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INSERM U1209, IAB Grenoble

Tabagisme maternel et méthylation de l'ADN placentaire

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iAB EPIGENETICS CHRONIC DISEASES CANCER

SFDOHaD Origines Développementales de la SANTÉ

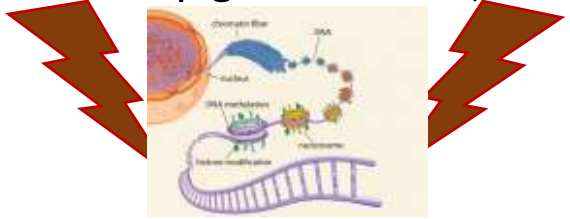
Early environmental exposures and the DOHaD

Origines Développementales de la SANTÉ

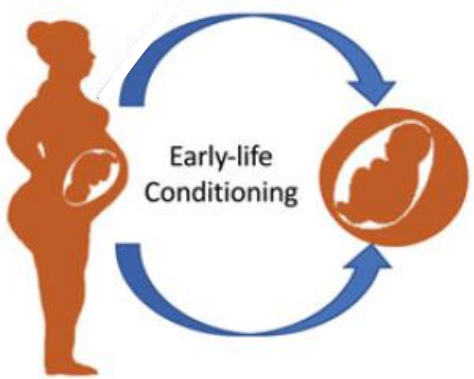
sf-dohad.fr

Urban exposome: air pollution, temperature, greeness | Endocrine disruptors | Smoking

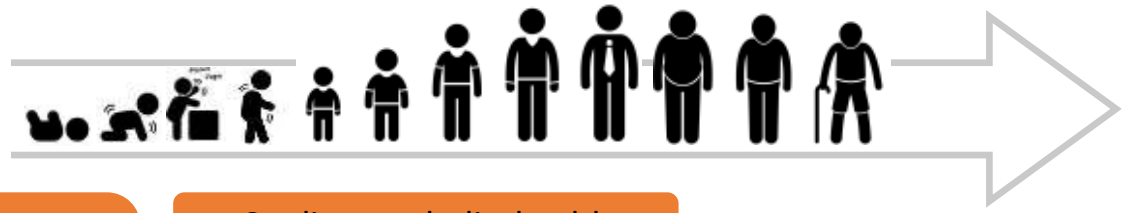
Epigenome : adaptation, deregulation



Modifiable (reprogrammation, environment)
Persist, even in the absence of the cause that established them



Environmental exposure in Infancy



Pregnancy outcomes (birth weight, etc)

Cardio metabolic health

respiratory health

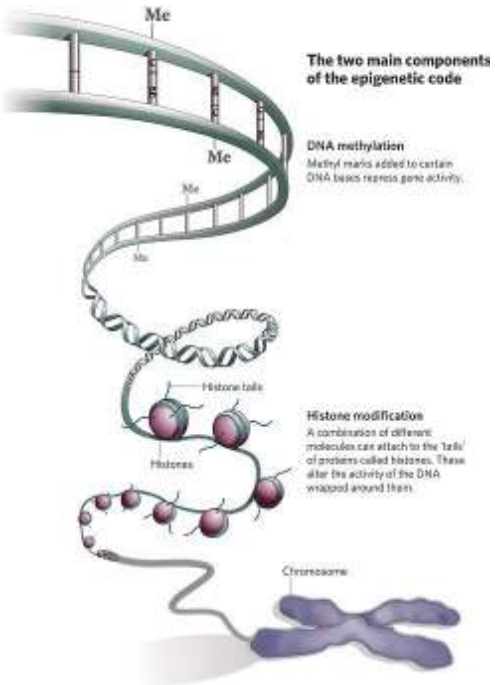
neurodevelopment

Adapté de Agarwal et al 2018

Epigenetic marks

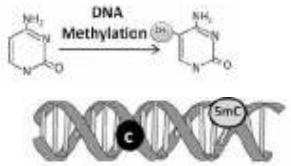
Gene expression mechanisms

- Does not depend on the DNA code
- Relatively stable over time
  - Mitosis (cell division)
  - Meiosis (transgenerational, limited evidence)



