

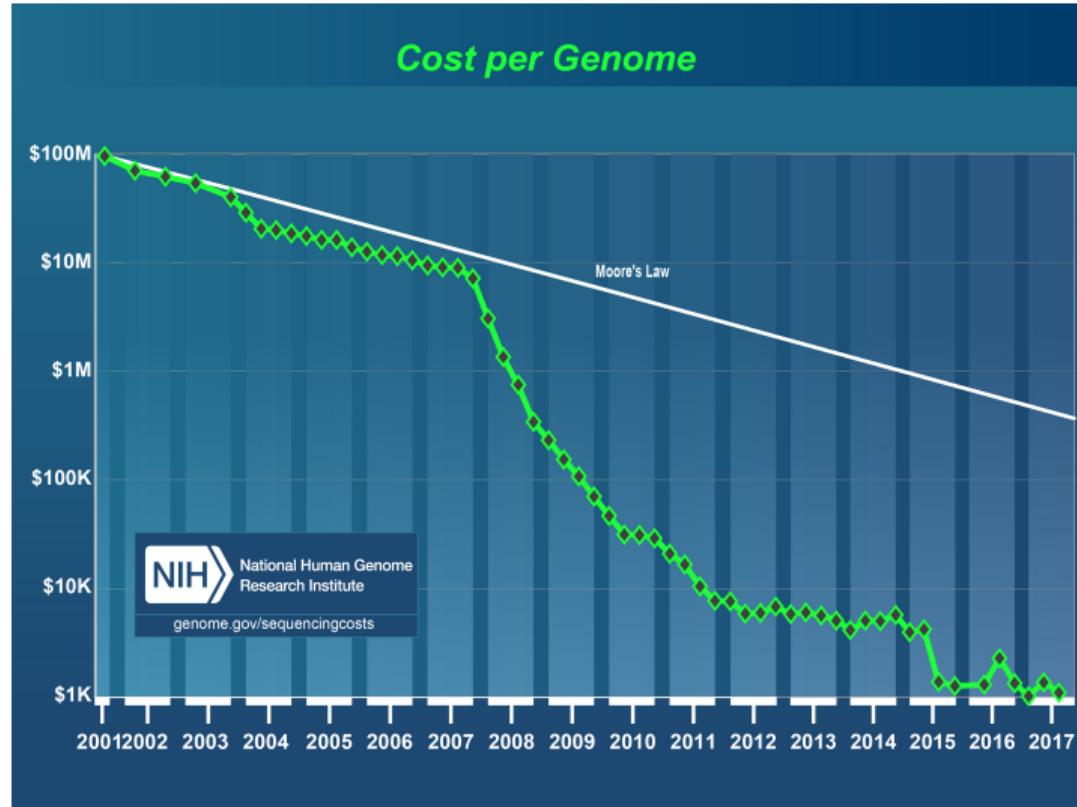
# Factorization Matrix Methods For Association Testing

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# Big data in genomic



## Genomic data

- ▶ The DNA is long sequence of nucleotide : A, C, T, G (3 billion for Human being)
- ▶ Data are single-nucleotide polymorphism called SNPs (10 millions for humain species)
- ▶ There are 2 alleles for each locus.

ADNs {

...	G	A	T	C	C	...
...	G	A	A	C	C	...
...	G	A	A	C	C	...
...	G	A	T	C	C	...
...	G	A	T	C	C	...

**Figure – SNPs illustration** The nucleotide differing between the DNA sequences is a SNP.

## Genomic matrix

Data are put into a matrix of size  $n \times p$  :

$$\mathbf{Y} = \begin{bmatrix} 0 & 1 & 2 & 2 & \cdots & \cdots & \cdots \\ 1 & 1 & 0 & 1 & \cdots & \cdots & \cdots \\ \vdots & \vdots & \vdots & \vdots & \cdots & \cdots & \cdots \\ \vdots & \vdots & \vdots & \vdots & \cdots & \cdots & \cdots \\ 0 & 0 & 2 & 0 & \cdots & \cdots & \cdots \end{bmatrix}$$

**Figure – Genomic matrix illustration.** Each entry of the matrix is number of time a mutant allele is observed for a given individual and locus.

## Problematic

### Latent Factor models for confounder adjustment in genome association studies

- ▶ Olivier François, Kevin Caye. Genome-wide association studies in geographically structured populations. *Molecular Ecology Resources*, papier invité. En révision.
- ▶ Kevin Caye, Olivier François. Optimal confounder adjustment in genome and epigenome-wide association studies. En préparation.
- ▶ Kévin Caye, Olivier François. `lfmm` : An R package for correcting association studies for confounding factors.

## Section 2

Confounders correction in association testing

# Association testing

## Goal

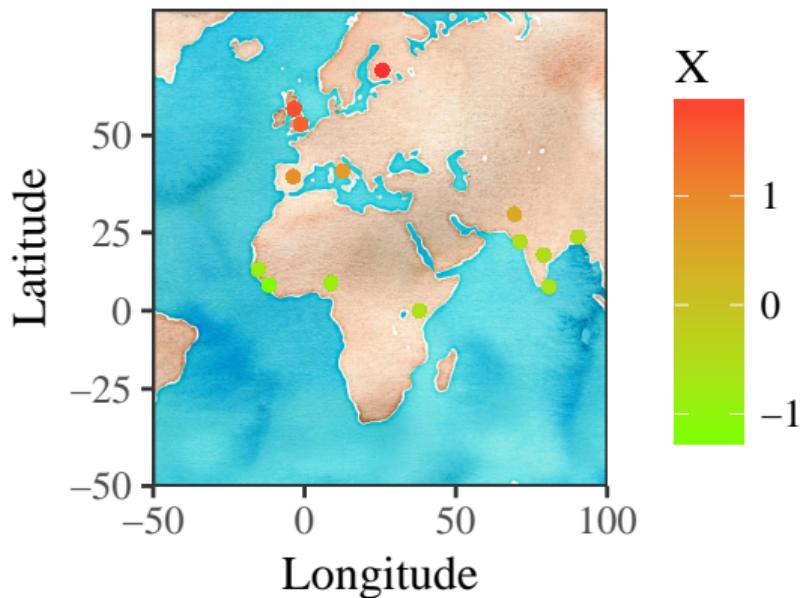
We want to detect SNPs correlated to a variable of interest.

