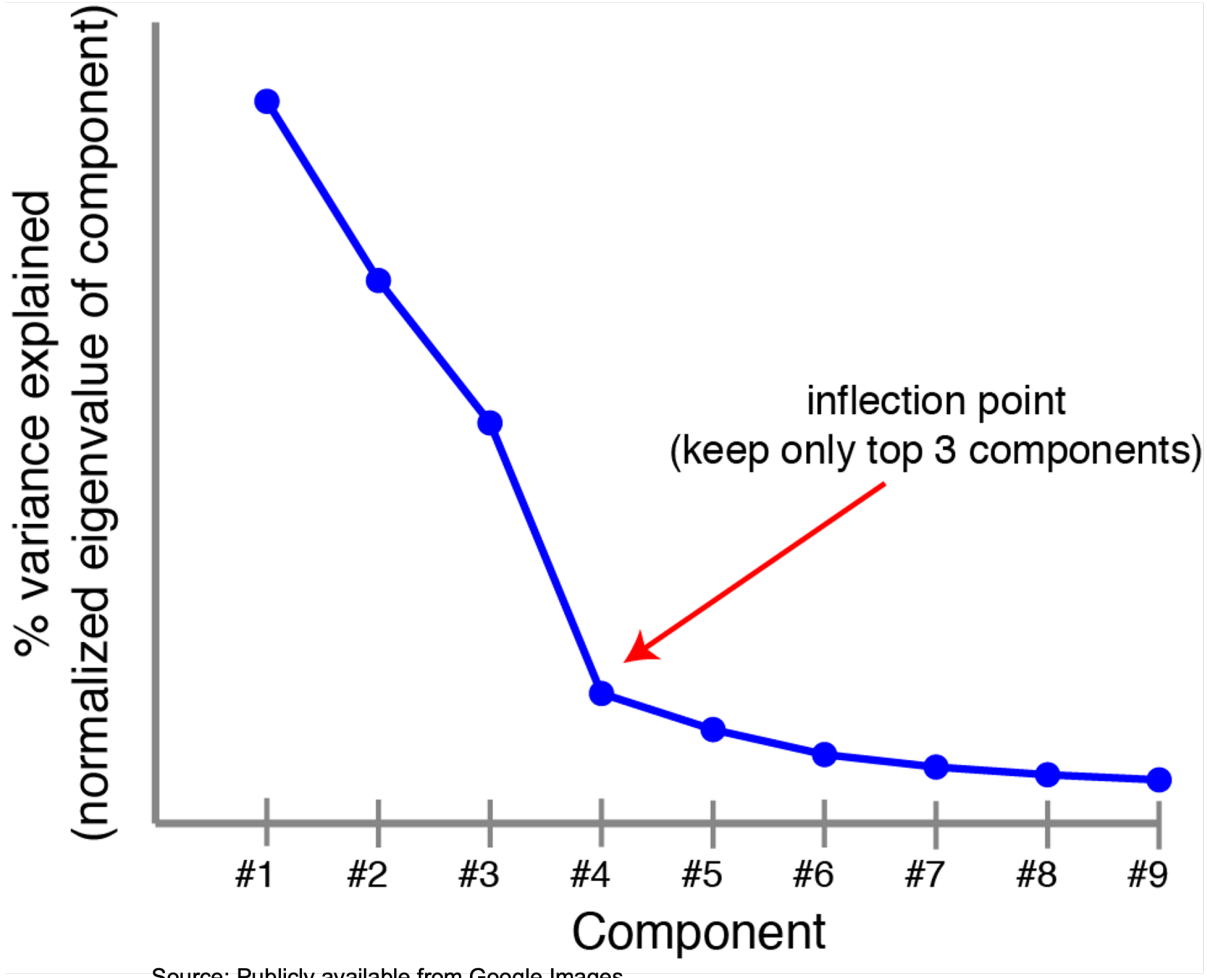
How Many PCs?

- Goal: use the fewest PCs that retain the relevant structure while avoiding noise/overfit.
- For data visualization, only a few are needed.
- To adjustment for stratification, the right number is data-dependent.
- Inspect explained variance / scree plot and add PCs until major structure is captured.



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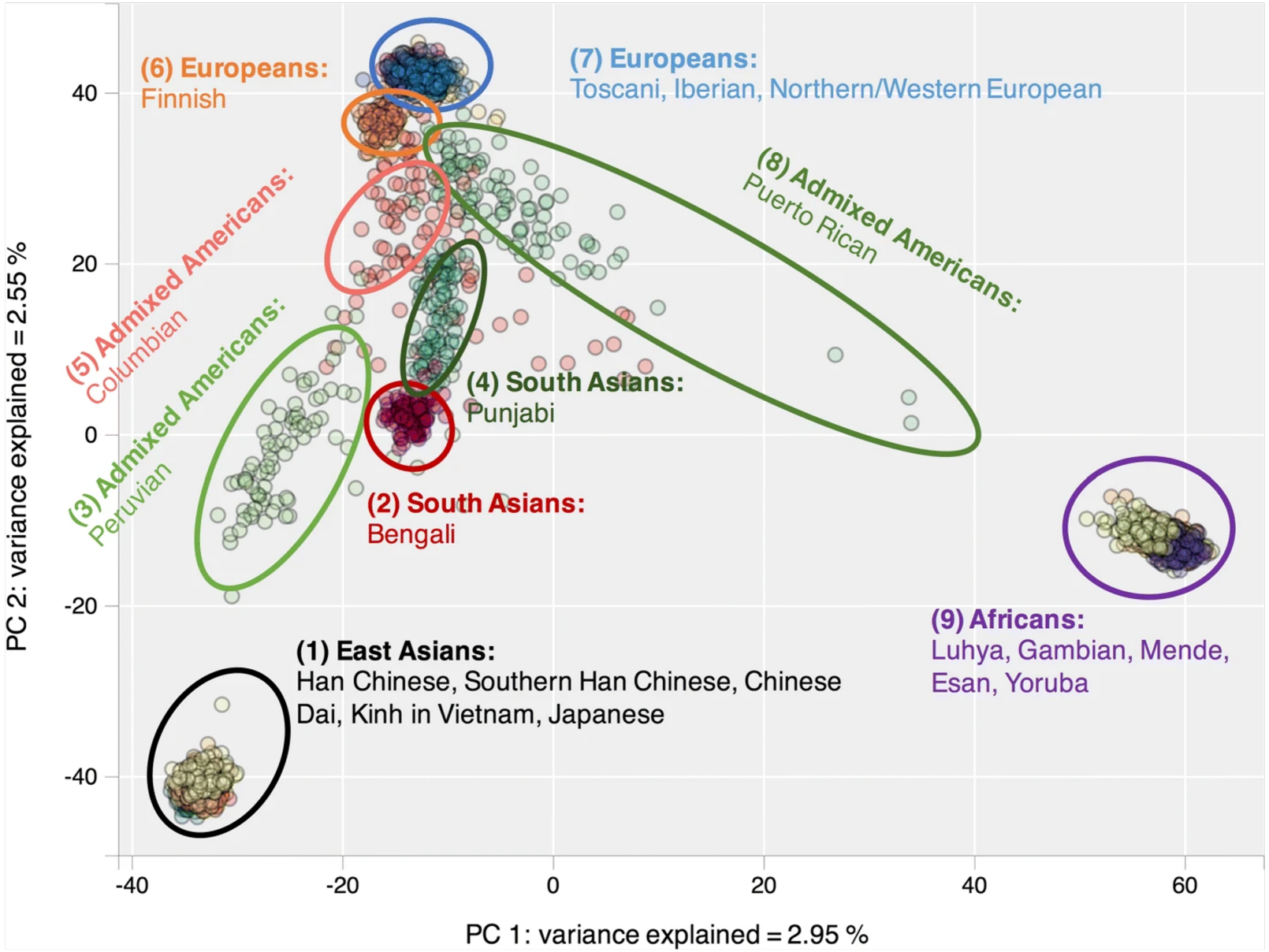
How can we use PCA?

