

Population Stratification & Genotype Imputation

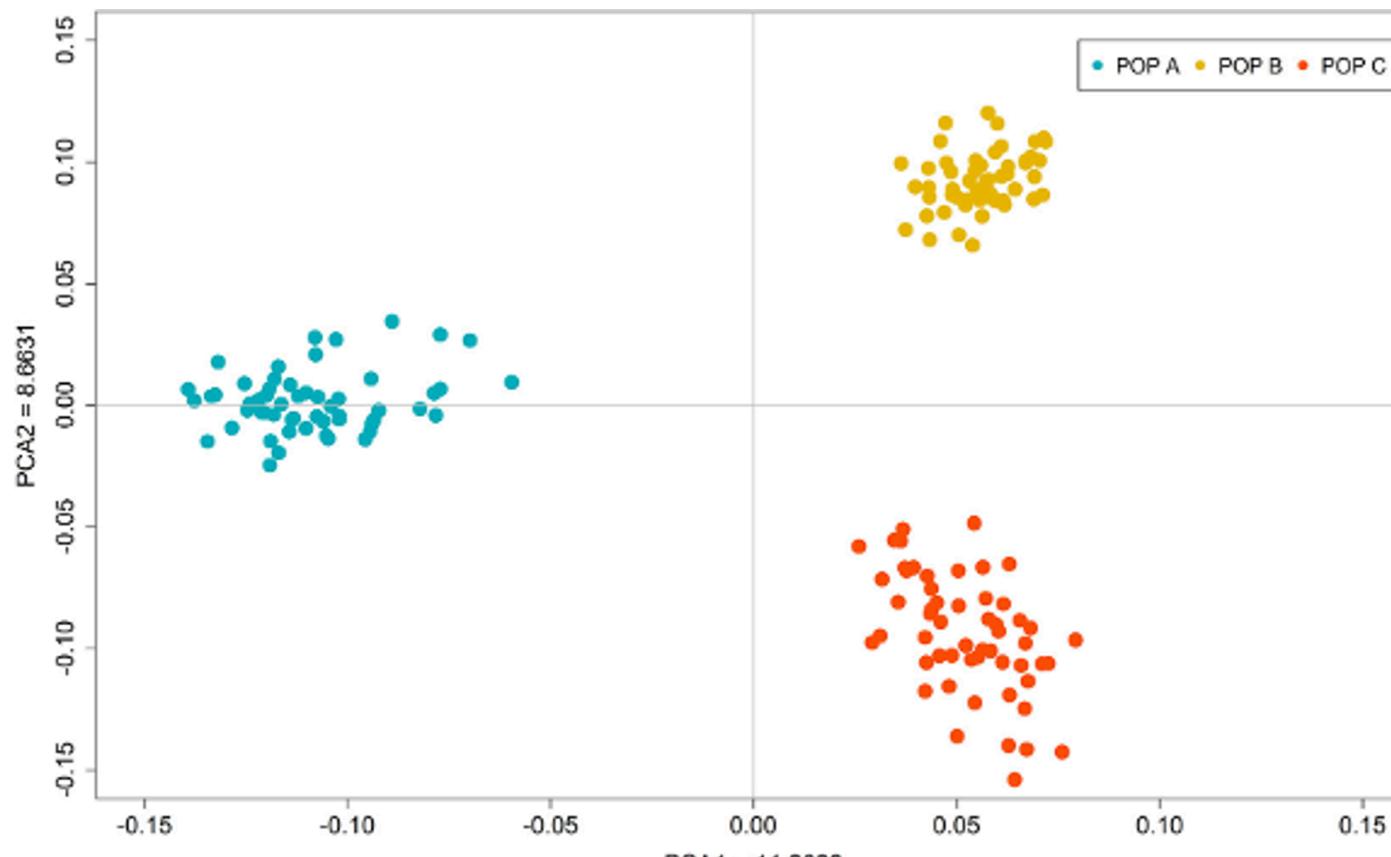
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$ echo "Data Sciences Institute"
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What You'll Learn Today

- **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.

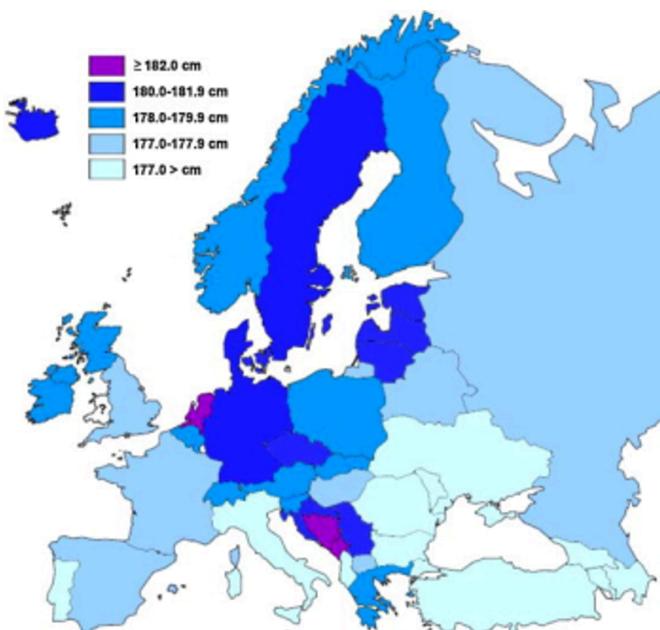


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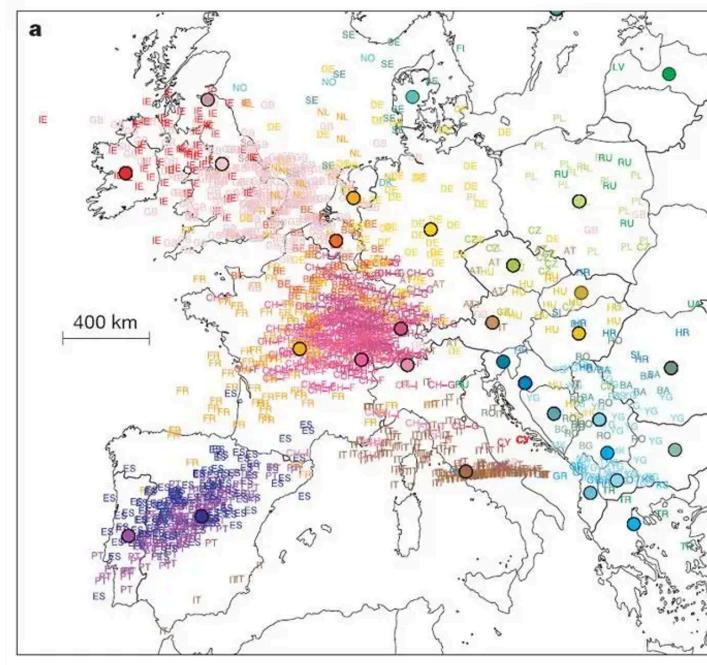
Population Stratification

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

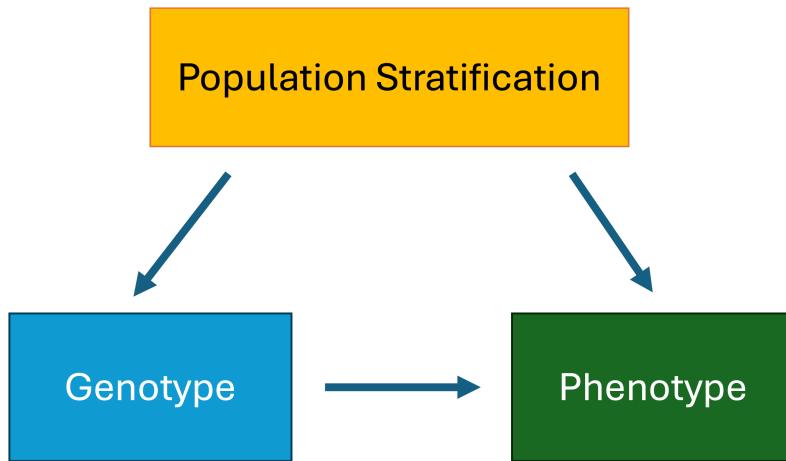
Height varies across countries



Allele frequencies vary as well



Population Structure Acts as a Confounder



Source: Created by Fan Wang

- Failing to account for population stratification results in incorrect variance estimates for the association test statistic → **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

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

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

$$\chi_{l,GC}^2 = \frac{\chi_l^2}{\lambda}, \quad \forall l = 1, \dots, L.$$

Key Principles of Genomic Control

- Most SNPs are expected not associated with the trait.
- If many SNPs show inflated test statistics → evidence of stratification.
- Compare **observed median χ^2** to 0.4549 (expected under the null).
- Ratio measures inflation due to stratification.
- Stratification should affect all loci equally → 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.