$$\begin{bmatrix} 0 & 1 & 2 & 2 & \dots & \dots & 0 & \dots \\ 1 & 1 & 0 & \dots & \dots & \dots & 1 & \dots \\ \vdots & \vdots & \vdots & \vdots & \dots & \dots & \vdots & \dots \\ \vdots & \vdots & \vdots & \vdots & \dots & \dots & \vdots & \dots \\ 0 & 0 & 2 & 0 & \dots & \dots & 1 & \dots \end{bmatrix} \sim \begin{bmatrix} 0.2 \\ 1.5 \\ \vdots \\ \vdots \\ 0 \end{bmatrix}$$

## Example of variable of interest

- ▶ Disease : diabetes, celiac disease (Dubois et al., 2010)
- ▶ Phenotype : la length (Wood et al., 2014)
- ▶ Environment : the temperature (Frichot et al., 2013)

# Genome wide association study with environmental variable



Genome data come from the 1000Genome project (Consortium, 2015)

- ▶ 1409 individuals from 14 populations
- ▶ 5397214 SNPs

Variable of interest

- ▶ climatic data from WordClim DataBase
- ▶ first principal component

We want to identify SNPs associated with the climate

## Linear regression model

Linear model for each SNP  $\mathbf{Y}_j$

$$\mathbf{Y}_j = \mathbf{X}\beta_j + \mathbf{E}_j,$$

where

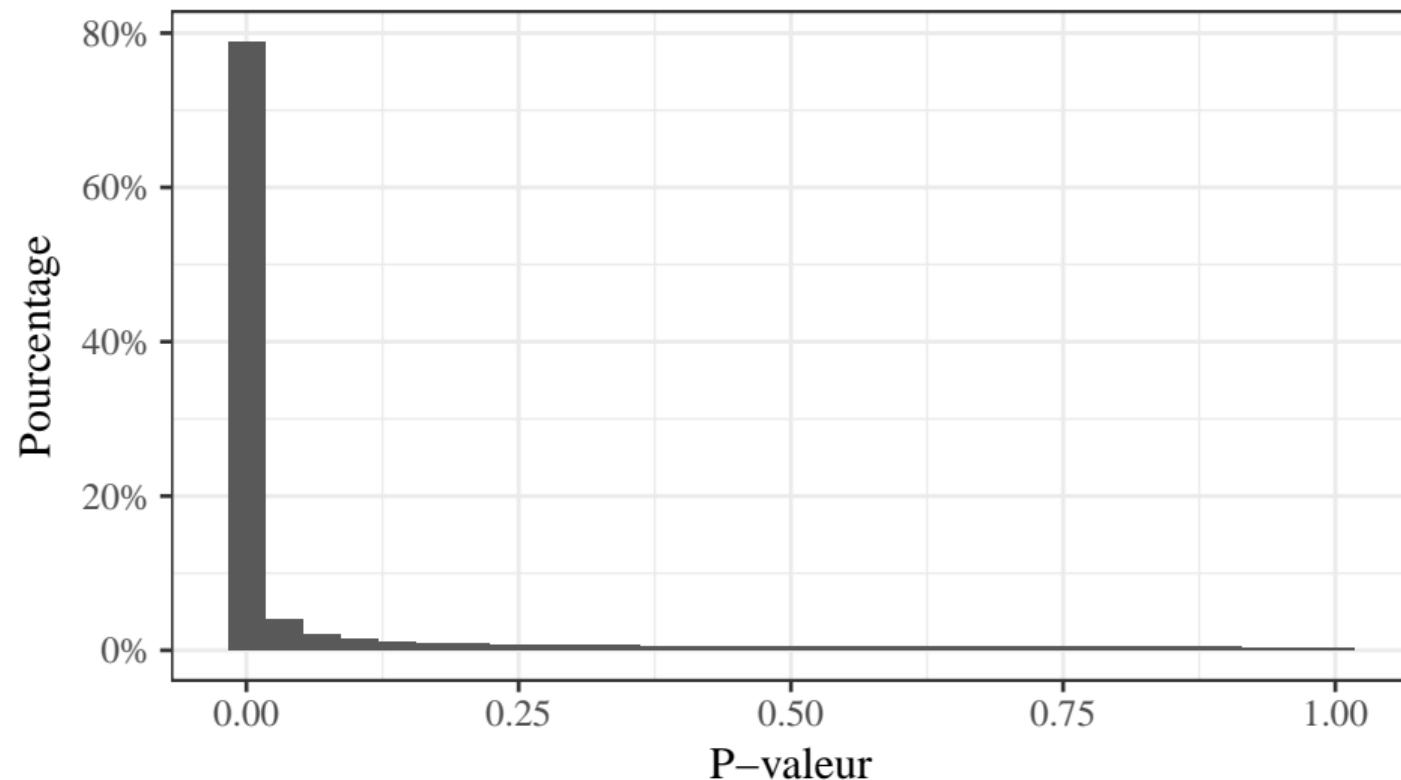
- ▶  $\beta_j$  stand for the effect of the variable of interest  $\mathbf{X}$  on the variable  $\mathbf{Y}_j$
- ▶  $\mathbf{E}_j$  is the residual noise