- Data Visualization: Project samples onto the leading PCs (e.g., PC1–PC2) and plot them to reveal clusters and gradients.
- Adjustment for confounding: Include the top PCs as covariates to control for population stratification in association analyses.
- 1000 Genomes PCA example: http://bwlewis.github.io/1000_genomes_examples/PCA_overview.html



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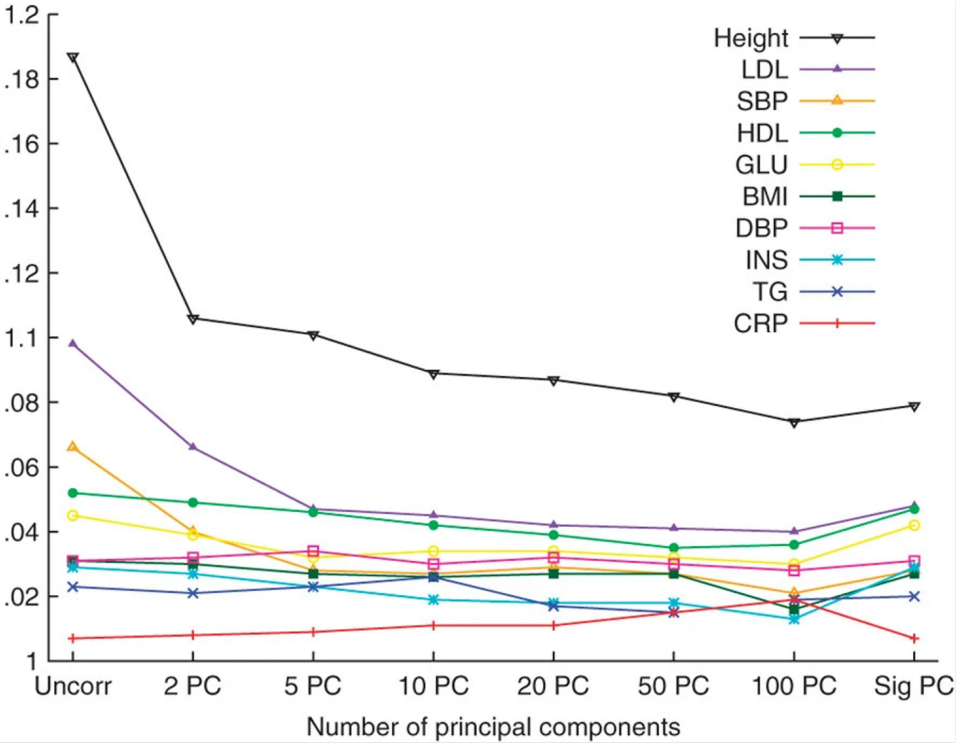
Correcting Stratification with Principal Components

- PC adjustment reduces inflation.
- Diminishing returns: Most correction occurs by ~10–20 PCs (or significant PCs).
- Trait-specific effect: Height shows the strongest inflation and benefits most from PC adjustment.



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Mixed Effect Models

- Mixed effect models can model population structure, family structure and cryptic relatedness.
- Model phenotypes using a mixture of fixed effects (SNPs) and random effects (family structure).
- An early approach EMMAX (Nature Genetics, 2010) is based on the mixed-effects model to account for hidden relatedness.
- Many other methods (BOLT-LMM, fastGWAS, SAIGE, REGENIE) have been proposed to improve the computational efficiency of these methods.

Linear Mixed Models (LMM) - Review

- **Ordinary linear model:** $Y = X\beta + C\alpha + \varepsilon$
- **Mixed linear model:** $Y = X\beta + C\alpha + u + \varepsilon$
  - u : genetic (heritable) random effect. $E(u) = 0, \mathrm{Var}(u) = \sigma_g^2 K.$
  - ε : residual, non-genetic noise. $\mathrm{Var}(\varepsilon) = \sigma_e^2 I.$
- **Genetic relationship matrix (GRM):** $K = \frac{GG^T}{M}$,
  where $G =$ genotype matrix ($N \times M$), $N =$ #individuals, $M =$# SNPs (both large in GWAS).

Linear Mixed effect Models (LMM) - Review

- K captures all kinds of relatedness:population stratification, family structure and hidden/cryptic relatedness.
- σ_g^2 : genetic variance that we want to estimate.
  - Estimation methods: REML or AI-REML.
- From the model, we can also derive estimates of random effects (u).