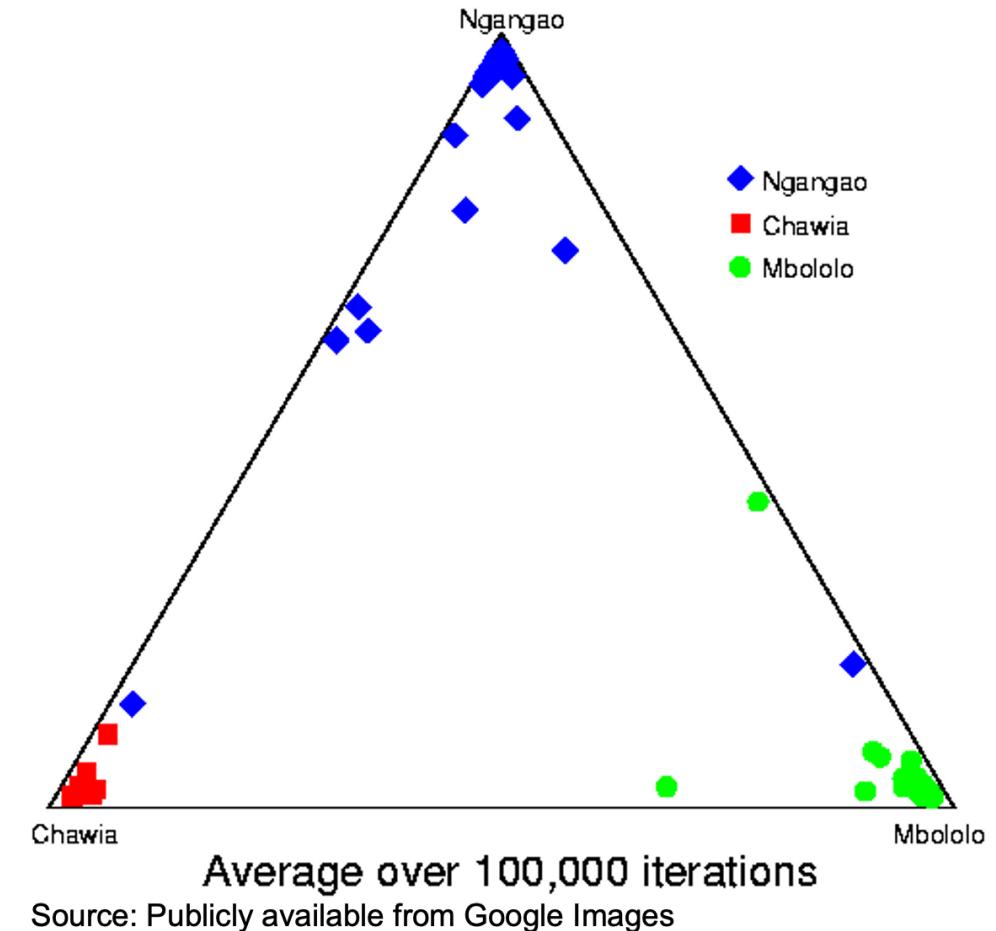
STRUCTURE

- 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

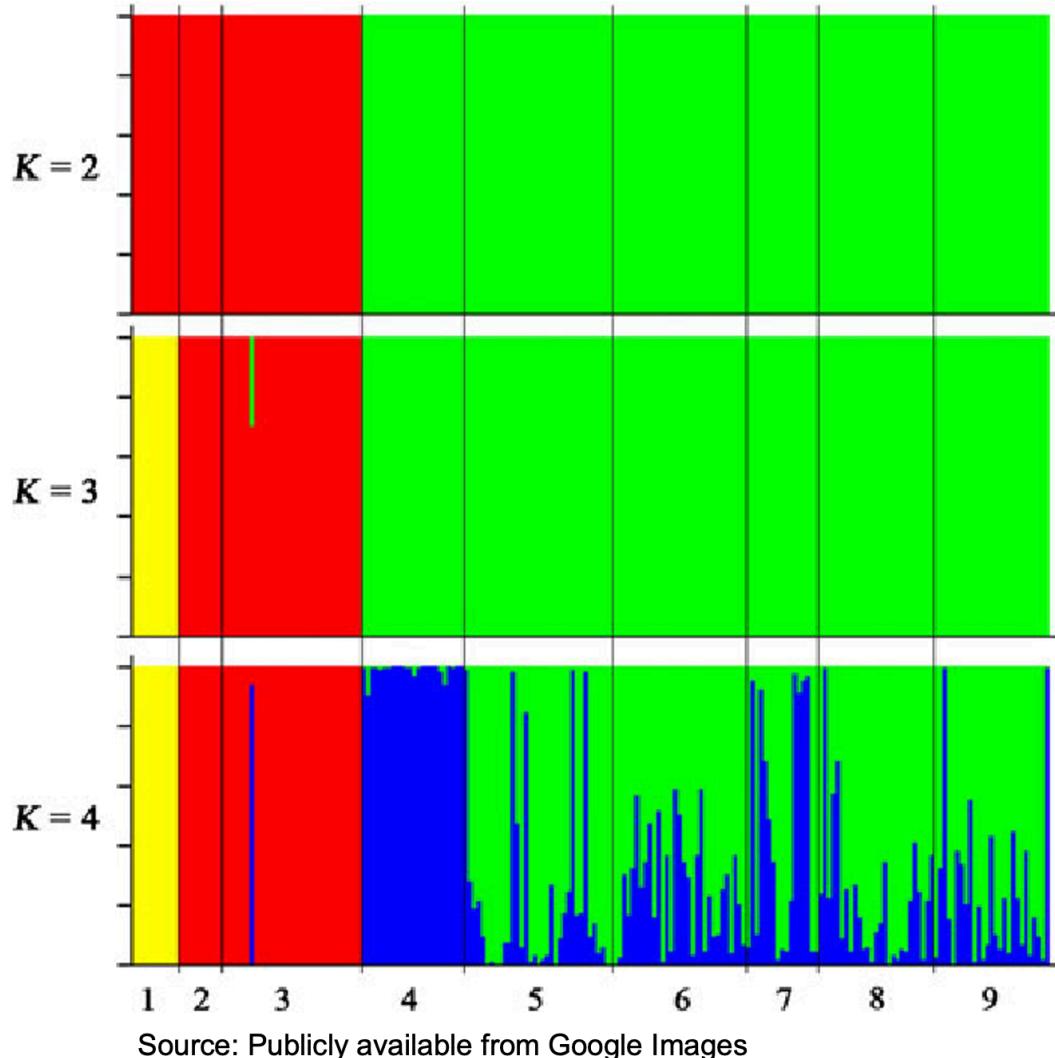
Convergence of Structure: Taita thrush data (K=3).

