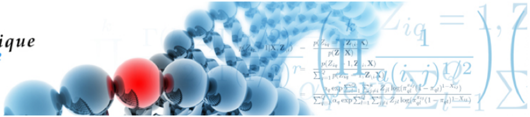

Estimation of a non-parametric variable importance measure of a continuous exposure

with Antoine Chambaz and Mark J. van der Laan
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Outline

1. Association between DNA copy number and gene expression

2. Targeted minimal loss estimation of association

3. Results

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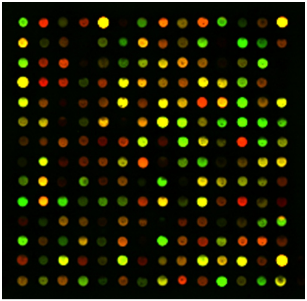
Cancers are characterized by changes at the molecular level

Different levels of biological information

- DNA copy number
- gene expression
- DNA methylation

Quantitative measurements can be obtained from DNA microarrays

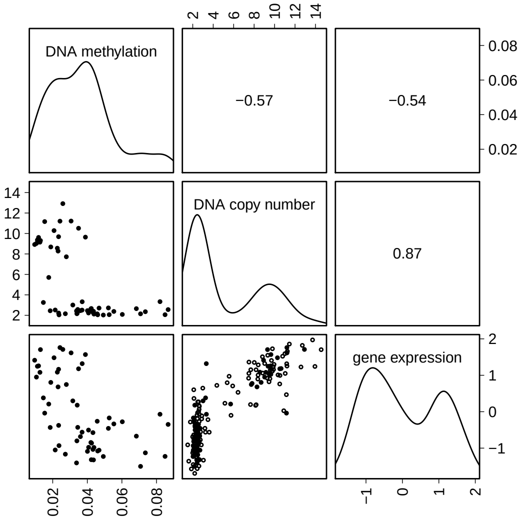


Goal : find genes that **drive** tumorigenesis

- to better understand cancer cells
- to help find new treatments

What gene-level data look like

187 GBM (brain cancer) samples from the Cancer Genome Atlas (TCGA)



Note : genomic data are complex

In the preceding plot, "gene-level data" refers to :

DNA methylation (W) : proportion of "methylated" signal at a CpG locus in the gene's promoter region.

DNA copy number (X) : smoothed normalized total copy number relative to a set of reference samples.

Expression (Y) : "unified" gene expression level across 3 platforms

Which genes are drivers ?

"Driver genes" are expected to show some **association** between DNA copy number and gene expression

⇒ **Test** for association, and **quantify** it

Methods for genome-wide scanning for gene-level associations

- linear correlations
- differential expression (T-tests) between copy number states
- canonical correlation analyses

Issues with existing methods

- they essentially identify genes that were already known to be implied
- associations may be non linear
- DNA methylation may down-regulate gene expression

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Definition of a parameter of interest

Observation $O = (W, X, Y) \sim P \in \mathcal{M}$ for a given gene :

- W : DNA methylation
- X : DNA copy number ; $X = 0$: **copy neutral state** (2 copies)
- Y : gene expression
- $\mathcal{M}$: non-parametric set of candidate data-gen. distributions of O

Parameter of interest (defined for all $P \in \mathcal{M}$)

$$\Psi(P) = \arg\min_{\beta \in \mathbb{R}} E_P \left[(E_P(Y|X, W) - E_P(Y|X = 0, W) - \beta X)^2 \right]$$

A non-parametric variable importance measure

Parameter of interest (defined for all $P \in \mathcal{M}$)

$$\Psi(P) = \arg\min_{\beta \in \mathbb{R}} E_P \left[(E_P(Y|X,W) - E_P(Y|X=0,W) - \beta X)^2 \right]$$

- $\Psi(P)$ **is a variable importance measure** of the "effect" of X (continuous) on Y (continuous) accounting for W
- $\Psi(P)$ **is non-parametric :** in a semi-parametric model where $E_P(Y|X,W) = E_P(Y|X=0,W) + \beta X$, we have $\Psi(P) = \beta$. By contrast, $\Psi : \mathcal{M} \to \mathbb{R}$ is defined **universally**