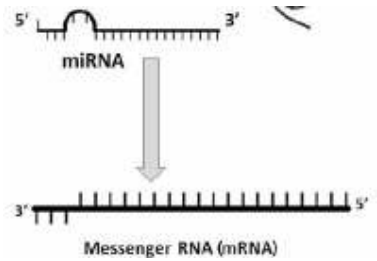
DNA methylation

Methyl marks added to certain DNA bases that repress gene transcription



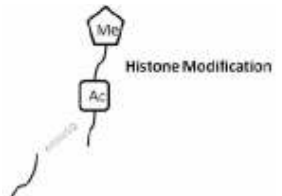
Micro RNA

Small non-coding RNA that block translation of messenger RNAs into proteins



Histone modifications

Different molecules that can attach to histone tails and alter the activity of DNA

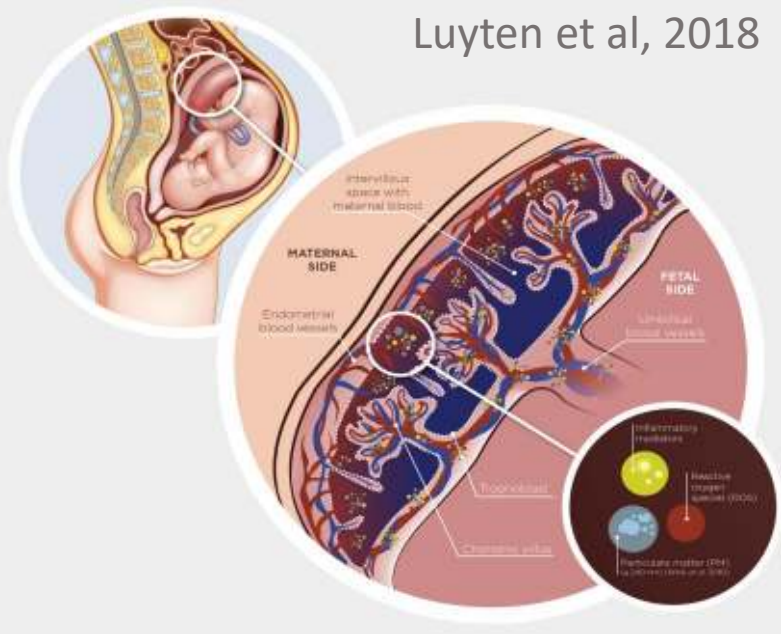


Usually, **lower methylation associated with higher expression**

- Most well known epigenetic mechanisms
- Reliable techniques
- Can use existing DNA collections

Adapted from Qiu, Nature, 2006

Why focusing on placenta ?



Luyten et al, 2018

- Tobacco smoking can cross the placenta and expose the developing fetus (Valentino et al., 2016; Wick et al., 2010)
- The placenta plays a key role in fetal programming:
  - support mother's health and the development of the fetus
  - conveys nutrients and oxygen to the fetus, regulates gas and waste exchanges, hormone interactions (Murphy et al., 2006)
- The placenta may provide a unique record of exposures occurring during pregnancy
- Most literature focused on cord blood (Joubert 2012, Joubert 2016, Kuppers 2015, Miyake 2018), a few epigenome wide studies on placenta (Morales et 2016, Cardenas 2019, Chhabra 2014) , reviewed by Nakamura 2021
- No study on women who stop smoking before pregnancy

Aim

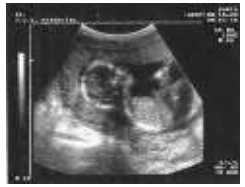
- identifying cigarette-induced alterations in placental DNA methylation
  - in women currently smoking during pregnancy
  - in women who quit in anticipation of pregnancy

EDEN mother-child cohort

Recruitment: 2003 to 2006 (University hospitals, Poitiers and Nancy)

Heude B, Int J Epid, 2015

Recruitment, n=2002
(before week 24)



- 5mm x 5mm samples in the middle of the placenta
- Fetal side
- Collected at birth and frozen -80°C
- n=568 samples
- Illumina 450k beadchip ≈ 480 000 CpG sites
- Global methylation, repetitive *Alu* and *LINE-1*

Conception

13, 26, 33 weeks of gestation

-Growth by repeated ultrasonography (13, 26, 33 weeks of gestation)

Birth

-Growth (weight, height, head circumference)
-Biological samples (placenta, cord blood, etc)