Association Testing with LMM (Step 1)

Step 1: Fit the null model

$$Y = C\alpha + u + \varepsilon$$

- Remove covariate effects:

$$\tilde{Y} = u + \varepsilon$$

- Using REML/AI-REML we estimate σ_g^2 and σ_e^2.
- Can also obtain **BLUPs** (best linear unbiased predictors) of u:

$$\hat{u} = \mathrm{K}\widehat{\sigma_g^2} \times \Sigma^{-1}\left(\mathrm{I} - \mathrm{C}\left(C^T\Sigma^{-1}\mathrm{C}\right)^{-1}C^T\Sigma^{-1}\right)\mathrm{Y}$$

- $\Sigma = \widehat{\sigma_g^2}\,\mathrm{K} + \widehat{\sigma_e^2}\mathrm{I}.$

Association Testing with LMM (Step 2)

Step 2: Test SNP effects

- Hypothesis: $H_0 : \beta = 0$
- Residual phenotype after removing random effect:

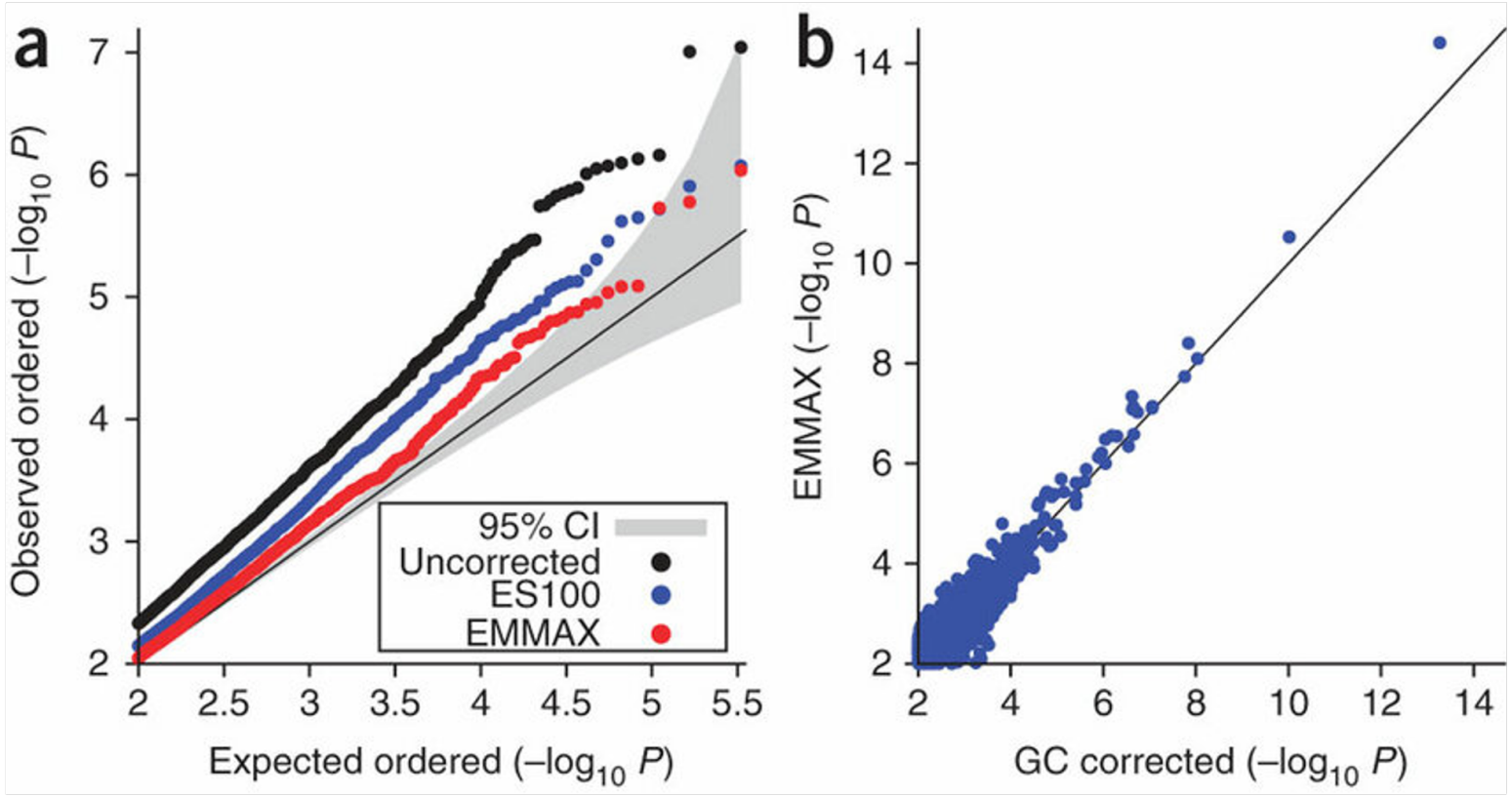
$$Y^*_{\text{resid}} = \tilde{Y} - \hat{u}$$

- Fit a standard linear regression:

$$Y^*_{\text{resid}} = X\beta + \varepsilon$$

- **Computational aspects**:
- Step 1 is expensive (matrix inversions, large-scale operations) but done only once.
- Step 2 is repeated across millions of SNPs (efficient).