We want to detect locus for which we can reject the null hypothesis

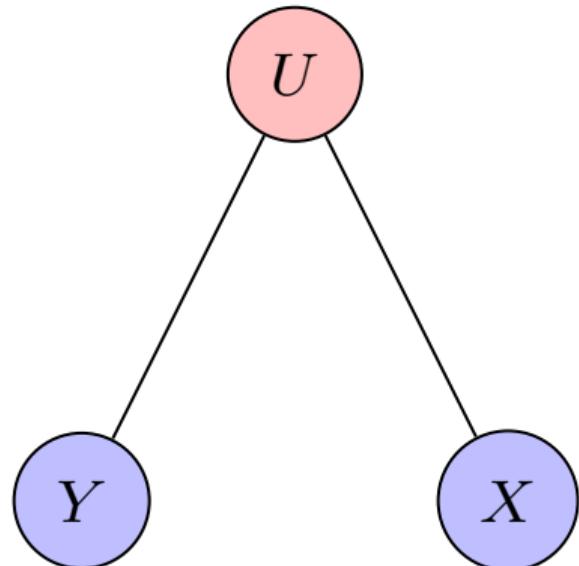
$$H_0 : \beta_j = 0$$

We use the **Student's t-test**

## Histogram of $p$ -valeurs genome-environment association testing



## Latent factor mixed model (LFMM)



$$\mathbf{Y} = \mathbf{XB}^T + \mathbf{UV}^T + \mathbf{E}$$

where

- ▶  $\mathbf{U}$  latent factor matrix of size  $n \times K$
- ▶  $\mathbf{V}$  latent factor effect matrix  $p \times K$
- ▶  $\mathbf{B}$  is the effect of the variable of interest  $\mathbf{X}$  on  $\mathbf{Y}$  of size  $p \times 1$
- ▶  $\mathbf{E}$  is the residual error matrix of size  $n \times p$

## Estimation method for regression model with latent factors

Méthode	Modèle	Algorithme	Référence
sva-twostep	ACP et régression linéaire	moindres carrés ordinaire et SVD	Leek et J. D. Storey, (2007)
sva-irw	<i>weighted</i> -ACP et régression linéaire	moindres carrés ordinaire et <i>weighted</i> -SVD	Leek et J. D. Storey, (2008)
cate	analyse factorielle et régression linéaire	EM ou SVD et estimation des moindres carrés généralisée	Wang et al., (2017)
ridgeLFMM	factorisation matricielle avec régularisation $L_2$	SVD et estimation des moindres carrés régularisée en norme $L_2$	
lassoLFMM	factorisation matricielle avec régularisation $L_1$	<i>soft-thresholded</i> SVD et estimation des moindres carrés régularisée en norme $L_1$	

## L2 regularized least-squares estimates

### Loss function

$$\mathcal{L}_{\text{ridge}}(\mathbf{U}, \mathbf{V}, \mathbf{B}) = \frac{1}{2} \left\| \mathbf{Y} - \mathbf{U}\mathbf{V}^T - \mathbf{X}\mathbf{B}^T \right\|_F^2 + \frac{\lambda}{2} \|\mathbf{B}\|_2^2$$

### Estimates

1. Compute

$$\hat{\mathbf{U}}\hat{\mathbf{V}}^T = \sqrt{\mathbf{P}_\lambda}^{-1} \text{svd}_K(\sqrt{\mathbf{P}_\lambda} \mathbf{Y})$$

where

$$\mathbf{P}_\lambda = \mathbf{Id}_n - (\mathbf{X}^T \mathbf{X} + \lambda \mathbf{Id}_n)^{-1} \mathbf{X}^T \mathbf{X}$$

2. Compute

$$\hat{\mathbf{B}}^T = (\mathbf{X}^T \mathbf{X} + \lambda \mathbf{Id}_d)^{-1} \mathbf{X}^T (\mathbf{Y} - \hat{\mathbf{U}}\hat{\mathbf{V}}^T),$$

## L2 regularized least-squares estimates

If  $\lambda \rightarrow 0$

- ▶  $P_\lambda = \text{Id}_n - (X^T X)^{-1} X^T X$
- ▶  $P_\lambda$  is not invisible
- ▶  $U$  et  $V$  are computed on the residual of the linear regression of  $Y$  by  $X$

Si  $\lambda \rightarrow \infty$

- ▶  $P_\lambda = \text{Id}_n$
- ▶  $U$  et  $V$  is given by the SVD of rank  $K$

## L2 regularized least-squares estimates

### Theorem 1

Let  $\lambda > 0$ . The estimates  $\hat{\mathbf{U}}$ ,  $\hat{\mathbf{V}}$  and  $\hat{\mathbf{B}}$  define a global minimum of the penalized loss function  $\mathcal{L}_{\text{ridge}}$ .

### Idea of the proof

$$\begin{aligned}\mathcal{L}_{\text{ridge}}(\mathbf{U}, \mathbf{V}, \mathbf{B}) &\geq \mathcal{L}_{\text{ridge}}(\mathbf{U}, \mathbf{V}, (\mathbf{X}^T \mathbf{X} + \lambda \mathbf{Id}_d)^{-1} \mathbf{X}^T (\mathbf{Y} - \mathbf{U} \mathbf{V}^T)) \\ &= \frac{1}{2} \left\| \sqrt{\mathbf{P}_\lambda} (\mathbf{Y} - \mathbf{U} \mathbf{V}^T) \right\|_F^2\end{aligned}$$

## L1 regularized least-squares estimates

### Loss function

$$\mathcal{L}_{\text{lasso}}(\mathbf{W}, \mathbf{B}) = \frac{1}{2} \left\| \mathbf{Y} - \mathbf{W} - \mathbf{X}\mathbf{B}^T \right\|_F^2 + \mu \|\mathbf{B}\|_1 + \gamma \|\mathbf{W}\|_*$$

where

- ▶  $\mathbf{W}$  is the latent matrix such that

$$\mathbf{W} = \mathbf{U}\mathbf{V}^T$$

- ▶  $\|\mathbf{W}\|_*$  is the nuclear norm equal to the sum of the matrix  $\mathbf{W}$  eigen values.