Age 3y

-Growth
-Neuro-development tests

Age 5 y

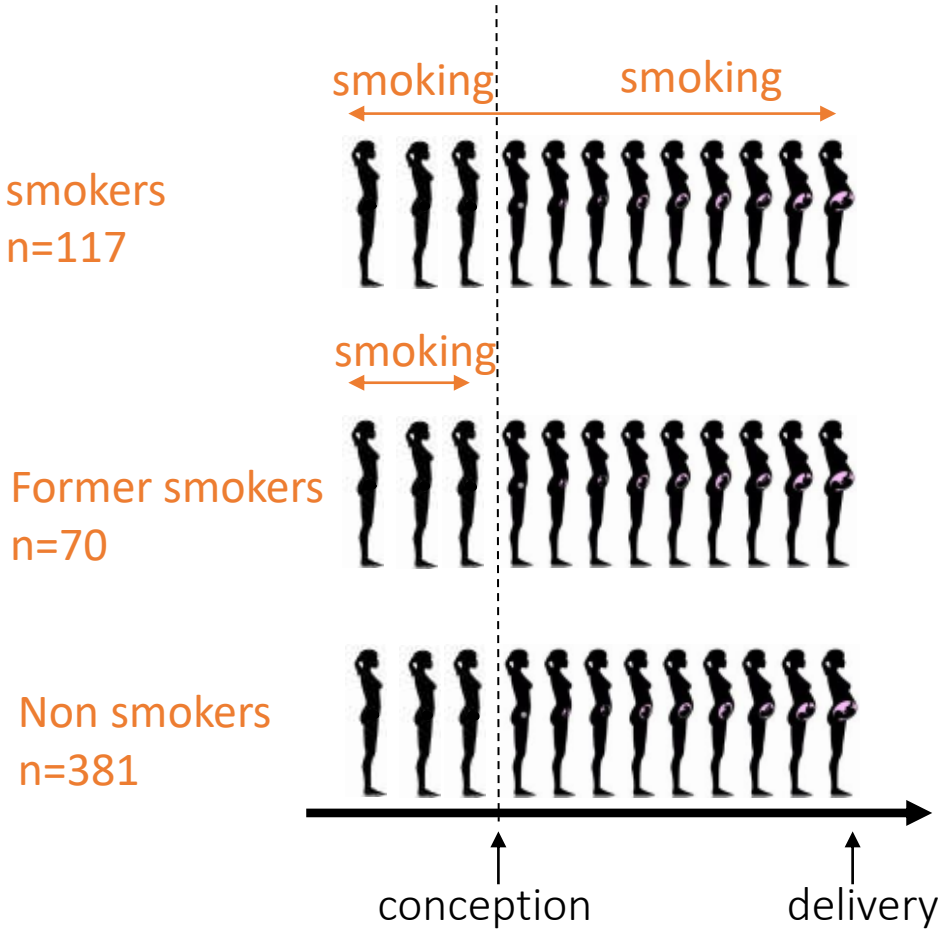
-Growth
-Neuro-development tests
-Lung function test

Age 10 y

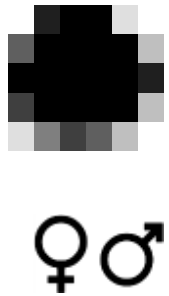
-Growth
-Neuro-development
-Lung function (n=200, as part of HELIX project)

Interviews and questionnaires : including data on socio-demographic factors

Study sample : 568 women with placental DNA methylation measured



	Mean	±SD
Birth weight (g)	3304	488
Gestational age (weeks)	39.8	1.7
Maternal age (yrs)	29	5
BMI	22.9	4.2
Cigarettes /day	1.7	3.5