Interpretation on the parameter of interest

Let $\theta(P)(X,W) = E_P(Y|X,W)$, then

$$\Psi(P) = corr(X, r_P(X,W))\sqrt{\frac{E_P[r_P(X,W)^2]}{E_P[X^2]}},$$

where $r_P(X,W) = \theta(P)(X,W) - \theta(P)(0,W)$

Case where X is binary

If $X \in \{0,1\}$, then

$$\Psi(P) = E_P[(\theta_P(1,W) - \theta_P(0,W))h(W)]$$

with weight $h(W) = P(X=1|W)/P(X=1)$

Targeted minimal loss estimation (TMLE) : motivation

Goal : estimate a parameter $\Psi(P)$ from observations arising from a distribution P. **Ψ is a known, smooth functional**.

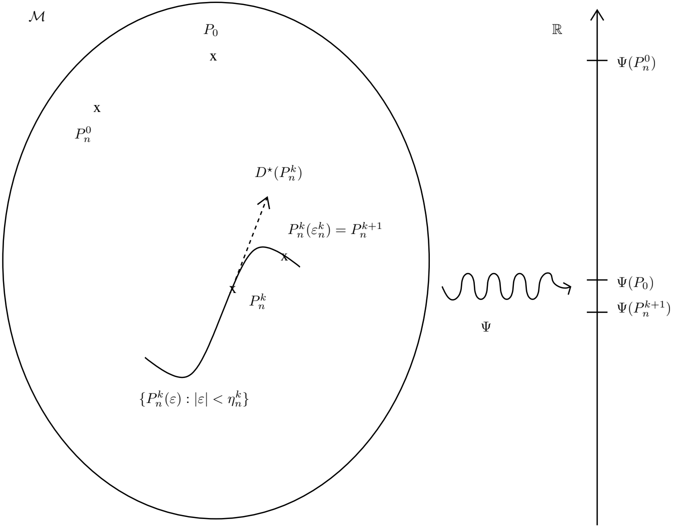
Naive strategy

1. Estimate P using $\hat{P}$
2. Plug-in : $\Psi(\hat{P})$

Our target parameter is $\Psi(P)$, not P !

- $\hat{P}$ aims at balancing bias and variance for the whole distribution
- $\Psi(\hat{P})$ **does not** balance bias and variance for $\Psi(P)$

Targeted minimal loss estimation : illustration



Targeted minimal loss estimation : algorithm

From an initial estimate P_n^0 :

1. Create a model $P_n^0(\varepsilon)$ parametrized by $\varepsilon \in \mathbb{R}$ whose score is the **efficient influence curve** of Ψ at P_n^0
2. Estimate ε using maximum likelihood : ε_n^0
3. Update accordingly : $P_n^1 = P_n^0(\varepsilon_n^0)$

Repeat as many times as necessary : $\forall k \geq 0, P_n^{k+1} = P_n^k(\varepsilon_n^k)$

... hence **final estimate** $P_n^\star$

Statistical properties

P_0 : true distribution of O

Consistency (double robustness)

TMLE is consistent if one of the following conditions holds :

- $\theta(P_n^\star)(0,\cdot)$ consistently estimates true $\theta(P_0)(0,\cdot)$
- $E_{P_n^\star}(X|W)$ and $P_n^\star(X=0|W)$ consistently estimate $E_{P_0}(X|W)$ and $P_0(X=0|W)$

Asymptotic normality

Under the same conditions with higher rates of convergence, TMLE is asymptotically Gaussian.
We can compute asymptotic *p*-values and thus **rank genes**

Note : comparison with a non-parametric estimator

Letting $\theta(P)(X, W) = E_P(Y|X, W)$, we have

$$\Psi(P) = \underset{\beta \in \mathbb{R}}{\arg\min}\, E_P \left[\theta(P)(X, W) - \theta(P)(0, W) - \beta X)^2\right]$$

An obvious substitution estimator of $\Psi(P_0)$ is

$$\hat{\psi}_n = \underset{\beta \in \mathbb{R}}{\arg\min}\, E_{P_n} \left\{ \left(\hat{\theta}_n(X, W) - \hat{\theta}_n(0, W) - \beta X\right)^2 \right\},$$

where $\hat{\theta}_n(X, W)$ is the Nadaraya-Watson estimator of $\theta(P_0)(X, W)$.