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

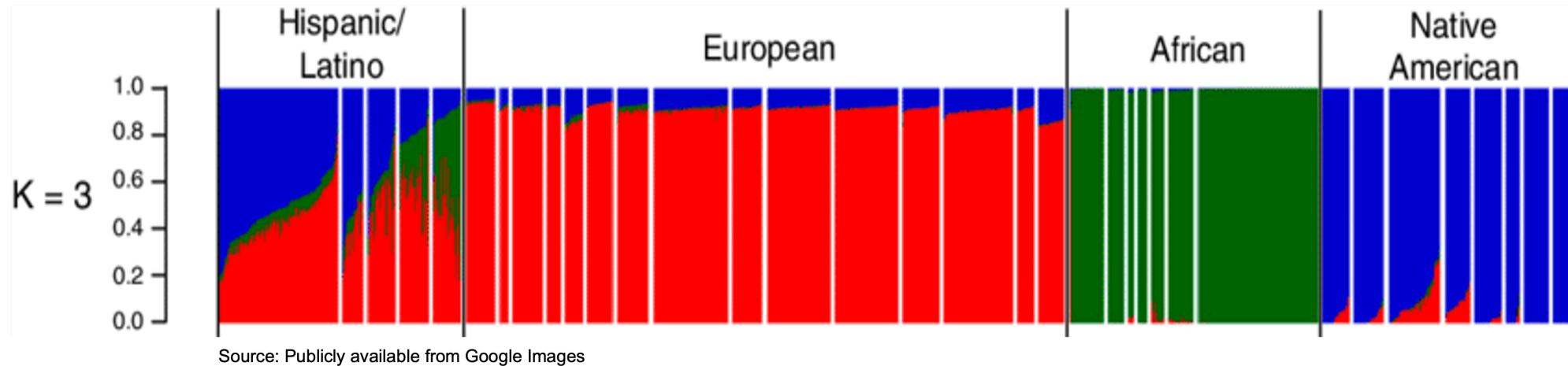


STRUCTURE Bar Plots

- 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.



Applying STRUCTURE to Admixed Populations



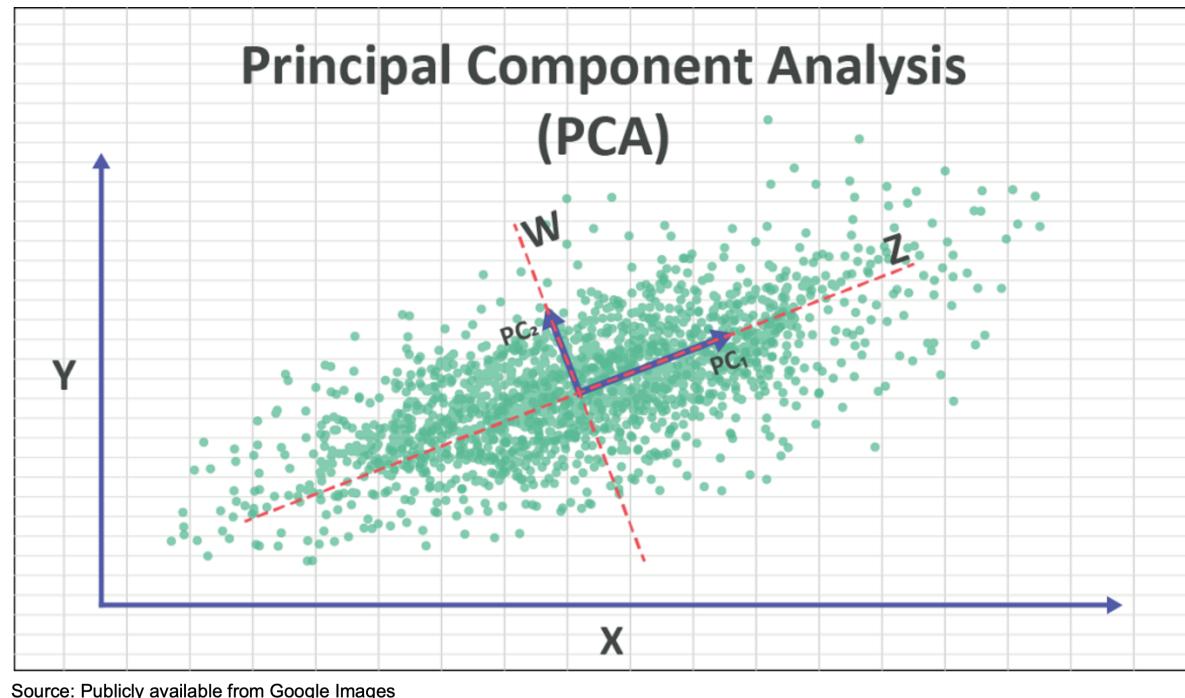
Principal Component Analysis

(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

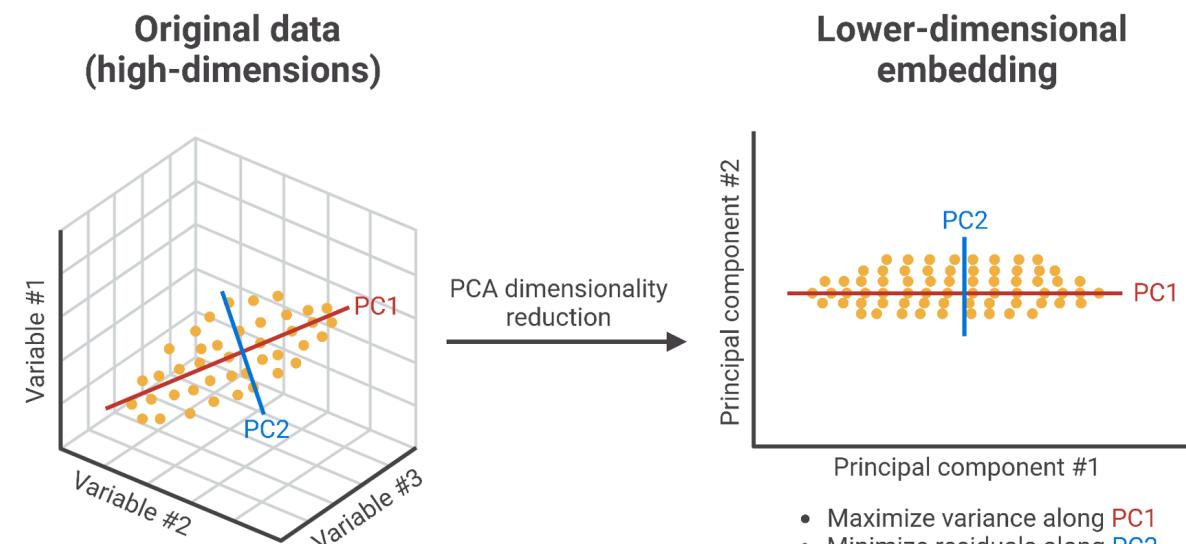
- Principal component analysis
(PCA) is a statistical procedure
that allows us to perform
dimension reduction.
 - Finds orthogonal directions
("principal components") that
capture the **largest possible**
variance in the data.



Geometric Picture

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

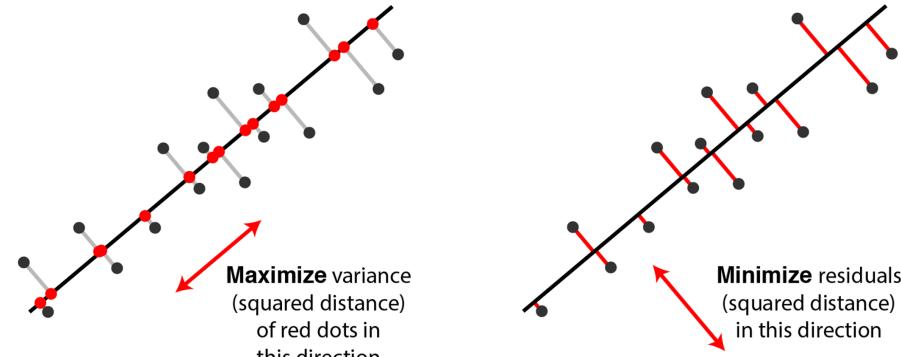
Principal Component Analysis (PCA) Transformation



Source: Publicly available from Google Images

Formal Objective

- Given $X = [x_1, \dots, x_n]^\top$, 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.