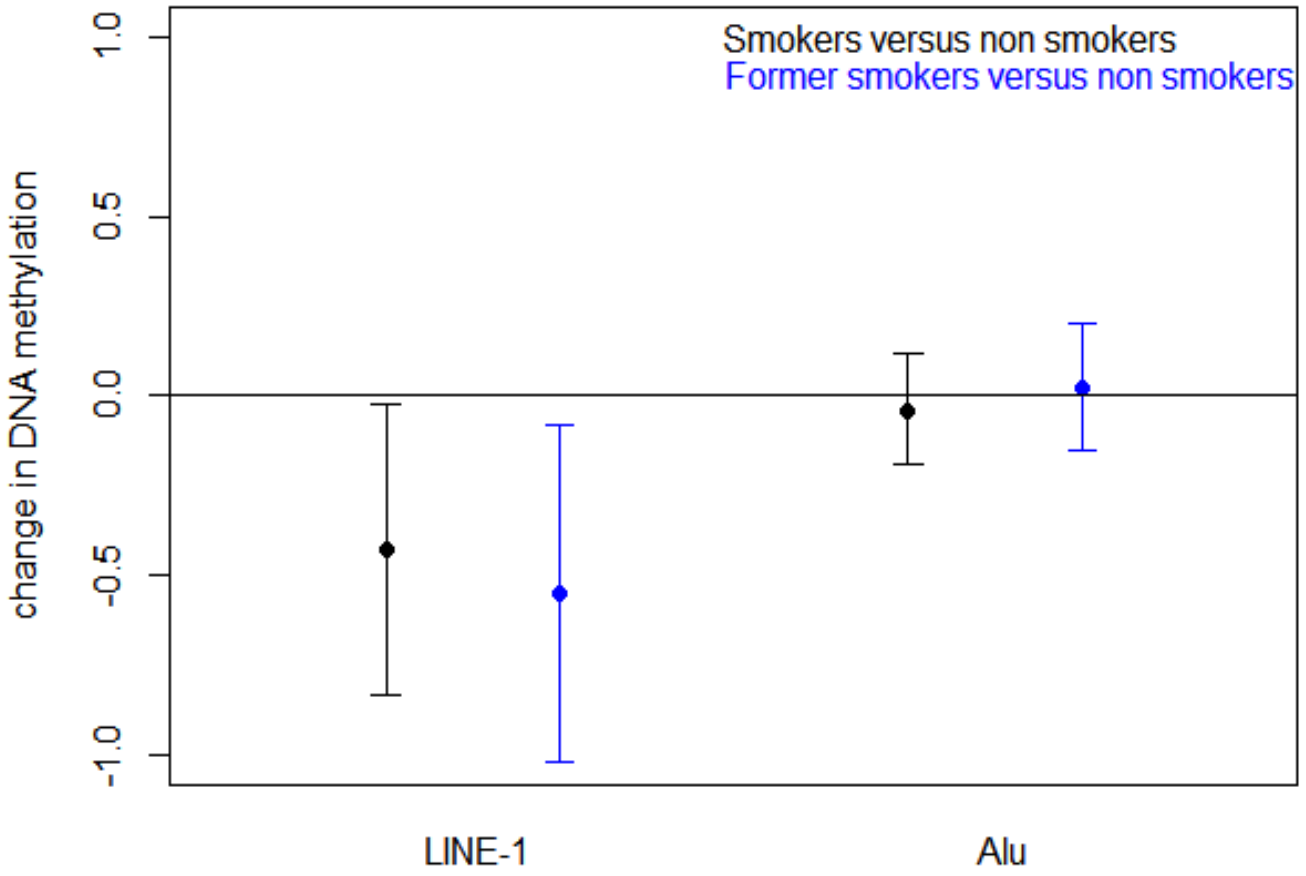
	Category	%
Centre	Poitiers vs. Nancy	43% vs. 57%
Sex	Male vs. Female	52% vs. 48%
Parity		
	Primipare	45
	1 child	38
	≥2 children	17
Education		
	<High school	37
	High school	23
	>=High school+2 years	41

Association between maternal smoking and DNA methylation

- 425 878 (after QC) CpG specific analyses
  - Robust linear regression
  - False Discovery Rate correction (Benjamini and Hochberg, 1995)
  - All models adjusted for:
    - Maternal age, BMI, education, parity, centre
    - Paternal smoking status at conception
    - Gestational duration, season of conception, sex of the baby
    - Technical factors: batch, plate, chip
    - Cell type proportions according to a ref-free method (Houseman et al 2016)

- Differentially Methylated Regions (DMR):
  - Comb-p (Pedersen et al., 2012)
  - Regions enriched for low p-values
  - Corrects for autocorrelation within 500 bp
  - Adjust for multiple testing
  - At least 2 probes per region

