XIV Conferencia Española de Biometría

Departamento de Estadística **Facultad de Biología**

Universitat de Barcelona

Table of Contents

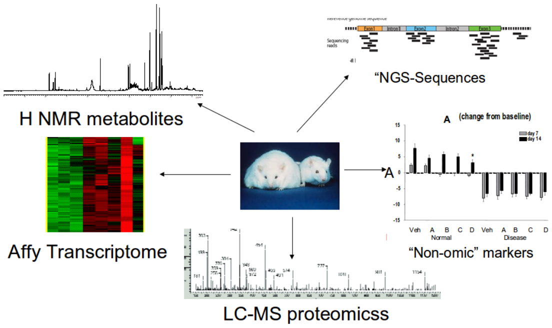
Contents

1 Introduction

1.1 Preliminaries

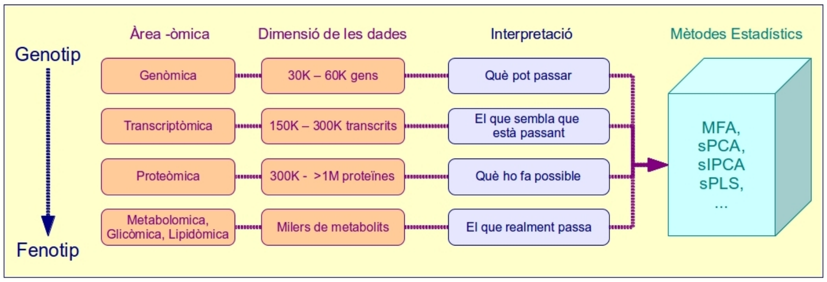
Post-genomics age: the next generation

- In a few years “new” ’omics’ technologies have started to be important:
  - The advent of *next generation sequencing*, providing powerful ways to yield improved information on many type of genomic, transcriptomic or epigenomic data.
  - The generalization of new high-throughput technologies allowing to study biological processes at the different levels at which they happen.



Data integration and systems biology -again

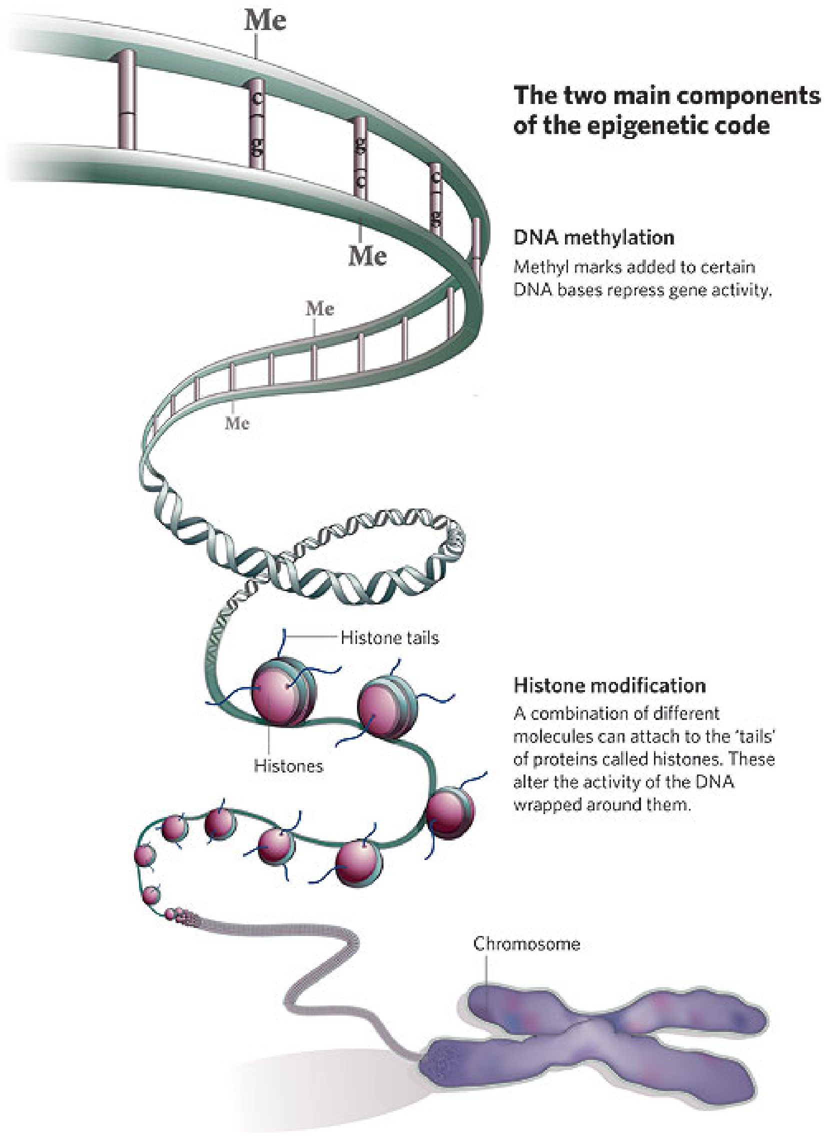
- Altogether this opens many fronts and opportunities
  - Statistics and bioinformatics are faced with the need for developing methods and tools for the for integrative analysis of big data sets of different sources and types
  - Biology and medicine are faced with the problem to pose the problems and use the results to improve their understanding in real systems biology approach.