Source: Publicly available from Google Images

Optimization Formulation

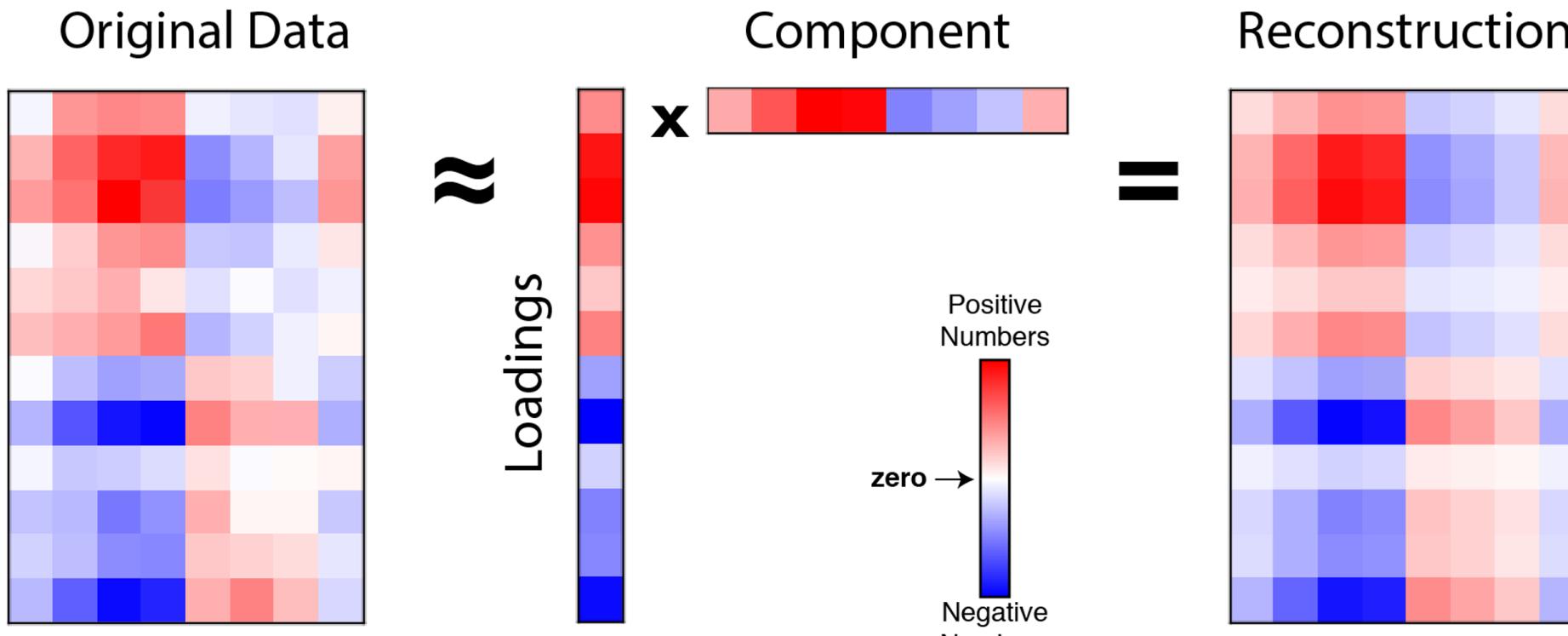
$$\min_{W,C} \|X - WC^\top\|_F \quad \text{s.t. } C^\top C = I_k$$

- 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
→ the top k principal component.
4. Low-rank reconstruction: $X \approx WC^\top$.