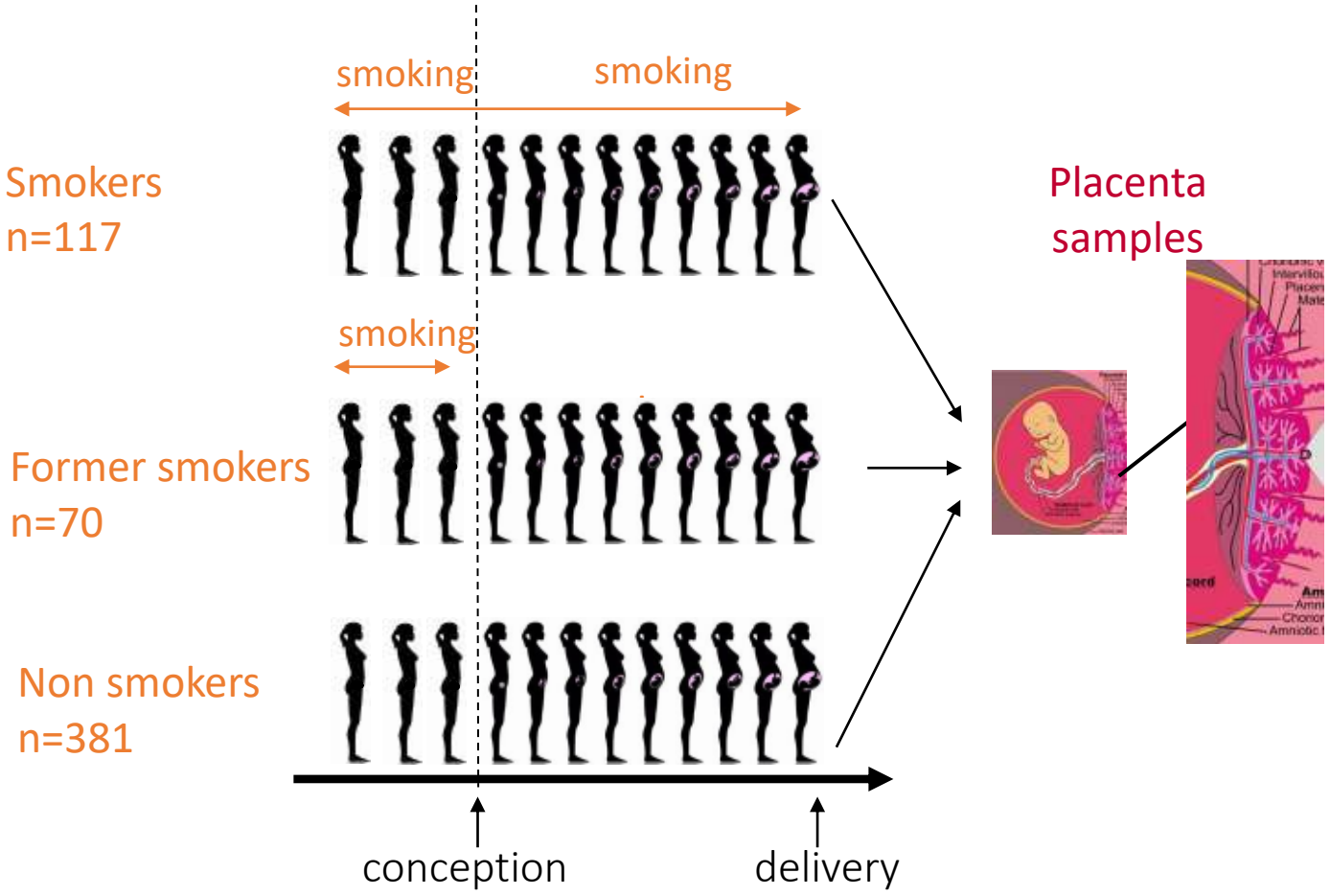
Maternal smoking status and global DNA methylation



Average methylation level
26.1 (±1.9) for *LINE-1*
16.1 (±1.0) for *Alu*

Armstrong et al 2016 (n=96, maternal side of placenta): no difference in placenta methylation for *LINE-1*

Epigenetic memory of former smoking



152 differentially methylated regions only present in the placenta of current smokers: **"reversible" alterations of DNA methylation,**

26 differentially methylated regions in former smokers : **"persistence"** in the placenta of former smokers, supports the hypothesis of an **"epigenetic memory"**