Such an estimator has non-parametric convergence rates ;
it **may not achieve $\sqrt{n}$-consistency**.

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Simulation strategy

Assumptions :

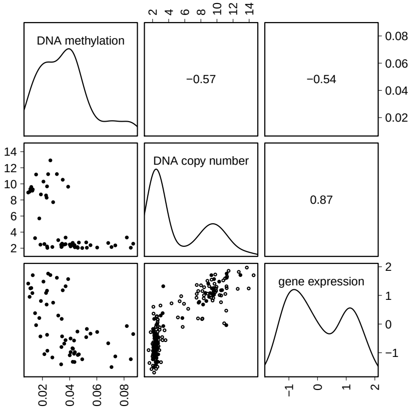
- up to 3 copy number classes : normal regions, and regions of copy number gains and losses
- in normal regions, expression is negatively correlated with methylation
- in regions of copy number alteration, copy number and expression are positively correlated

GBM data used as a baseline for simulation :

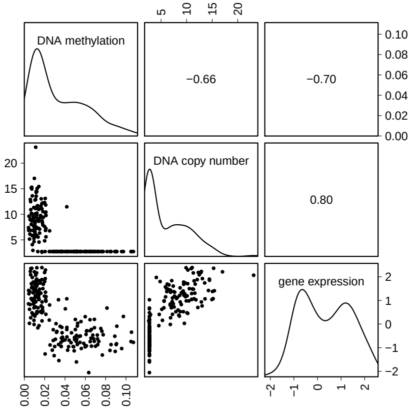
Sample name	Methylation	Copy number	Expression
TCGA-02-0001	0.05	2.72	-0.46
TCGA-02-0003	0.01	9.36	1.25

Simulated data set mimics real data set

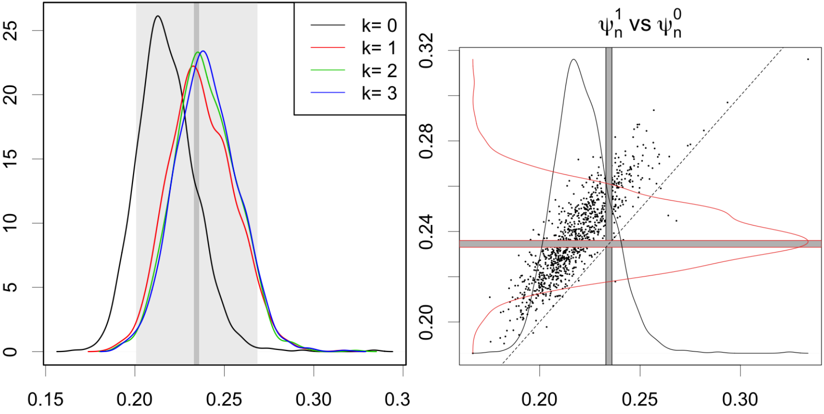
Real data (GBM, n=187)



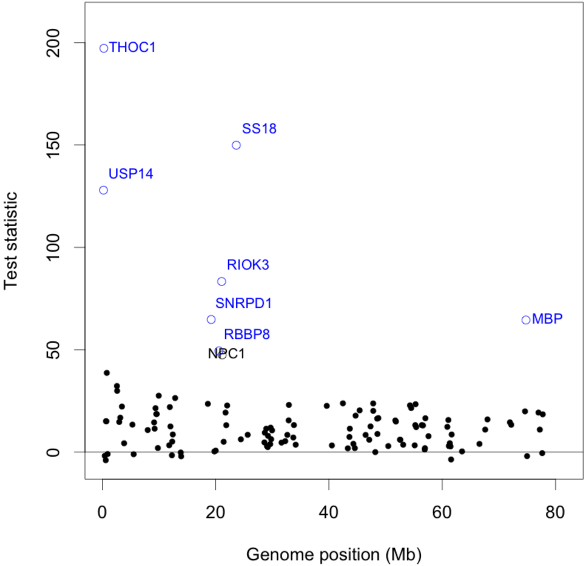
Simulated data (n=200)



Simulated data : TMLE corrects initial estimation



Real data analysis : TCGA OV data set



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