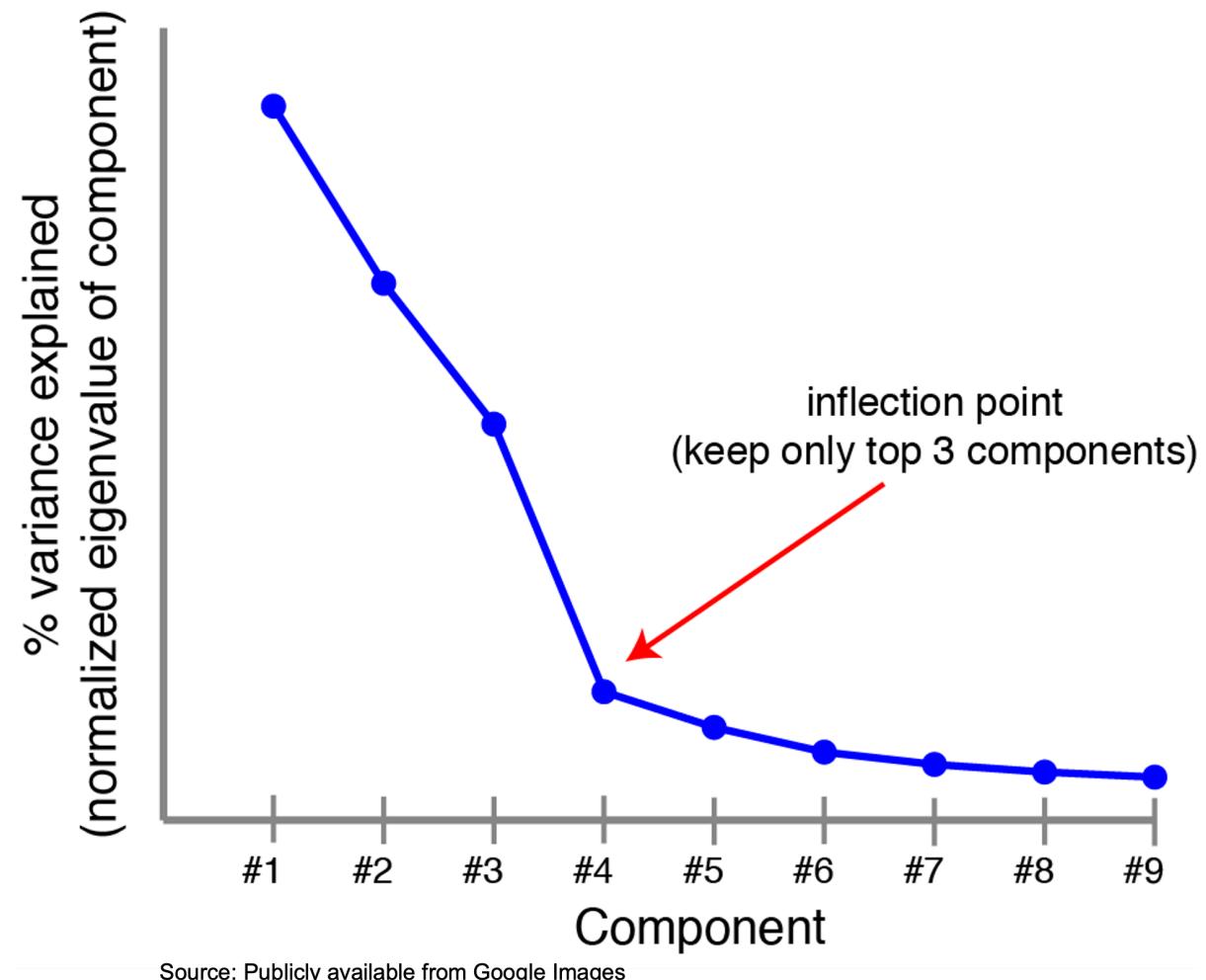
Lower-Dimensional Representation



Source: Publicly available from Google Images

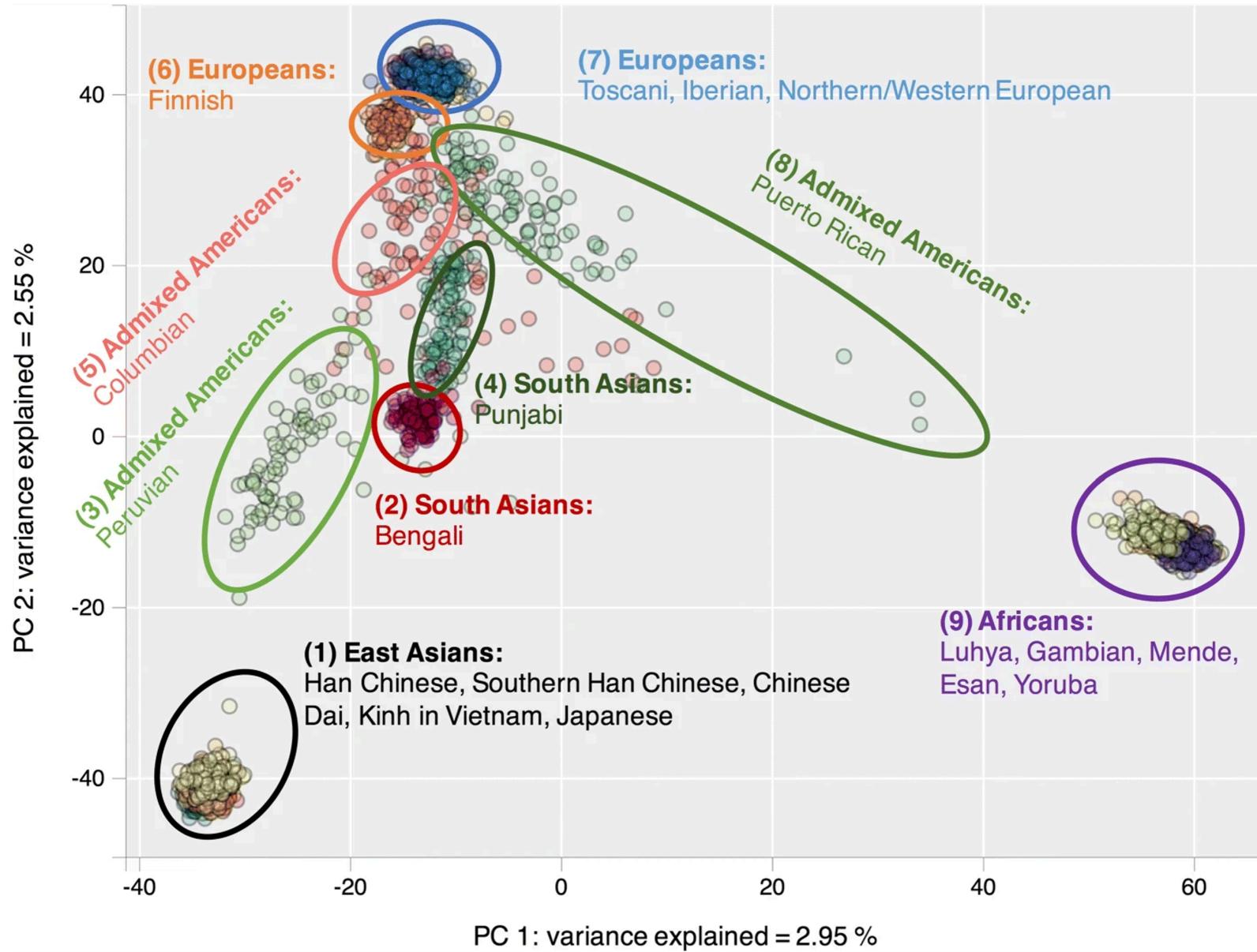
How Many PCs?

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



How can we use PCA?

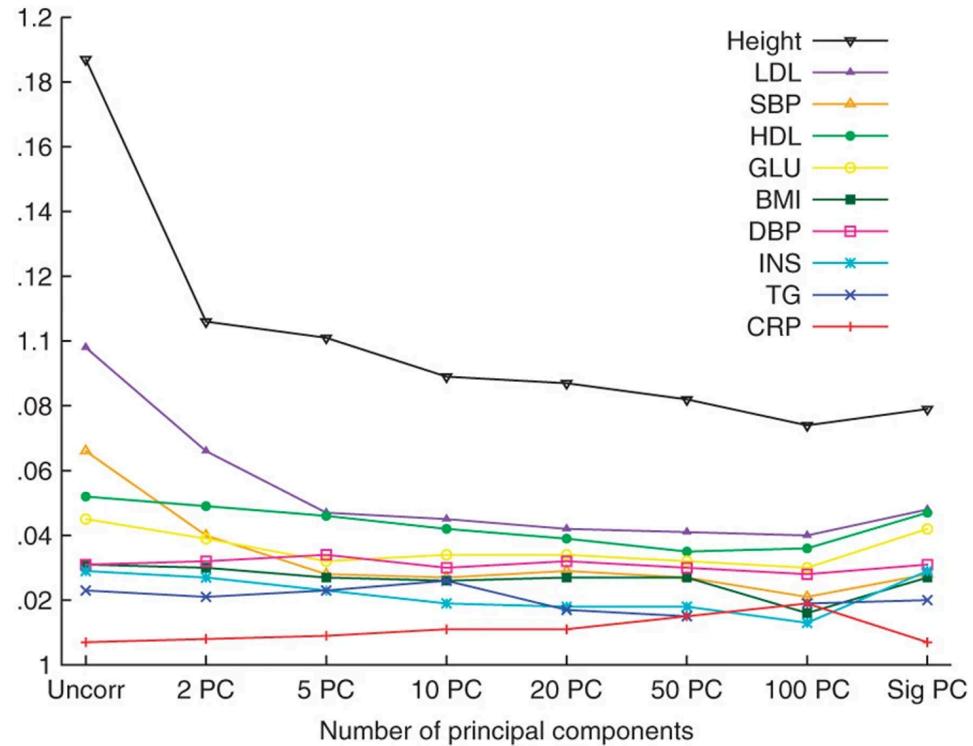
- Data Visualization: Project samples onto the leading PCs (e.g., PC1–PC2) and plot them to reveal clusters and gradients.
- Adjustment for confounding: Include the top PCs as covariates to control for population stratification in association analyses.
- 1000 Genomes PCA example:
http://bwlewis.github.io/1000_genomes_examples/PCA_overview.html



Source: Publicly available from Google Images

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.



Source: Publicly available from Google Images

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. $E(u) = 0$, $\text{Var}(u) = \sigma_g^2 K$.
 - ε : residual, non-genetic noise. $\text{Var}(\varepsilon) = \sigma_e^2 I$.
- 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 :

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

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

Association Testing with LMM (Step 2)

Step 2: Test SNP effects

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

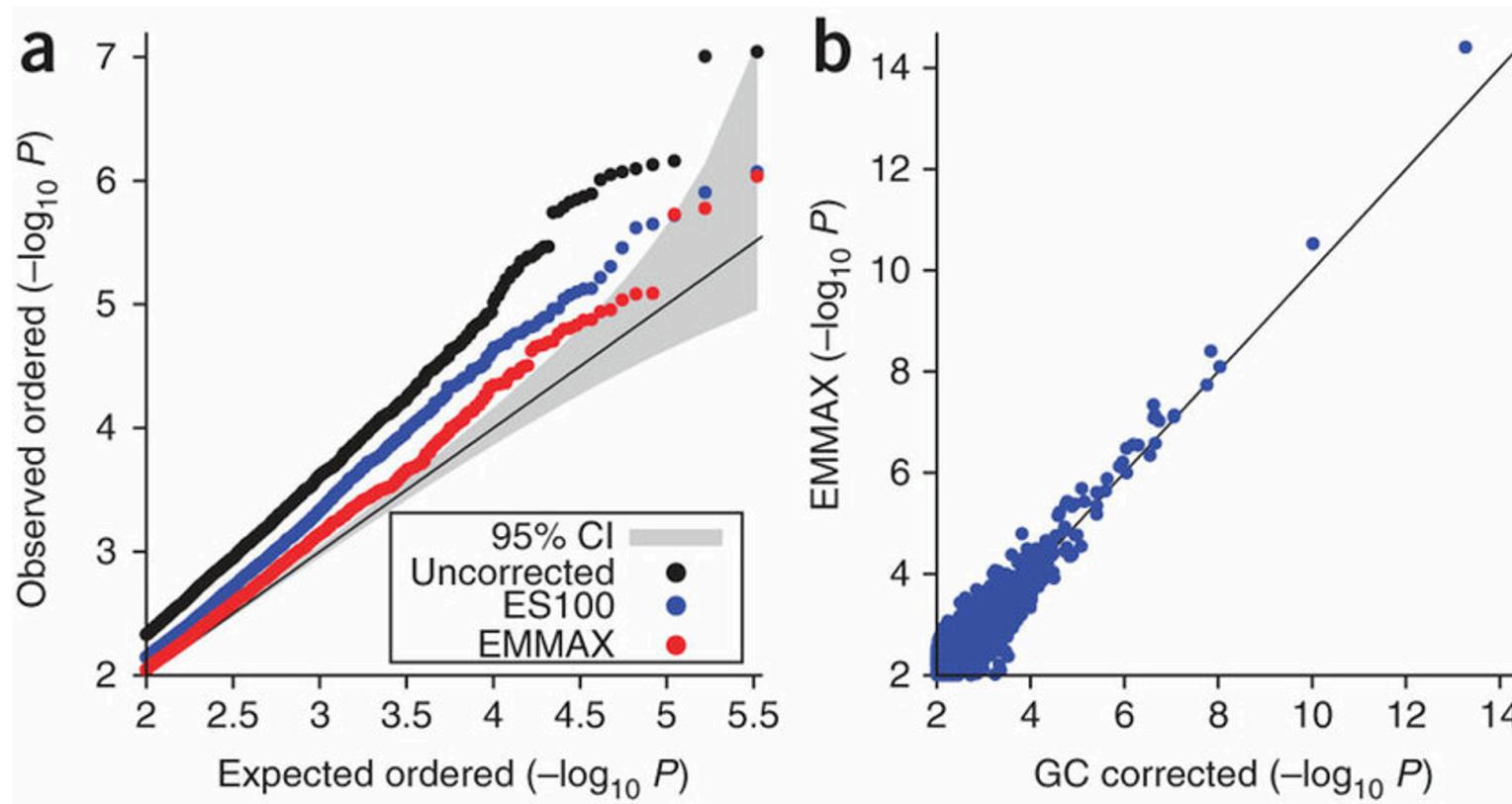
$$Y_{\text{resid}}^* = \tilde{Y} - \hat{u}$$