BMC Medicine

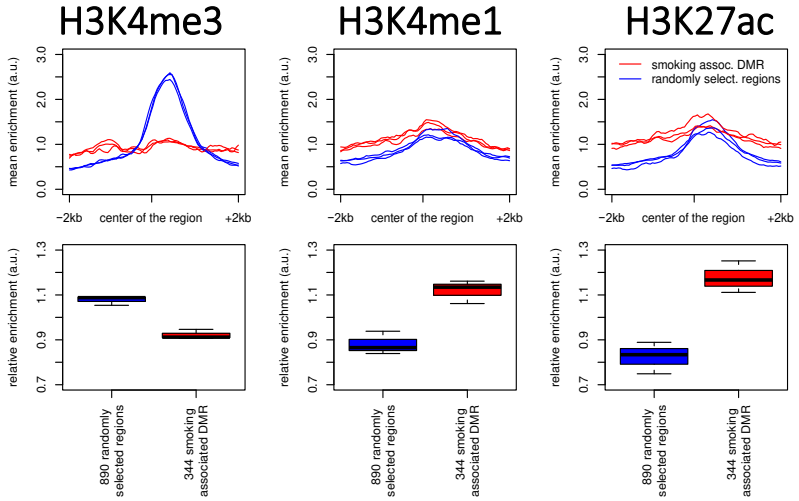
Rousseaux et al. 2020

DMR sensitive to exposure to tobacco are enriched in enhancer histone marks

203 differentially methylated regions

(with at least 2 CpGs within a 2000 bp window) including 1023 CpGs

Using available ENCODE ChIPseq data obtained for placenta.