## L1 regularized least-squares estimates

block-coordinate descent algorithm

Initialize

$$\hat{\mathbf{W}}_{t=0} = \mathbf{0}$$

$$\hat{\mathbf{B}}_{t=0} = \mathbf{0}$$

Then alternate

1. Compute  $\hat{\mathbf{B}}_t$  the optimum of

$$\mathcal{L}'_{\text{lasso}}(\mathbf{B}) = \frac{1}{2} \|(\mathbf{Y} - \hat{\mathbf{W}}_{t-1}) - \mathbf{X}\mathbf{B}^T\|_F^2 + \mu \|\mathbf{B}\|_1 \quad (1)$$

2. Compute  $\hat{\mathbf{W}}_t$  the optimum of

$$\mathcal{L}''_{\text{lasso}}(\mathbf{W}) = \frac{1}{2} \|(\mathbf{Y} - \mathbf{X}\hat{\mathbf{B}}_t^T) - \mathbf{W}\|_F^2 + \gamma \|\mathbf{W}\|_* \quad (2)$$

# L1 regularized least-squares estimates

## Theorem 1

The block-coordinate descent algorithm for estimating the L1 regularized parameters converge toward a global minimum of the loss function  $\mathcal{L}_{\text{lasso}}$ .

The proof rely on a work of Tseng, (2001)

The main hypothesis are :

- ▶  $\mathbf{W}, \mathbf{B} \mapsto \|\mathbf{Y} - \mathbf{W} - \mathbf{X}\mathbf{B}^T\|_F^2$  is convex and differentiable (the term of attach to data in  $\mathcal{L}_{\text{lasso}}$ )
- ▶  $\mathbf{B} \mapsto \|\mathbf{B}\|_1$  is continuous and convex (regularization term in  $\mathcal{L}_{\text{lasso}}$ )
- ▶  $\mathbf{W} \mapsto \|\mathbf{W}\|_*$  is continuous and convex (regularization term in  $\mathcal{L}_{\text{lasso}}$ )

hypothesis testing corrected for confounders (Price et al., 2006)

For each explained variable  $\mathbf{Y}_j$

$$\mathbf{Y}_j = \hat{\mathbf{U}}\gamma_j^T + \mathbf{X}\beta_j + \mathbf{E}_j,$$

where  $\hat{\mathbf{U}}$  is an estimates of the latent variable matrix.

We test the following hypothesis

$$H_0 : \beta_j = 0$$

We want to the list  $\Gamma = \{1, \dots, J\}$  such that

$$p(\beta_j = 0 | j \in \Gamma) = T$$

where  $T$  is the expected false discovery rate (FDR).

We used the  $q$ -valeur John D Storey et Tibshirani, 2003

## Section 3

### Methods Comparison On Simulations

# Methods comparison on dataset simulated from 1000Genomes dataset

## Simulated Dataset

- ▶ Compute the  $K$  first principal components

$$\mathbf{Y} = \mathbf{U}\mathbf{V}^T + \mathbf{\epsilon}$$

- ▶ Simulate  $\mathbf{U}'$  and  $\mathbf{X}'$  by controlling the correlation.
- ▶ Then create a new matrix

$$\mathbf{Y}' = \mathbf{U}'\mathbf{V}^T + \mathbf{X}'\mathbf{B}'^T + \mathbf{\epsilon}$$