Àrea -òmica	Dimensió de les dades	Interpretació
Genòmica	30K – 60K gens	Què pot passar
Transcriptòmica	150K – 300K transcrits	El que sembla que està passant
Proteòmica	300K - >1M proteïnes	Què ho fa possible
Metabolomica, Glicòmica, Lipidòmica	Milers de metabolits	El que realment passa

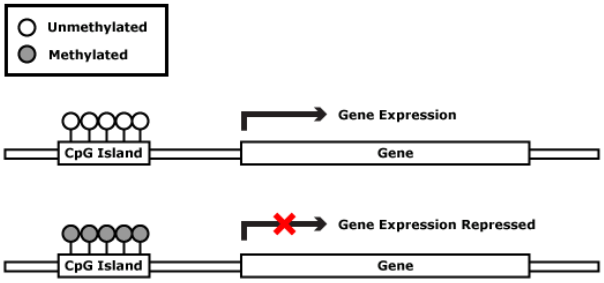
Epigenetics and epigenomics

- Epigenetics, *the study of environmental factors on gene expression in DNA*, shows a renewed impetus:
  - NGS allows in-deep analysis of regulatory mechanisms such as *methylation* or *histone modifications*.
  - There is increasing evidence that many differentiation processes are triggered and maintained through epigenetic mechanisms.


Me
The two main components of the epigenetic code
c
g
g
c
c
g
Me
DNA methylation
Methyl marks added to certain DNA bases repress gene activity.
Me
Me
Histone tails
Histone modification
A combination of different molecules can attach to the 'tails' of proteins called histones. These alter the activity of the DNA wrapped around them.
Histones
Chromosome

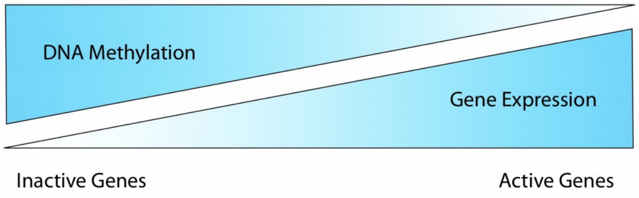
Methylation

- One main epigenetic regulatory mechanisms is methylation a process by which a gene's behavior is altered, but the gene itself isn't changed.
- Essentially methylation acts by inhibiting gene expression that is, the more methylated is a gene the more repressed is its expression



Methylation and gene expression

- Although the relation between methylation and gene expression is probably continuous ("*the more...the less...*"),



- methylation is, in practice, seen as a dual phenomenon
  - A methylated gene is "off"
  - An unmethlated gene is "on"
- Practical problem: ***at which methylation level a gene is seen as "methylated" (that is, it is "turned off")?***