PCA VS. Mixed Model



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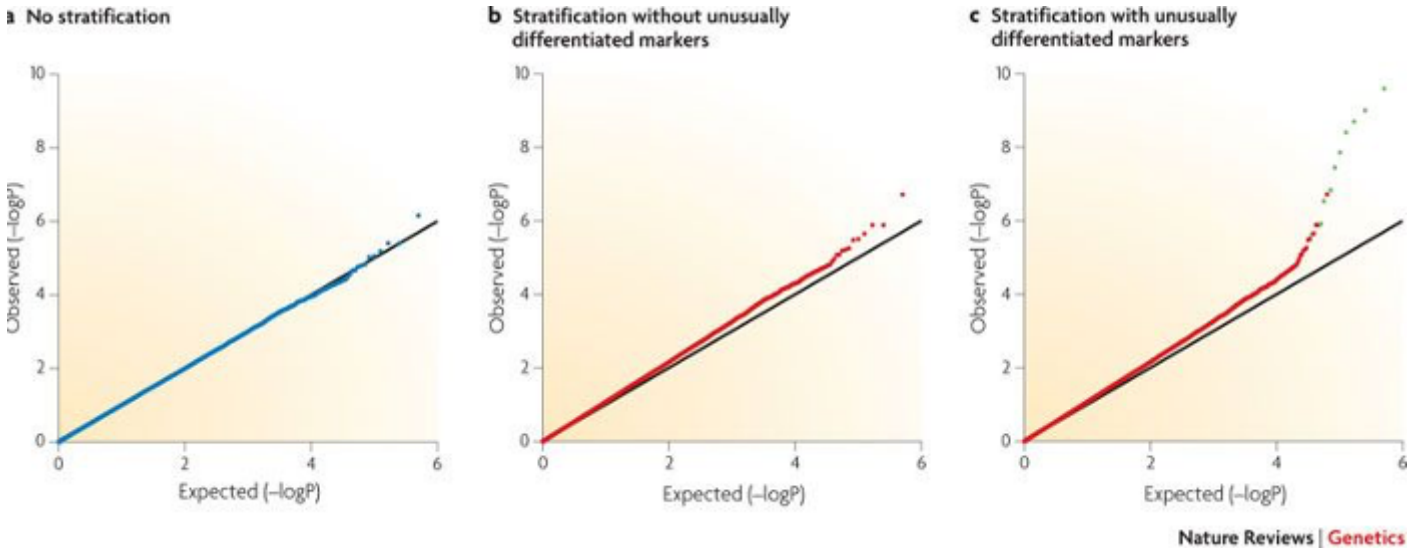
Q–Q plot as a Diagnostic Tool

- Order SNPs according to their p-values.
- Compare the distribution of observed vs. expected test statistics or p-values under the null hypothesis.
- Deviations from the diagonal line indicate potential population stratification or true associations.



Is population stratification fully resolved?

- The answer is no — existing correction methods may fail or be insufficient in some scenarios.
- Good epidemiological study design is still the most important way to avoid confounding.
- Relying on convenience controls can introduce substantial bias.
- Family-based designs are gaining renewed interest due to their robustness to population stratification.

Genotype Imputation

- Missing data in genotypes can arise in two ways:
  - The genotype matrix contains missing values, similar to classical missing data.
  - Genotyping arrays only assay a subset of SNPs, leaving many unmeasured.
    - E.g. A genotyping platform may capture ~500K SNPs, while tens of millions of common SNPs exist across the genome. For association studies, we are often interested in testing many more of these.
- Question: **Can we statistically recover ("impute") these untyped SNPs?**

Genotype Imputation

Genotype data with missing data at untyped SNPs (grey question marks)

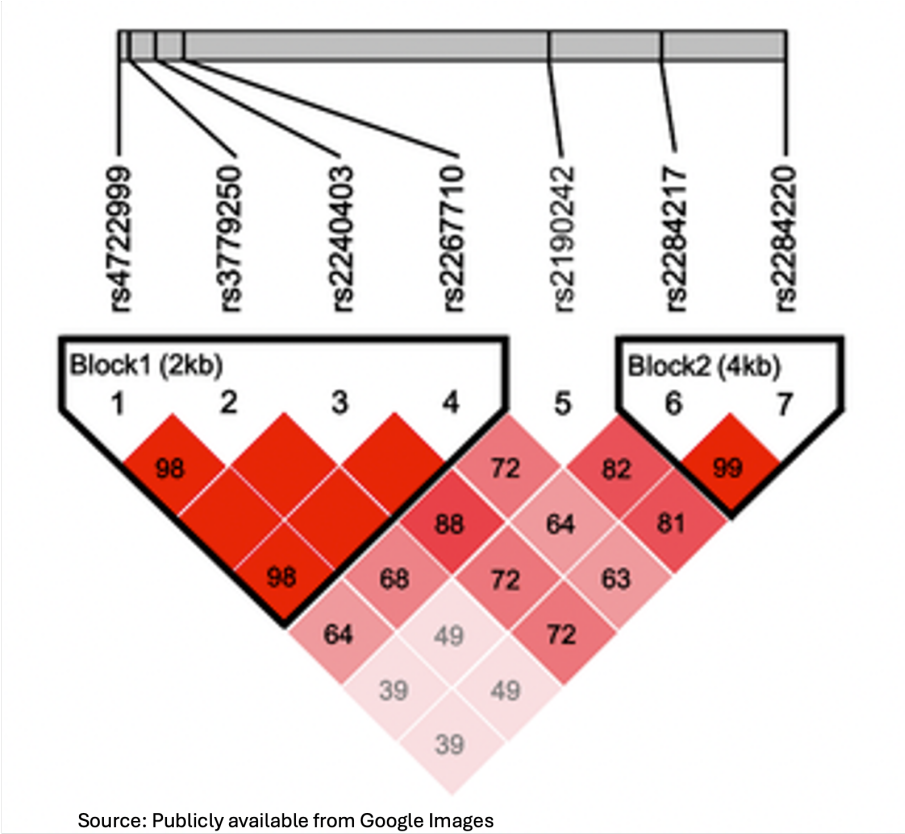
1	**?**	**1**	**?**	**1**	**?**	**0**	**2**	**2**	**?**	**2**	**?**	**0**
0	?	2	?	2	?	0	2	2	?	2	?	0
1	?	2	?	2	?	0	2	1	?	2	?	0
1	?	2	?	1	?	1	2	2	?	2	?	0
2	?	2	?	2	?	1	2	1	?	2	?	0
1	?	1	?	1	?	1	2	2	?	2	?	0
1	?	2	?	2	?	0	2	1	?	2	?	1

UK Biobank Imputation

- UK Biobank released data on $\sim 500,000$ individuals, including genotypes and thousands of phenotypes.
- Each sample was initially genotyped with < 1 million SNPs.
- Using haplotype reference panels, imputation expanded this to ~ 80 million variants.
- The resulting dataset is massive: ~ 2 TB of imputed genetic information.

Leveraging LD for Imputation

- Correlations among SNPs, known as linkage disequilibrium (LD), allow us to infer untyped variants from observed ones.