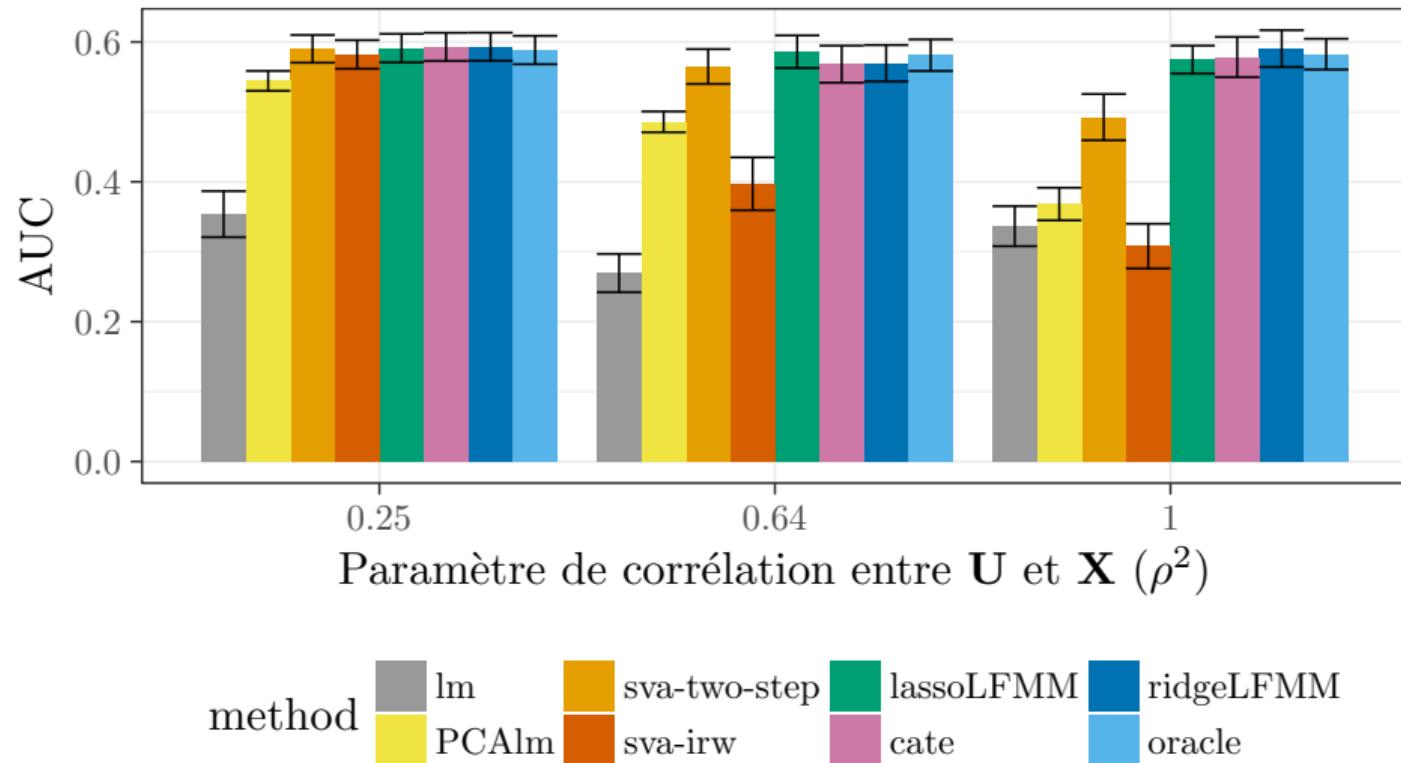
## Criteria

- ▶ AUC : Area under the curve  $(1 - \text{FDR}) \times \text{recall}$  (or power)

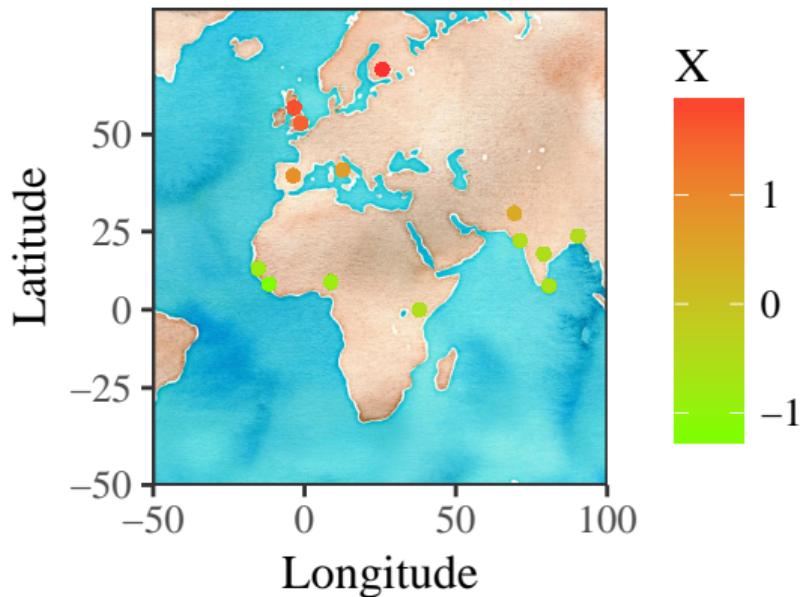
We compared the following method

- ▶ lm
- ▶ lmPCA
- ▶ sva-twostep
- ▶ sva-irw
- ▶ cate
- ▶ oracle
- ▶ ridgeLFMM
- ▶ lassoLFMM

## Result of methods comparison on simulated dataset



# Gene-environment association study (GEA)



Genome data come from the 1000Genome project (Consortium, 2015)