- 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).

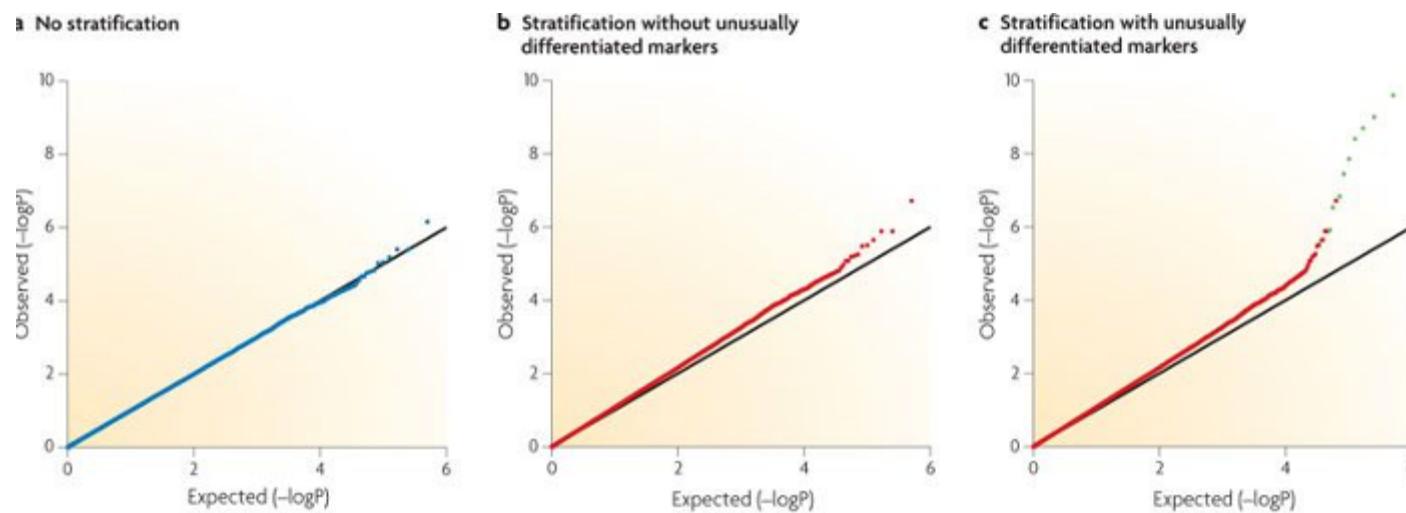
PCA VS. Mixed Model



Source: Publicly available from Google Images

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)

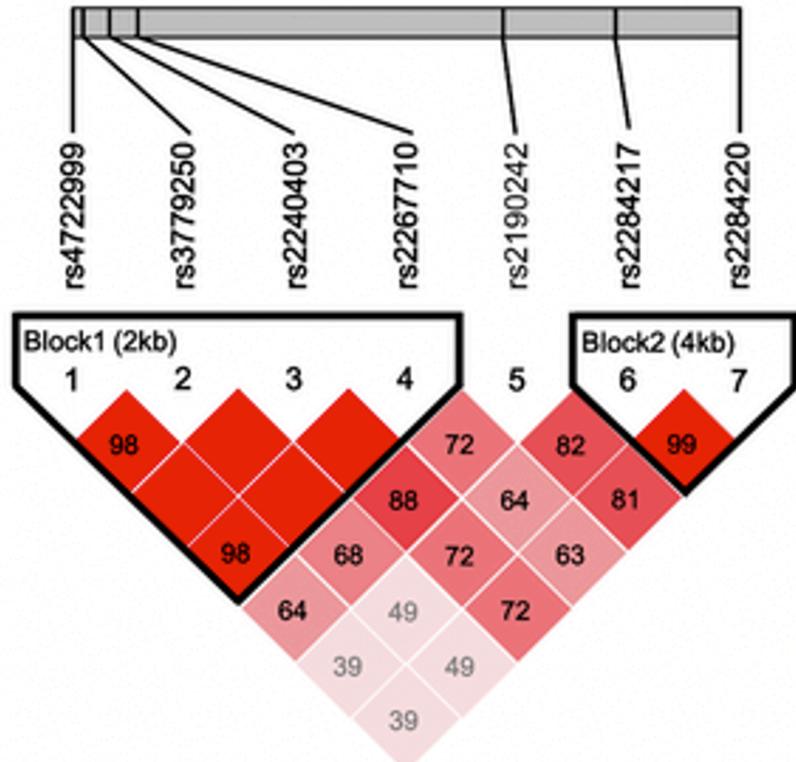
1	?	1	?	1	?	0	2	2	?	2	?	0
0	?	2	?	2	?	0	2	2	?	2	?	0
1	?	2	?	2	?	0	2	1	?	2	?	0
1	?	2	?	1	?	1	2	2	?	2	?	0
2	?	2	?	2	?	1	2	1	?	2	?	0
1	?	1	?	1	?	1	2	2	?	2	?	0
1	?	2	?	2	?	0	2	1	?	2	?	1

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.