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Principles of Imputation

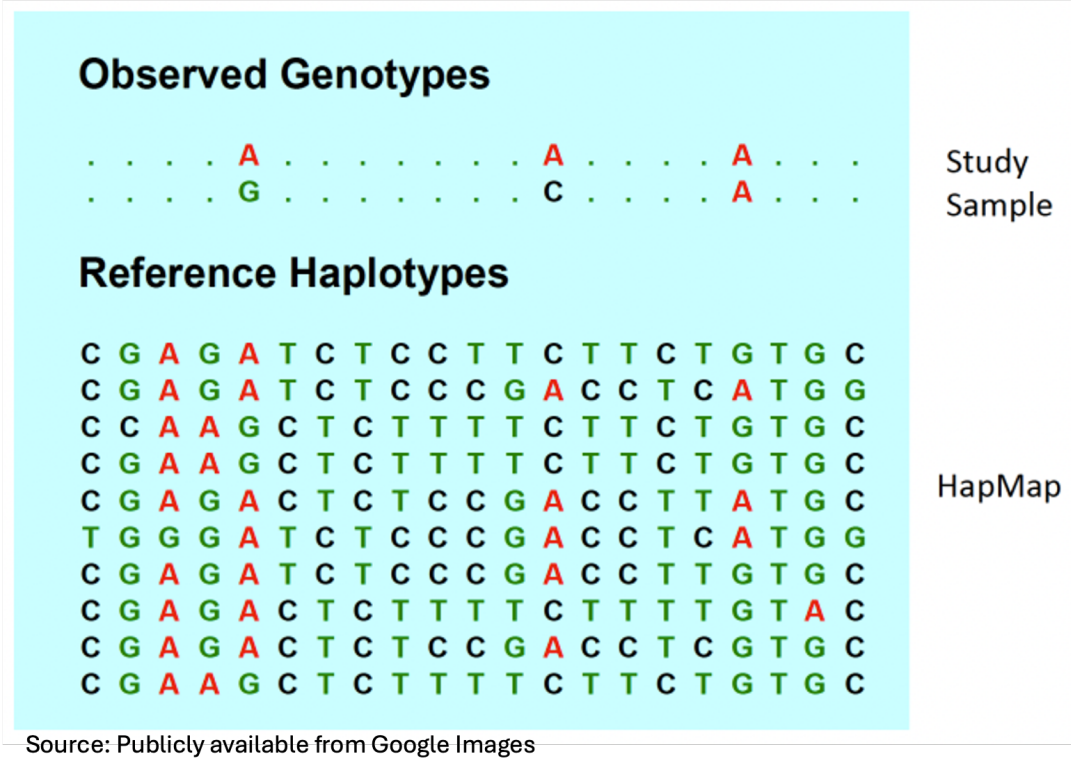
- Imputation methods infer genotypes at untyped sites using patterns from reference panels.
- Often described as **in-silico genotyping.**
- Widely applied in GWAS for:
  - Increasing statistical power.
  - Enabling meta-analysis by harmonizing across studies.

Reference Panels

- In GWAS, we typically start with ~500K genotyped SNPs.
- To impute additional SNPs, we require a **reference dataset** containing observed genotypes at those untyped sites.
- Examples of reference resources: HapMap, 1000 Genomes, UK Biobank, TOPMed.
- **Haplotypes:** each individual genotype can be decomposed into two haplotypes, inherited from each parent.

Matching Haplotypes

- The reference panel must be sufficiently large and diverse to include a comprehensive set of haplotypes, enabling reconstruction of any individual in the population.



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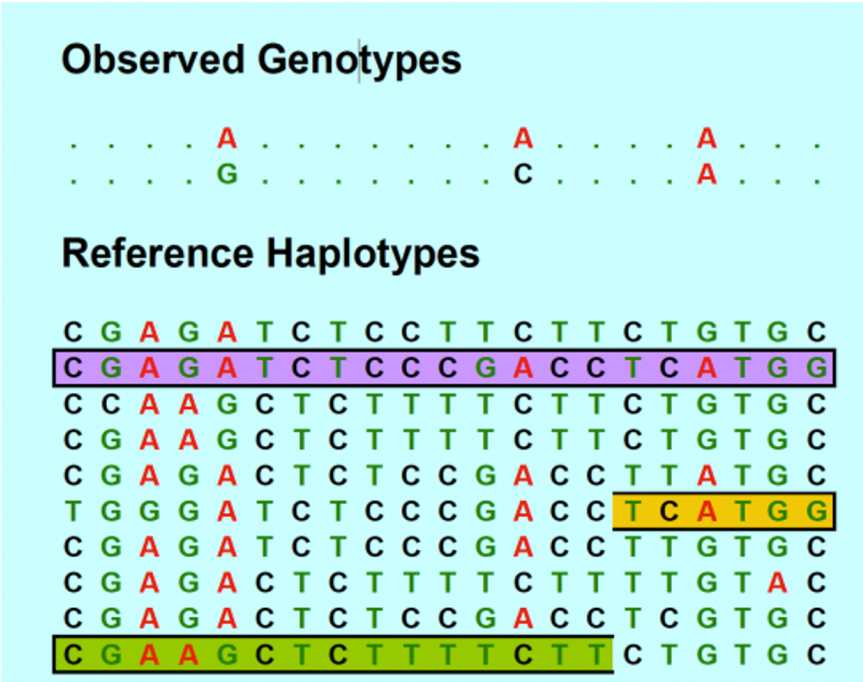
Inferring Missing Genotypes

- Once suitable haplotypes are identified, missing alleles can be filled in.
- The imputed genotype data is a probabilistic reconstruction, guided by LD and haplotype structure.