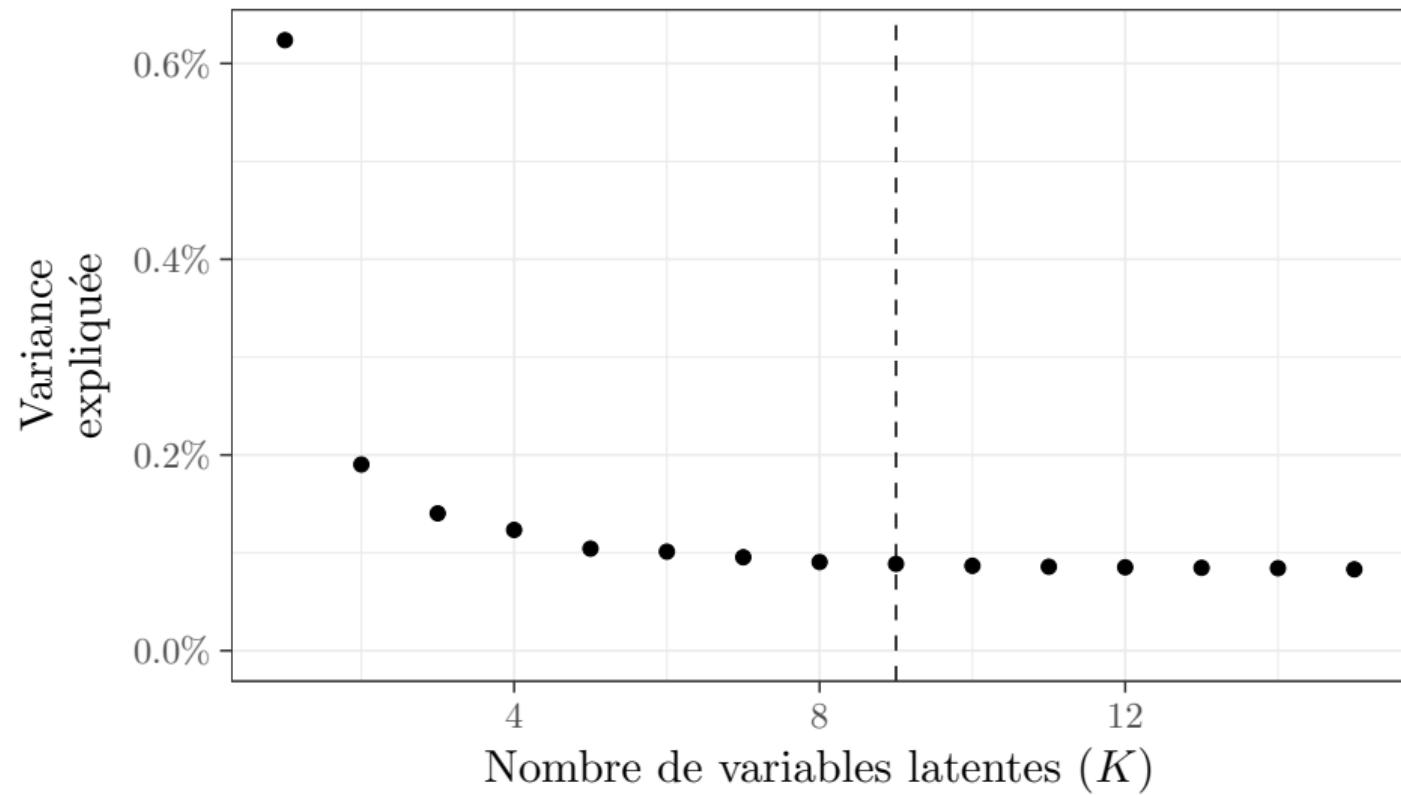
- ▶ 1409 individuals from 14 populations
- ▶ 5397214 SNPs

Variable of interest

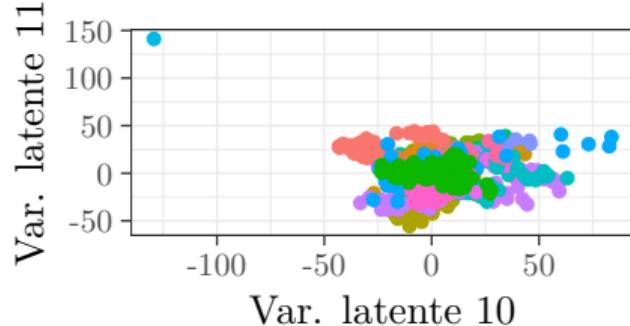
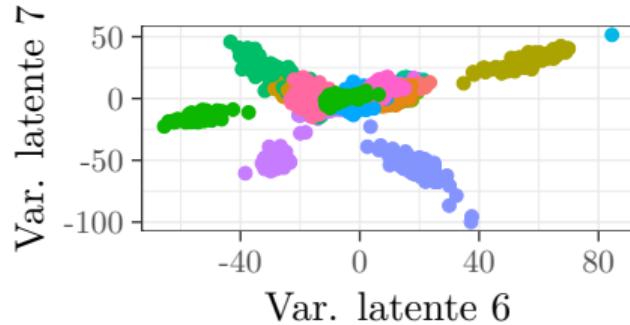
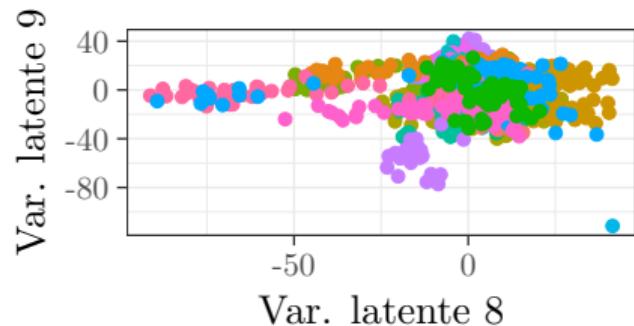
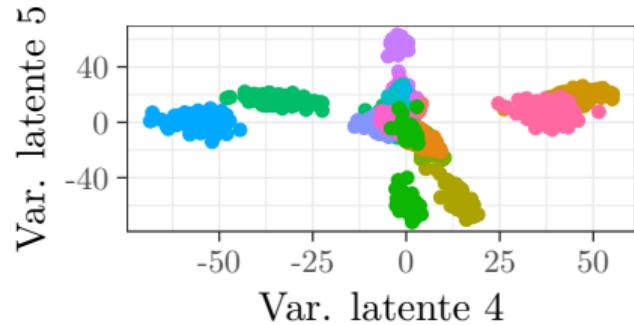
- ▶ climatic data from WordClim DataBase
- ▶ first principal component