Source: Publicly available from Google Images

Principles of Imputation

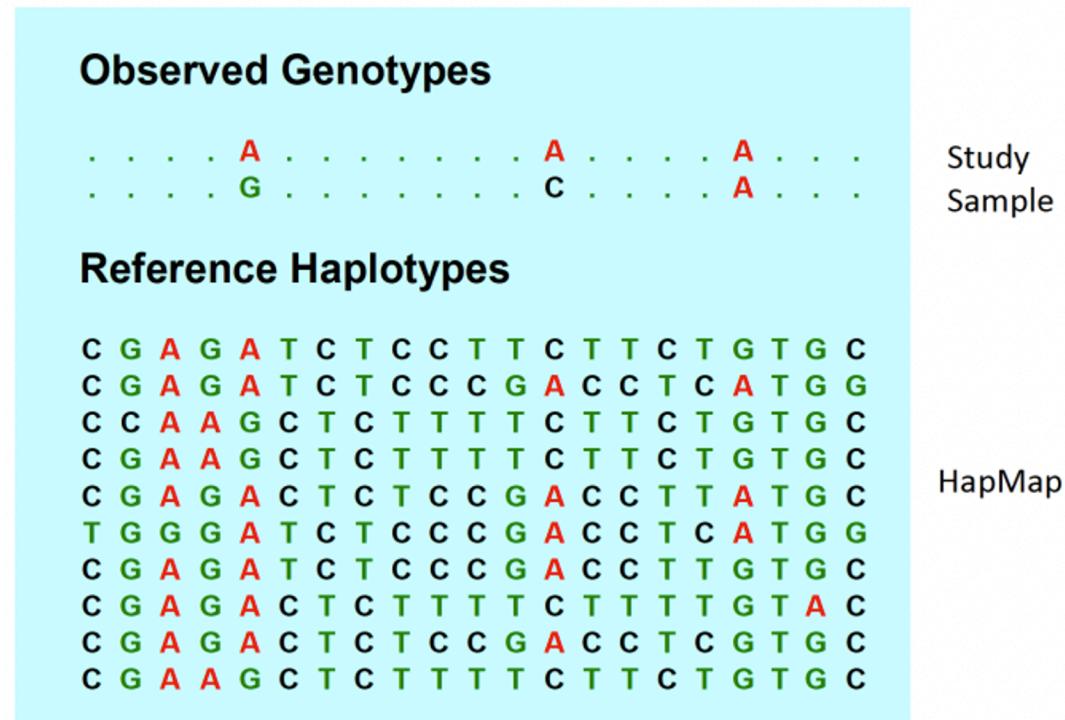
- Imputation methods infer genotypes at untyped sites using patterns from reference panels.
- Often described as **in-silico genotyping**.
- Widely applied in GWAS for:
 - Increasing statistical power.
 - Enabling meta-analysis by harmonizing across studies.

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

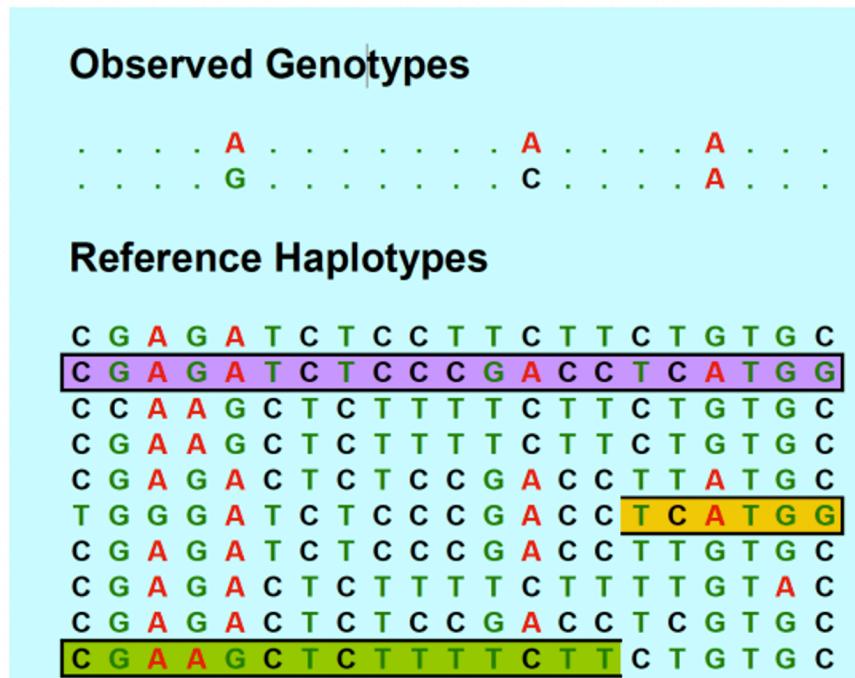
- The reference panel must be sufficiently large and diverse to include a comprehensive set of haplotypes, enabling reconstruction of any individual in the population.



Source: Publicly available from Google Images

Inferring Missing Genotypes

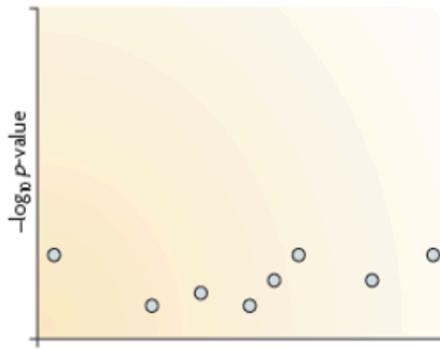
- Once suitable haplotypes are identified, missing alleles can be filled in.
- The imputed genotype data is a probabilistic reconstruction, guided by LD and haplotype structure.



Source: Publicly available from Google Images

Imputation Workflow

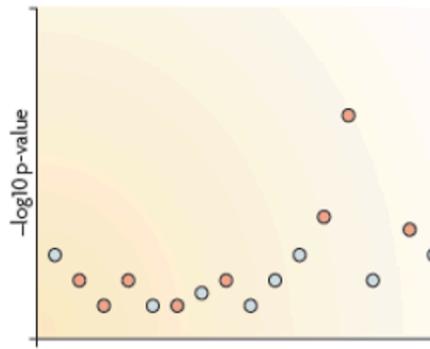
b Testing association at typed SNPs may not lead to a clear signal



d Reference set of haplotypes, for example, HapMap

0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0
1	1	1	1	1	1	1	0	0	1	0	0	1	0	1	1
0	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	0	1	1	1	0	0	1	1	1	1	0	1	1
0	0	1	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	1	1	0	1	0	0	1	0	0	1	0	1	0
1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	0
0	0	0	0	1	1	1	0	0	1	1	1	1	1	1	0
1	1	1	0	0	1	0	0	1	1	1	0	1	1	1	0

f Testing association at imputed SNPs may boost the signal

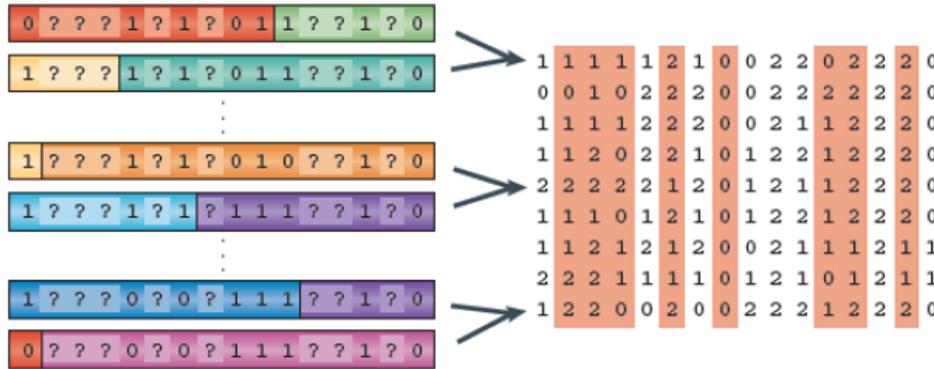


a Genotype data with missing data at untyped SNPs (grey question marks)

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

c Each sample is phased and the haplotypes are modelled as a mosaic of those in the haplotype reference panel

e The reference haplotypes are used to impute alleles into the samples to create imputed genotypes (orange)



Source: Publicly available from Google Images

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 :

$$P(G_i | H, \theta, \rho) = \sum_Z P(G_i | Z, \theta)P(Z | H, \rho)$$

- 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 .