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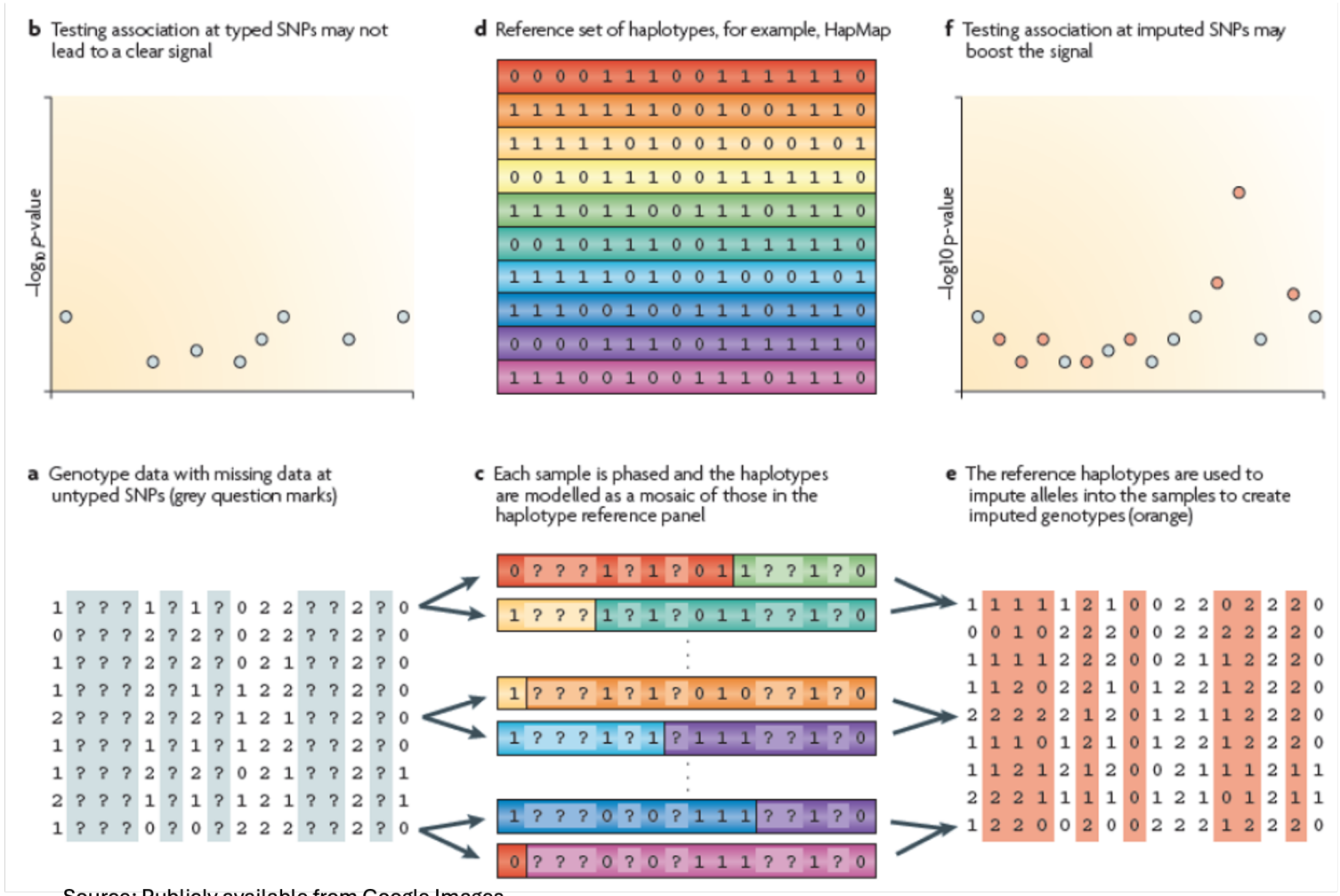
Imputation Workflow



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How Imputation Works

- Starting point: a reference set of haplotypes.
- Using the linkage disequilibrium (LD) structure in a reference population, together with the LD among observed SNPs in a dataset, we can impute the alleles of an untyped (hidden) SNP.
- Critical assumption: reference samples and study participants come from the same (or very similar) population.
- If mismatched, imputation accuracy declines significantly.

Reference Panel Resources and Limitations

- 1000 Genomes Project: ~7.7M SNPs.
- Haplotype Reference Consortium (HRC): 64,976 haplotypes, > 39 M SNPs (minor allele count ≥ 5).
- TOPMed Reference Panel: ~97K deeply sequenced genomes, >300M variants.
- Imputation accuracy depends on:
  - Reference panel size. Small panels can overfit and yield unstable imputations.
  - Population match. The study cohort should resemble the reference.
- Whole-genome sequencing (WGS) data with large sample sizes are very useful as reference datasets for imputation, especially for low-frequency variants.

Imputation Model

- Think of an individual's genotype as a mosaic of two haplotypes drawn from a reference set – this is what enables imputation.
- Let H be the reference haplotypes and G_i the genotype vector for individual i.
- Model the likelihood by summing over latent haplotype pairs Z :

$$P\left(G_i \mid H, \theta, \rho\right) = \sum_Z P\left(G_i \mid Z, \theta\right) P(Z \mid H, \rho)$$

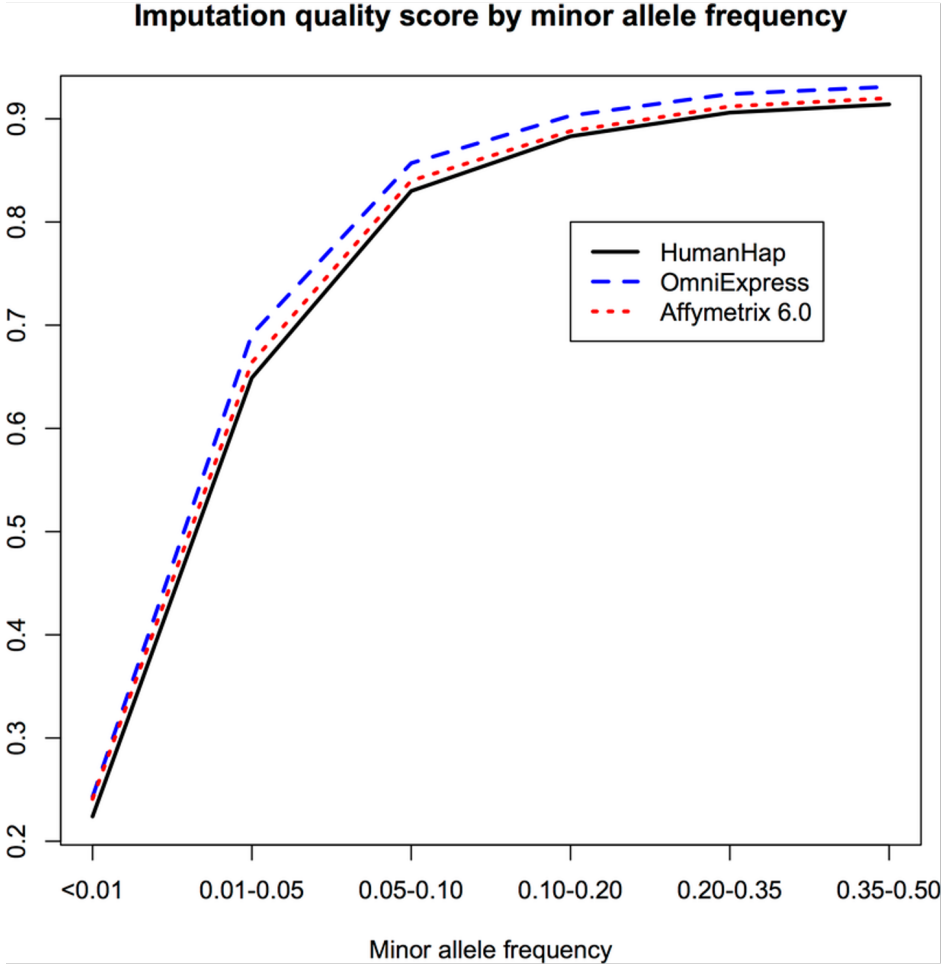
- Each Z is a pair of haplotype sequences assembled from the reference, allowing for recombination (ρ).
- Include a small mutation rate (θ) so Z can generate G.