We want to identify SNPs associated with the climate

## Choice of K for the gene-environment association study



# Choice of K for the gene-environment association study



# Results of the gene-environment association study

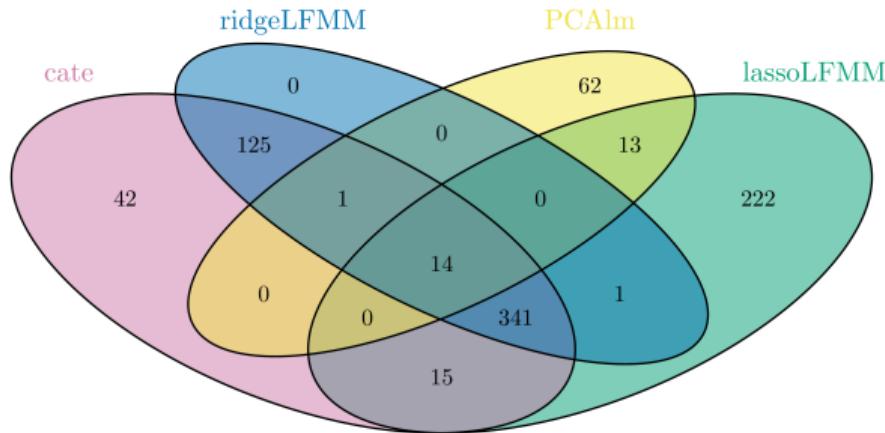


Figure – Venn diagram for an expected false discovery rate of 1%.

## Results of the gene-environment association study

SNPs	Détecté par les méthodes	Description du phénotype	
rs10908907	ridgeLFMM, cate	Alcoholism (heaviness of drinking)	
rs10496731	lassoLFMM	Body Height	
rs2472297	ridgeLFMM, cate, lassoLFMM	Caffeine metabolism	
rs2256175	ridgeLFMM, cate, lassolFMM	Cholesterol total	
rs2472297	ridgeLFMM, cate, lassoLFMM	Coffee consumption (cups per day)	
rs2278544, rs2322659	lassoLFMM	Congenital lactase deficiency	
rs4954218	ridgeLFMM, cate, lassoLFMM	Corneal structure	
rs882300	ridgeLFMM, cate, lassoLFMM	Electrocardiographic traits	
rs882300	ridgeLFMM, cate, lassoLFMM	Electrocardiography	
rs2256175	ridgeLFMM, cate, lassolFMM	Giant cell arteritis	
rs2256175, rs2104012, rs2853977	rs6085576, rs1983716,	ridgeLFMM, cate, lassoLFMM	Height
rs6430549	ridgeLFMM, cate, lassoLFMM	Hematocrit	
rs2278544, rs2322659	lassoLFMM	Lactose intolerance	
rs882300	ridgeLFMM, cate, lassoLFMM	Multiple sclerosis	
rs1123848	ridgeLFMM, cate, lassoLFMM	Neuroblastoma	
rs17158483	lassoLFMM	Obesity-related traits	

# Association study between DNA methylation level and the rheumatoid arthritis (EWAS)

Dataset (Liu et al., 2013)

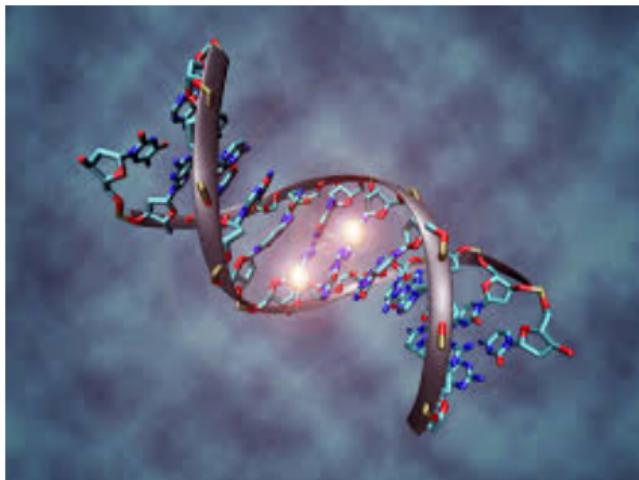
- ▶  $\mathbf{Y}$  contains methylation level for 485577 DNA location for 699 individuals (354 case and 335 control)
- ▶  $\mathbf{X}$  stand for the rheumatoid arthritis

Confounding factors

- ▶ cellular composition
- ▶ age
- ▶ gender
- ▶ tobacco consummation

Goal

Find the methylation sites associated with the rheumatoid arthritis



(Wikipedia, 2017)

## Results of the EWAS

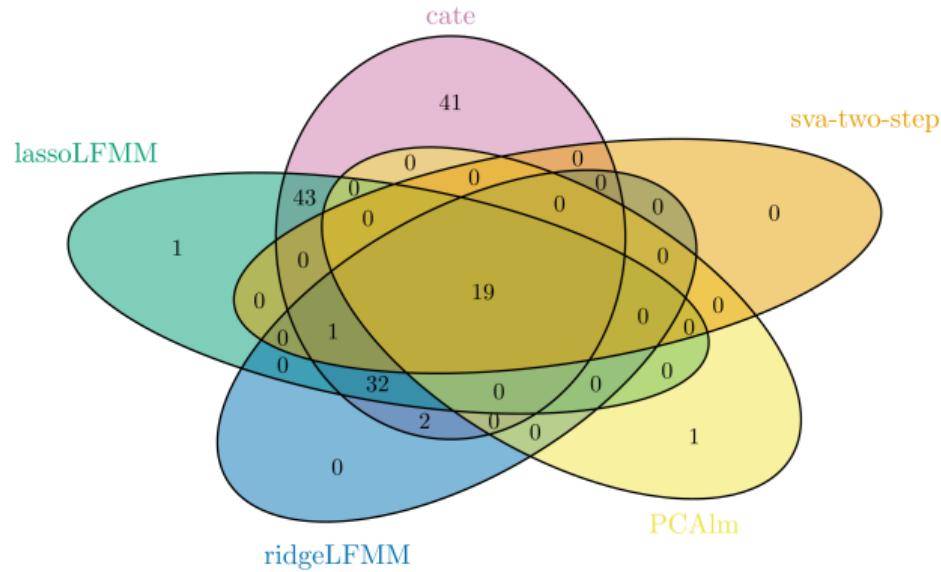


Figure – Venn diagram for an expected false discovery rate of 1 %.