Gene-specific methylation on-off threshold

- Since measurements for methylation and expression are both continuous, a biaxial plot of these two signals will exhibit an L-shape pattern.
- If we truly believe that methylation is binary, there are two implications:
  1. the reflection point of the L-shape is an appropriate choice to binarize methylation data, and
  2. conditioning on the binarized on-off methylation status, the continuous valued methylation data and expression data should be independent,

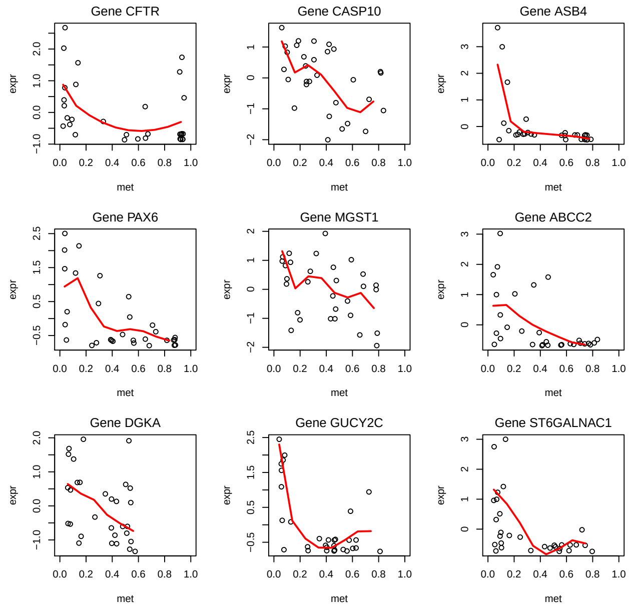
  which motivates Liu(2012) to quantify the L-shape pattern using conditional mutual information (MI).

1.2 Motivation

A colon cancer study

- This study originates in a work searching for colon cancer biomarkers.
- 30 cell lines characterized by increasing sensitivity to a drug were analyzed using several high-throughput methods: *transcriptomics*, *methylation*, *miRNAs*, *SNPs*, and *proteomics*.
- In this work we consider the problem of stablishing which genes were regulated by methylation.
- For each gene/methylation locus one has 30 points and a scatterplot showing the relation so we need methods to find patterns of scatterplots

Scatterplot patterns



1.3 Objectives

Objectives

- Study how gene expression is regulated by methylation in a set of colon cancer cell lines.
- Set up a method to detect the level of methylation at which a gene can be considered regulated by methylation (to be ”on”).

- Compare this method with other existing that have been developed to
  - detect methylation thresholds
  - detect patterns in scatterplots

2 Methods for pattern selection

2.1 Based on Conditional Mutual Information

Conditional Mutual Information

Two questions: which genes exhibit L-shape, and what is the optimal threshold for binarizing methylation data for each L-shape gene.

The key

To determine whether methylation and expression of a gene exhibit an L-shape, we compute the conditional Mutual Information (MI) for different choices of threshold to binarize the methylation data.

If we consider the continuous valued methylation and expression data as two random variables X and Y, and denote a nominal threshold as t, the conditional MI can be written as a weighted sum of MIs on the two sides of the threshold.

$$cMI(t) = I(X, Y|X > t)P(X > t) + I(X, Y|X \leq t)P(X \leq t)$$

Optimal threshold

When t is 0 or 1, cMI equals to the mutual information derived from all data points.

For an L-shape gene, as t moves from 0 to 1, $cMI(t)$ first decreases and then increases, and its value approaches zero when t coincides with the reflection point. Therefore,

Optimal threshold

The ratio $r = \frac{\min\{cMI(t)\}}{cMI(0)}$ for an L-shape gene is small, and $t^* = \text{argmin}\{cMI(t)\}$ is the optimal threshold for dichotomizing the methylation data of this gene.