Imputation Model

- Goal: given θ, ρ, and H, evaluate $P\left(G_i \mid H, \theta, \rho\right)$ for each individual.
- This is computationally heavy and is typically implemented with Hidden Markov Models (HMMs).
- Common software: IMPUTE, MACH, BEAGLE.
- The Michigan Imputation Server: https://imputationserver.sph.umich.edu/index.html.

Minor Allele Frequency (MAF) Effect

- Common SNPs are imputed more accurately than rare SNPs.



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Using Imputed Genotypes

- Accuracy matters $\rightarrow$ **Poor imputation** can create **false positives**.
- SNPs flagged as associated are often **regenotyped*** for confirmation.
- Imputation yields probabilities for the three genotypes; use them in downstream association analyses.
    - For each SNP and each individual: $P(AA), P(Aa), P(aa)$.

Example

SNP	p_0	p_1	p_2	AF1
1	0.85	0.14	0.01	0.35
2	0.01	0.98	0.01	0.44
3	0.96	0.039	0.001	0.02
4	0.87	0.11	0.02	0.04

- In the example, SNPs shows high confidence in the imputed genotype.

Choices for Association Analyses

1. **Multiple imputations** (draws from genotype posteriors).
2. **Best-guess genotype**: $\arg\max\{P(AA), P(Aa), P(aa)\}$.
3. **Expected allele count (dosage)**: E [alleles] $= 2P(AA) + P(Aa)$.

- Quality metric: r^2 between best-guess genotype (or true genotype when masked) and the expected allele count.
- Validation approach: hide some SNP genotypes, run imputation, and compare the predictions to the masked truth.
- Filter low-quality sites, e.g., exclude SNPs with $r^2 < 0.9$ (threshold can vary by study).

Information Measure After Imputation

- How much information did imputation add for SNP l ?

$$\mathrm{INFO}_l = 1 - \frac{1}{n}\sum_{i=1}^{n}\frac{v_{il}}{w_l}$$

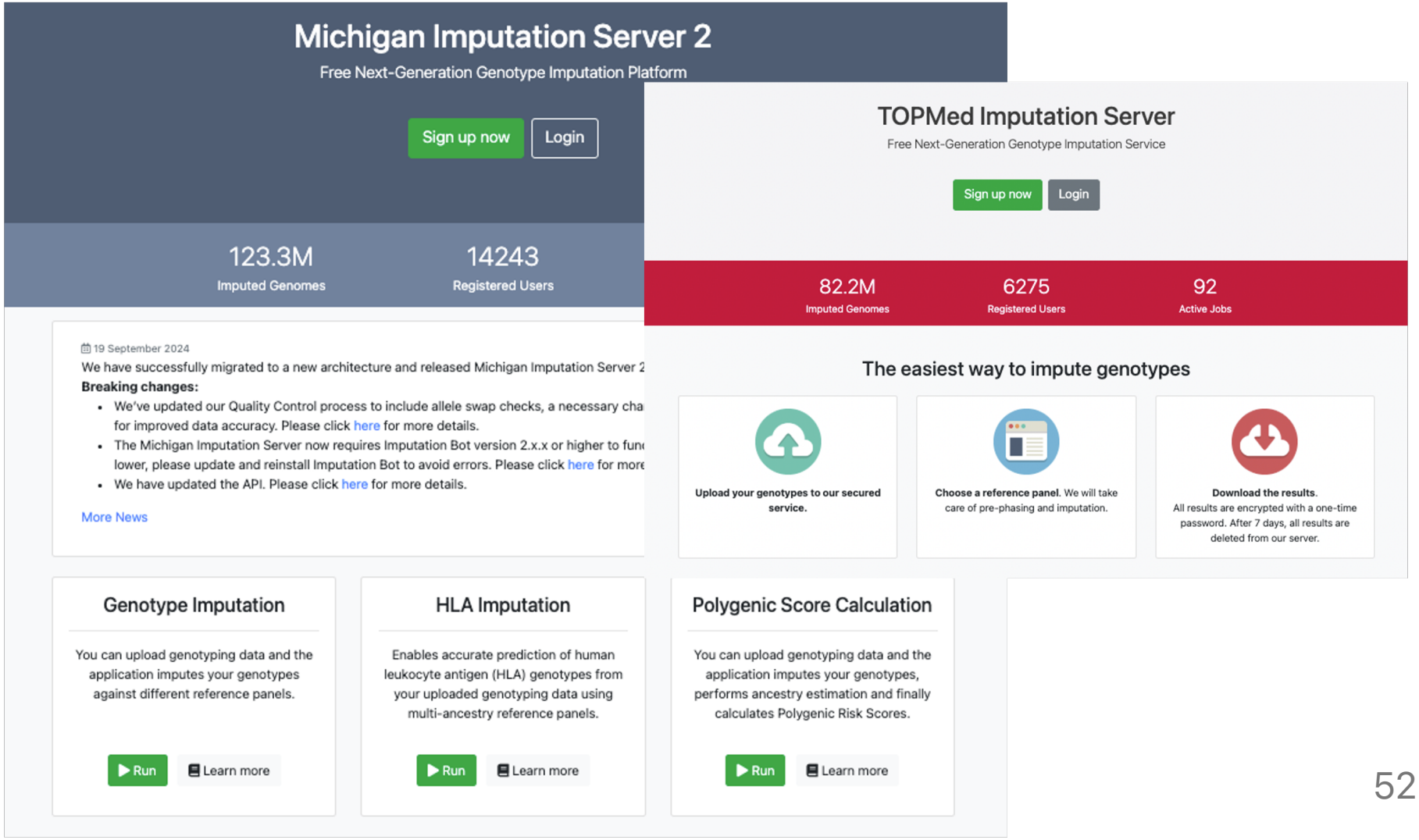
where $v_{il} = \mathbb{E}\left(x_{il}^2 \mid p_i\right) - \left[\mathbb{E}\left(x_{il} \mid p_i\right)\right]^2$ is the variance of the imputed genotype for person i, and $w_l = 2f_l\left(1 - f_l\right)$ is the expected variance under HWE (allele frequency f_l).