## Methylation sites found out in other studies (Rahmani et al., 2016 ; Zou et al., 2014) (EWAS)

ID	Chr	Position	Gene	PCAlm	lassoLFMM	cate	ridgeLFMM
<b>cg16411857</b>	<b>16</b>	<b>57023191</b>	<b>NLRC5</b>	<b>9.2e-13</b>	<b>2.4e-12</b>	<b>6.6e-12</b>	<b>5.3e-12</b>
<b>cg07839457</b>	<b>16</b>	<b>57023022</b>	<b>NLRC5</b>	<b>1.9e-11</b>	<b>4.5e-11</b>	<b>1.1e-10</b>	<b>9.7e-11</b>
<b>cg05428452</b>	<b>6</b>	<b>32712979</b>	<b>HLA-DQA2</b>	<b>5.4e-11</b>	<b>4.6e-11</b>	<b>8.5e-11</b>	<b>8.8e-11</b>
<b>cg02508743</b>	<b>8</b>	<b>56903623</b>	<b>LYN</b>	<b>2.9e-08</b>	<b>2.7e-08</b>	<b>2.7e-08</b>	<b>2.8e-08</b>
<b>cg20821042</b>	<b>6</b>	<b>32709158</b>	<b>HLA-DQA2</b>	<b>6.5e-08</b>	<b>6.1e-08</b>	<b>9.6e-08</b>	<b>1.0e-07</b>
<b>cg13081526</b>	<b>6</b>	<b>32449961</b>		<b>1.5e-07</b>	<b>1.2e-07</b>	<b>2.0e-07</b>	<b>2.2e-07</b>
<b>cg18052547</b>	<b>6</b>	<b>32552547</b>	<b>HLA-DRB1</b>	<b>1.8e-07</b>	<b>1.8e-07</b>	<b>3.0e-07</b>	<b>3.1e-07</b>
<b>cg25372449</b>	<b>6</b>	<b>32490350</b>	<b>HLA-DRB5</b>	<b>2.5e-07</b>	<b>2.6e-07</b>	<b>4.5e-07</b>	<b>4.6e-07</b>
<b>cg02030958</b>	<b>13</b>	<b>110386267</b>		<b>4.0e-07</b>	<b>7.8e-08</b>	<b>6.0e-08</b>	<b>1.1e-07</b>
<b>cg16171858</b>	<b>3</b>	<b>58472734</b>		<b>4.6e-07</b>	<b>1.6e-07</b>	<b>2.7e-08</b>	<b>3.8e-08</b>
<b>cg03280622</b>	<b>8</b>	<b>145023013</b>	<b>PLEC1</b>	<b>4.7e-07</b>	<b>5.0e-09</b>	<b>5.8e-09</b>	<b>3.8e-08</b>
<b>cg24150157</b>	<b>19</b>	<b>51891210</b>	<b>LIM2</b>	<b>6.2e-07</b>	<b>3.1e-07</b>	<b>1.6e-07</b>	<b>2.1e-07</b>
<b>cg26244575</b>	<b>12</b>	<b>76354015</b>		<b>6.9e-07</b>	<b>2.7e-09</b>	<b>5.0e-10</b>	<b>4.2e-09</b>
<b>cg05370853</b>	<b>6</b>	<b>32606634</b>	<b>HLA-DQA1</b>	<b>7.1e-07</b>	<b>3.0e-07</b>	<b>3.3e-07</b>	<b>4.4e-07</b>
<b>cg14989316</b>	<b>10</b>	<b>80757927</b>	<b>LOC283050</b>	<b>7.3e-07</b>	<b>6.1e-08</b>	<b>7.8e-08</b>	<b>2.1e-07</b>
<b>cg17360552</b>	<b>6</b>	<b>32725332</b>	<b>HLA-DQB2</b>	<b>8.1e-07</b>	<b>6.1e-07</b>	<b>1.1e-06</b>	<b>1.2e-06</b>
<b>cg01373248</b>	<b>3</b>	<b>18480297</b>	<b>SATB1</b>	<b>8.1e-07</b>	<b>1.4e-07</b>	<b>1.1e-07</b>	<b>2.5e-07</b>
<b>cg26164488</b>	<b>2</b>	<b>64440295</b>		<b>9.3e-07</b>	<b>3.5e-09</b>	<b>1.6e-09</b>	<b>1.4e-08</b>
<b>cg05874806</b>	<b>2</b>	<b>102350276</b>	<b>MAP4K4</b>	<b>1.1e-06</b>	<b>1.1e-06</b>	<b>4.7e-07</b>	<b>5.6e-07</b>

## Section 5

### Conclusions

- ▶ Two new methods to estimate the confounding factor for correcting association studies
- ▶ Theoretical convergence of the algorithms
- ▶ Same power than oracle on simulation
- ▶ On true dataset methods based on the latent factor mixed model discover more association
- ▶ On true dataset association discovered can be very different between methods
- ▶ We deliver a R package `lfmm` which implement these methods

Thank you for your attention !

## Choix du nombre de variables latentes

On projette  $\mathbf{Y}$  sur l'espace orthogonal à  $\mathbf{X}$  en prenant  $\lambda = 0$

$$\mathbf{D}_0 \mathbf{Q}^T \mathbf{Y} = \mathbf{D}_0 \mathbf{Q}^T \mathbf{U} \mathbf{V}^T + \mathbf{D}_0 \mathbf{Q}^T \mathbf{E}.$$

On calcule les valeurs singulières pour visualiser la variance expliquée par chaque variable latente (scree plot).