Joint distribution estimator

To estimate the MI terms we use a kernel-based estimator, which constructs a joint probability distribution by applying a Gaussian kernel to each data point, and estimates the MI based on the joint distribution. The estimator is as follows:

$$I(X, Y) = \frac{1}{M} \sum_{i=1}^{M} \log \frac{M \sum_{j=1}^{M} e^{-\frac{1}{2h^2}((x_i - x_j)^2 + (y_i - y_j)^2)}}{\sum_{j=1}^{M} e^{-\frac{1}{2h^2}(x_i - x_j)^2} \sum_{j=1}^{M} e^{-\frac{1}{2h^2}(y_i - y_j)^2}}$$

where h is a tuning parameter for the kernel width and empirically set $h = 0.3$.

2.2 Based on Spline regression

Clustering using Spline regression

We implemented regression based on B-splines because they are particularly efficient due to the block-diagonal basis matrices that result.

Let

- $\varsigma = \{t_1 < \cdots < t_N\}$ non decreasing knot sequence
- $[t_m, t_{m+1})$ half open interval
- B_{mp} p-th order polynomial (degree $p-1$) with finite support over the interval and 0 everywhere else so that $\sum_{m=1}^{N-p} B_{mp}(x) = 1$
- then $s(x) = \sum_{m=1}^{N-p} B_{mp}(x) c_m$

Clustering using Spline regression (2)

To represent the curve we set:

$$y_{ij} = s(x_{ij})$$

So

$$\mathbf{y}_i = \mathbf{B}_i \mathbf{c}$$

with

- $\mathbf{B}_i = [B_{1p}\mathbf{x}_i, B_{2p}\mathbf{x}_i, \ldots, B_{Lp}\mathbf{x}_i]$ the spline basis matrix
- $\mathbf{c}$ the vector of spline coefficients.

Clustering using Spline regression (3)

Algorithm

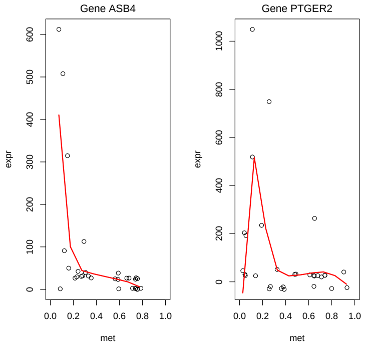
1. Selection of the genes with a negative significant correlation
2. Fit cubic regression splines
3. Data to cluster: splines coefficients
4. Calculation of a distance matrix between genes as $1 - \rho$
5. Hierarchical clustering

3 Results

Results (1) Splines–based regression

- After the previous selection of genes we worked with 191 genes
- We decided to choose 5 clusters

- The 2 first clusters included the genes with an L-shape



Results (2) Conditional Mutual Information

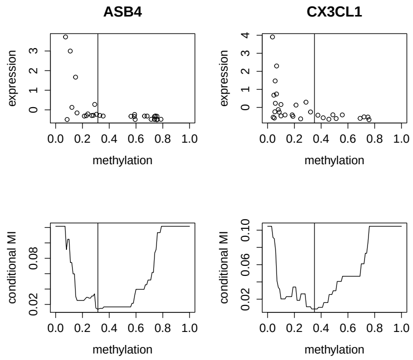
- No previous selection of the genes was needed
- We filtered for L-shapes using a combination of three criteria:
  - the ratio $r < 0.25$
  - unconditioned MI $cMI(0) > 0.1$
  - the median expression on the left side of the optimal threshold t^* is higher than the median expression on the right side.
- The parameters are chosen according to a random permutation test (see Liu(2012)).
- According to the above criteria, a total of 641 genes are selected to be L-shape genes.

Results (3) Comparison between the methods

The results of both methods that can be summarized in the following table:

Initial selection	191	641

Cluster	Splines	cMI
1	140	102
2	22	16
Total	162	118



Conclusions

- We have found similar results between both methods.
- Biological interpretation is still being done by biological researchers although results are consistent with the hypothesis (we have found genes regulated by methylation).
- Sample size is a limiting factor: cMI works better with hundreds of samples but one may have a very small number (real cases: 30, 12)

Acknowledgments

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