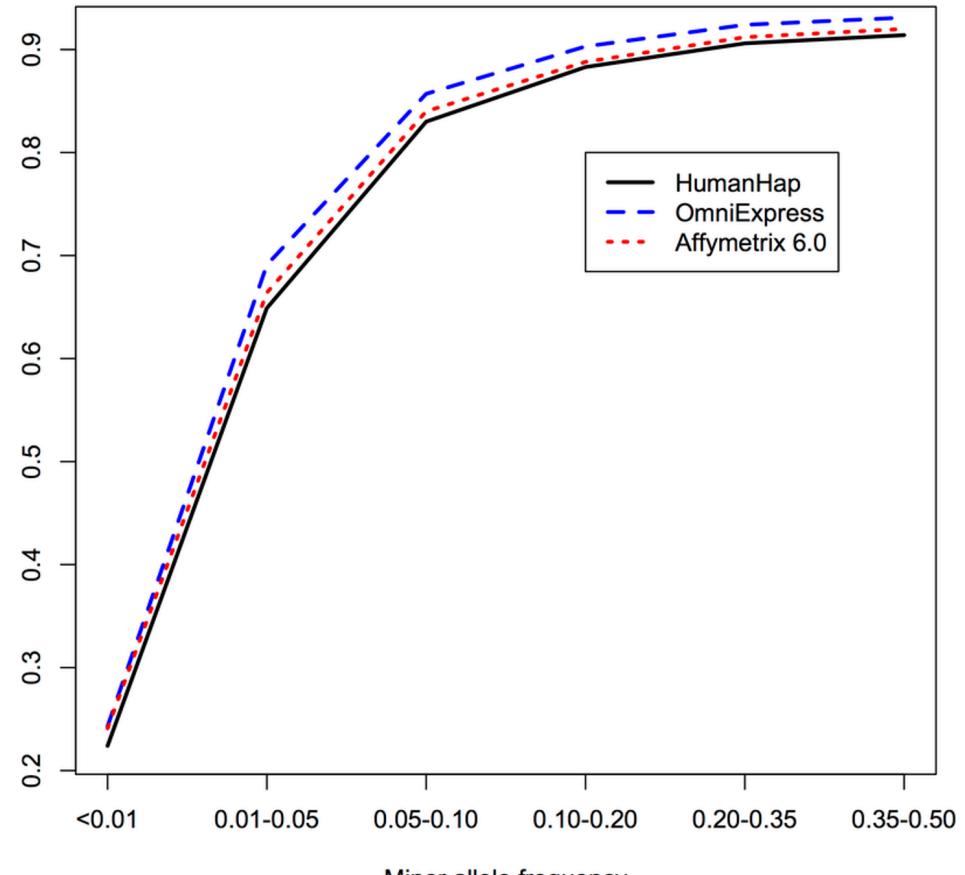
Imputation Model

- Goal: given θ , ρ , and H , evaluate $P(G_i \mid H, \theta, \rho)$ 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.

Imputation quality score by minor allele frequency



Source: Publicly available from Google Images

Using Imputed Genotypes

- Accuracy matters → 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)**: $E[\text{alleles}] = 2P(AA) + P(Aa)$.
 - 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 ?

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

where $v_{il} = \mathbb{E}(x_{il}^2 | p_i) - [\mathbb{E}(x_{il} | p_i)]^2$ is the variance of the imputed genotype for person i , and $w_l = 2f_l(1 - f_l)$ 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

Michigan Imputation Server 2

Free Next-Generation Genotype Imputation Platform

[Sign up now](#) [Login](#)

123.3M
Imputed Genomes

14243
Registered Users

19 September 2024
We have successfully migrated to a new architecture and released Michigan Imputation Server 2.
Breaking changes:

- We've updated our Quality Control process to include allele swap checks, a necessary change for improved data accuracy. Please click [here](#) for more details.
- The Michigan Imputation Server now requires Imputation Bot version 2.x.x or higher to function correctly. If you are using a lower version, please update and reinstall Imputation Bot to avoid errors. Please click [here](#) for more details.
- We have updated the API. Please click [here](#) for more details.

[More News](#)

Genotype Imputation

You can upload genotyping data and the application imputes your genotypes against different reference panels.

[Run](#) [Learn more](#)

HLA Imputation

Enables accurate prediction of human leukocyte antigen (HLA) genotypes from your uploaded genotyping data using multi-ancestry reference panels.

[Run](#) [Learn more](#)

Polygenic Score Calculation

You can upload genotyping data and the application imputes your genotypes, performs ancestry estimation and finally calculates Polygenic Risk Scores.

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TOPMed Imputation Server

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82.2M
Imputed Genomes

6275
Registered Users

92
Active Jobs

The easiest way to impute genotypes

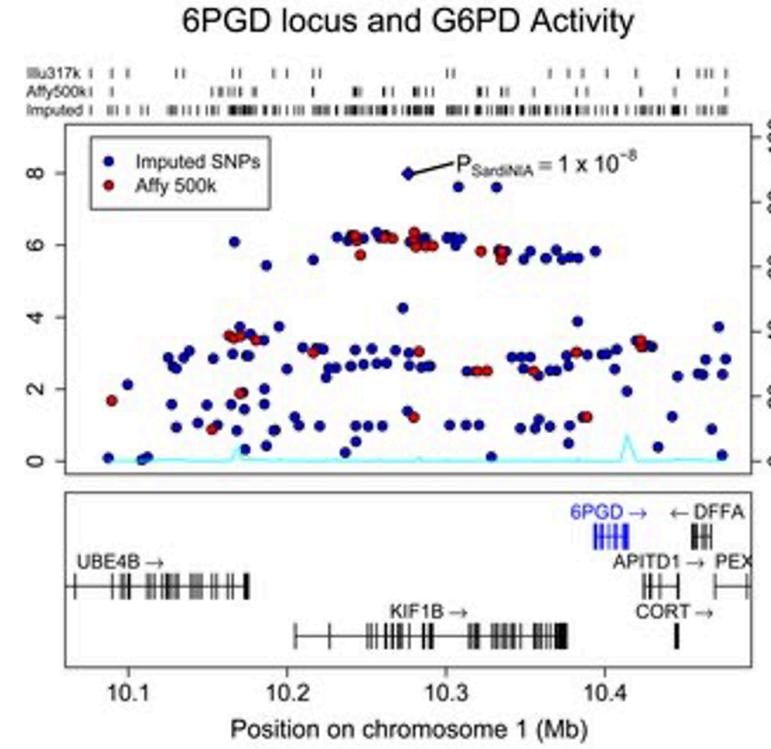
 Upload your genotypes to our secured service.

 Choose a reference panel. We will take care of pre-phasing and imputation.

 Download the results.
All results are encrypted with a one-time password. After 7 days, all results are deleted from our server.

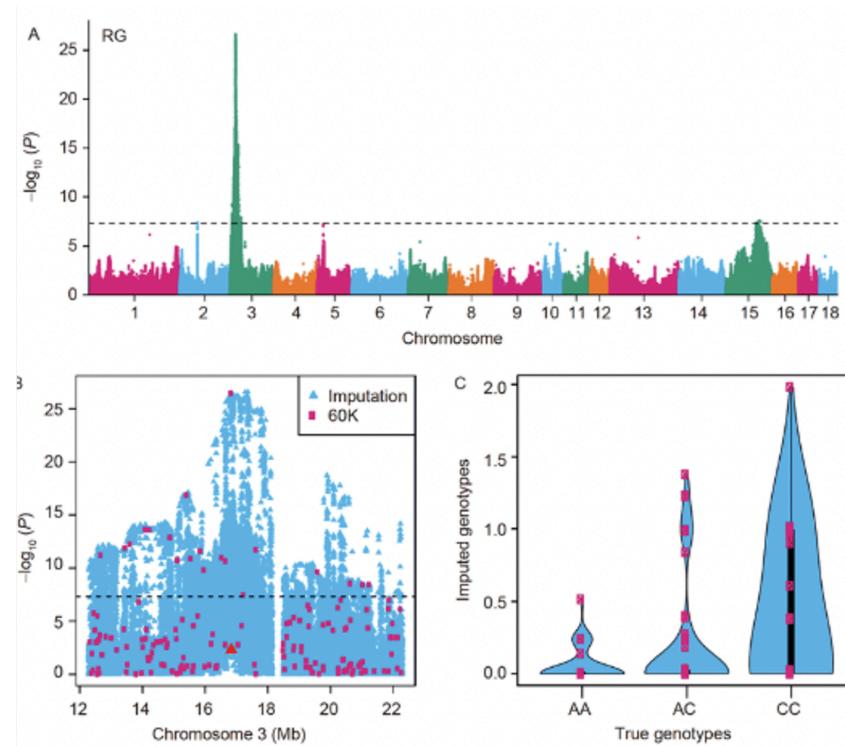
Application

- 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.



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?