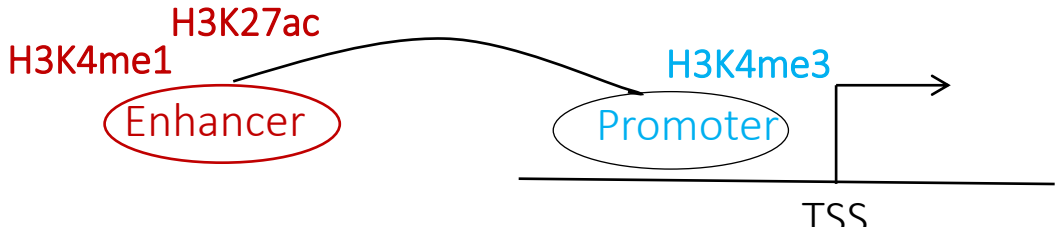


203 DMR associated with pregnancy smoking status

420 randomly selected regions

TSS less represented

Enrichment in enhancers histone marks

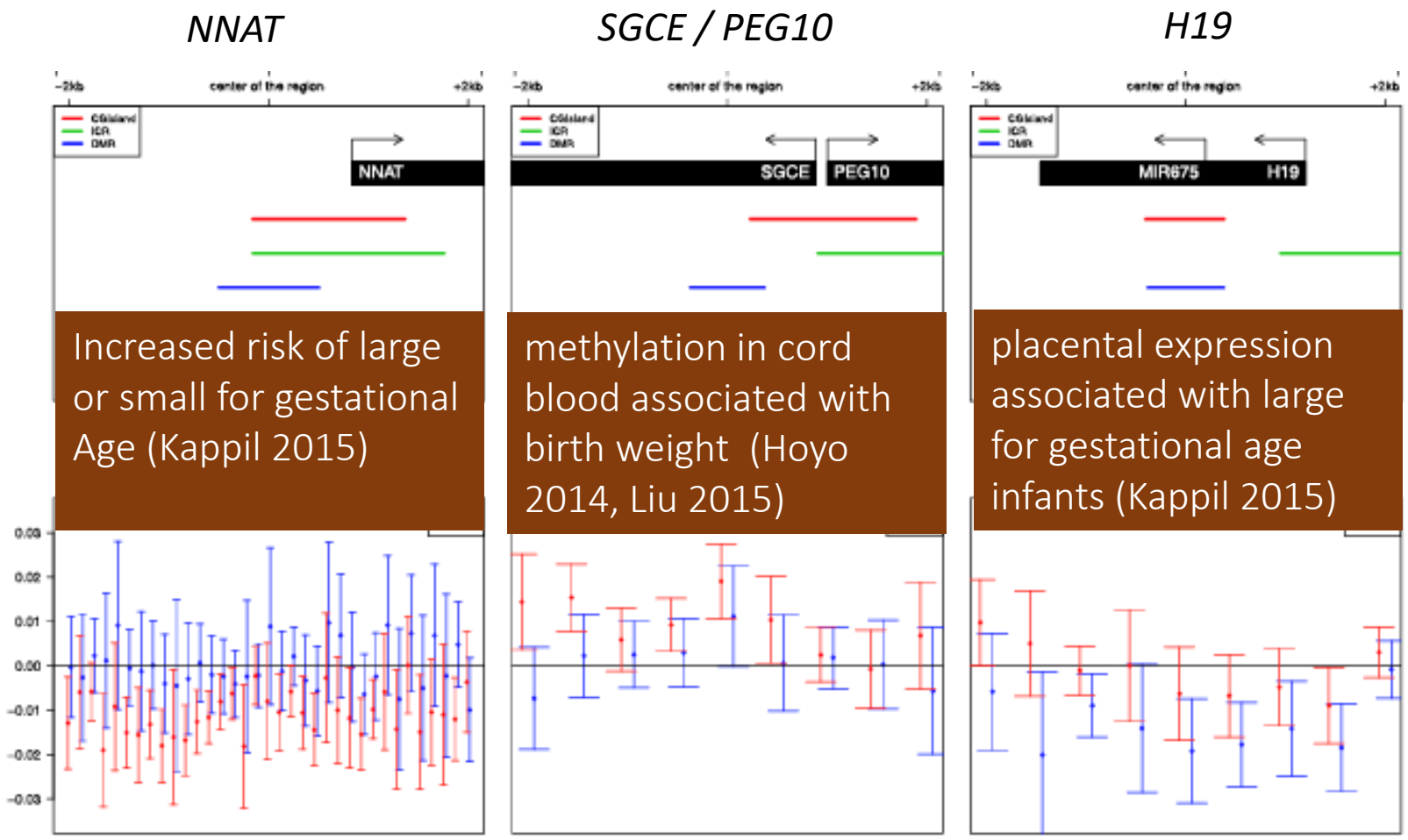


BMC Medicine

Rousseaux et al. 2020

Enrichment in imprinted genes

The 203 DMRs included 10 imprinted genes



BMC Medicine

Rousseaux et al. 2020

Take-home messages

- Suggest epigenetic memory of maternal tobacco smoking
- Gene promoter regions little impacted by maternal smoking
- Imprinted genes and enhancers preferentially affected by maternal smoking

Conclusions

- Epigenetic epidemiology : investigate and suggest biological mechanisms behind epidemiological associations
  - Biomarker of exposure and / or disease, mediator, adaptative mechanisms
  - In collaboration with experimental studies
  - Association ➔mechanisms➔causation

- Limits :
  - Evidence for causation is limited
  - Subtle signal
  - Functional consequences, whole epigenetic machinery: histones, micro RNAs
  - Covering parts of the genome (450k, EPIC)
  - Consortium

Future steps

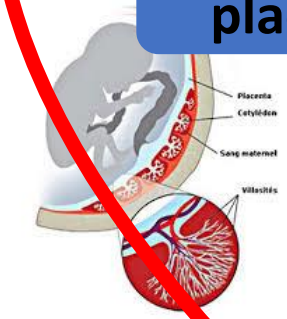
Médiateur des effets

Mécanismes épigénétiques

de l'exposition sur la santé ?

(Bio)marqueurs d'exposition

Méthylation de l'ADN **placentaire**

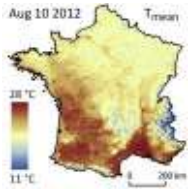


(Bio)marqueurs de risque ?

Expositions environnementale pendant la **grossesse**

Pollution atmosphérique

Climat

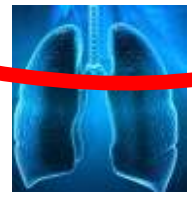


Tabac

Développement et santé de **l'enfant**

Issues de grossesse, croissance foetale

Santé respiratoire



Neuro-développement

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eden
Étude des Déterminants pré et post natals du développement et de la santé de l'ENfant

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