- Interpretation:
  - INFO = 1: complete certainty in imputation (one of the p's is 1).
  - INFO = 0: no gain beyond what we could have imputed using only allele frequency.

Imputation Portals

- Michigan Imputation Server: https://imputationserver.sph.umich.edu/index.html#!:
  - upload GWAS genotypes (VCF/PLINK), select a reference panel, receive phased + imputed data.
- TOPMed Imputation Server: https://imputation.biodatacatalyst.nhlbi.nih.gov/#!

Imputation Portals



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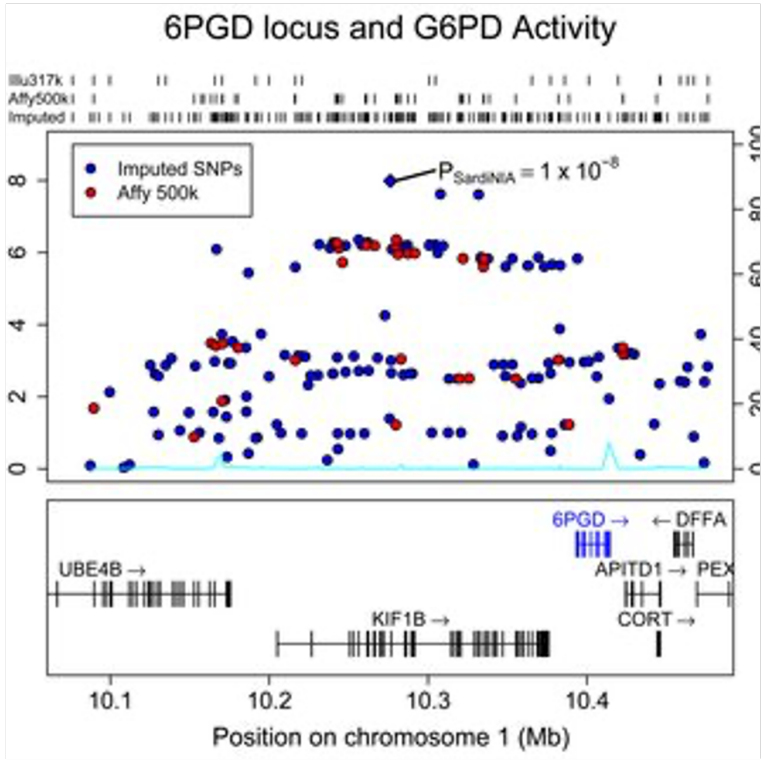
Application

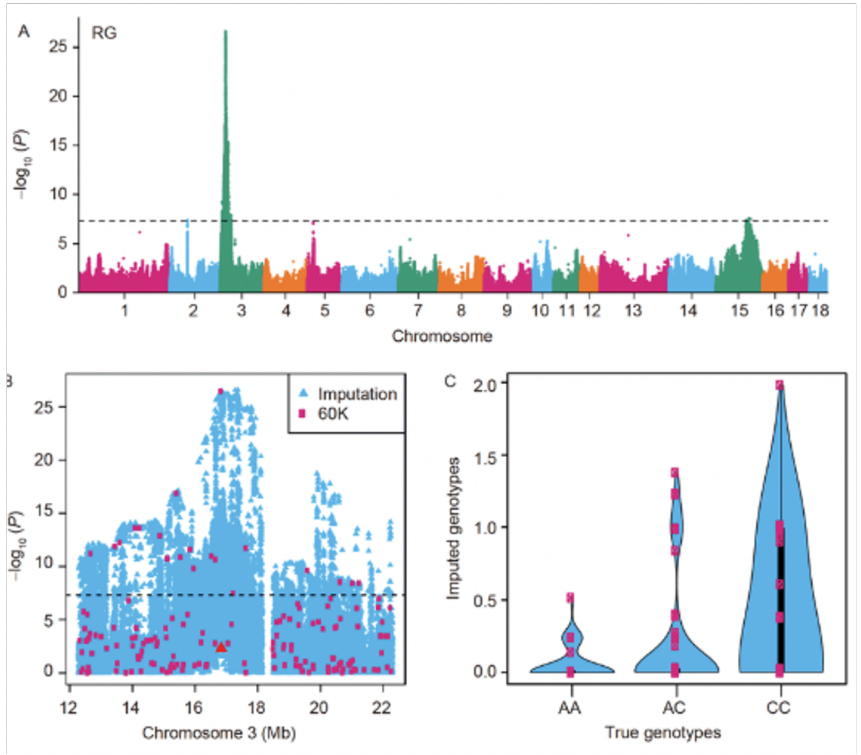
- Imputed vs. array SNPs: Imputation adds dense coverage across the region, beyond the 500k array.
- Stronger signal: The top association reaches $P \approx 1 \times 10^{-8}$, clearer than with typed SNPs alone.



Source: Publicly available from Google Images

Application

- Imputed variants densely tile the region and highlight signals missed by the 60k typed set.
- Imputed dosages separate well by true genotypes (AA/AC/CC), indicating high accuracy.



Source: Publicly available from Google Images

Take-Home Point

- Imputation reliably recovers common variants because they sit on long-range linkage disequilibrium (LD) patterns that are well represented in current reference panels.
- For low-frequency and rare alleles, accuracy drops sharply as MAF decreases- posterior dosage r^2 / INFO deteriorates
    - Such alleles are sparsely observed, have weaker LD tags, and are often population-specific.
- Extending the same reliability to rare variants requires larger, ancestry-representative WGS panels, improved phasing, and ancestry-aware algorithms, with stringent quality control and experimental confirmation of key findings.

What's Next

- Quality Control
- GWAS Tutorial
- Linkage and Association
- Post-GWAS Analyses

What questions do you have about anything from today?