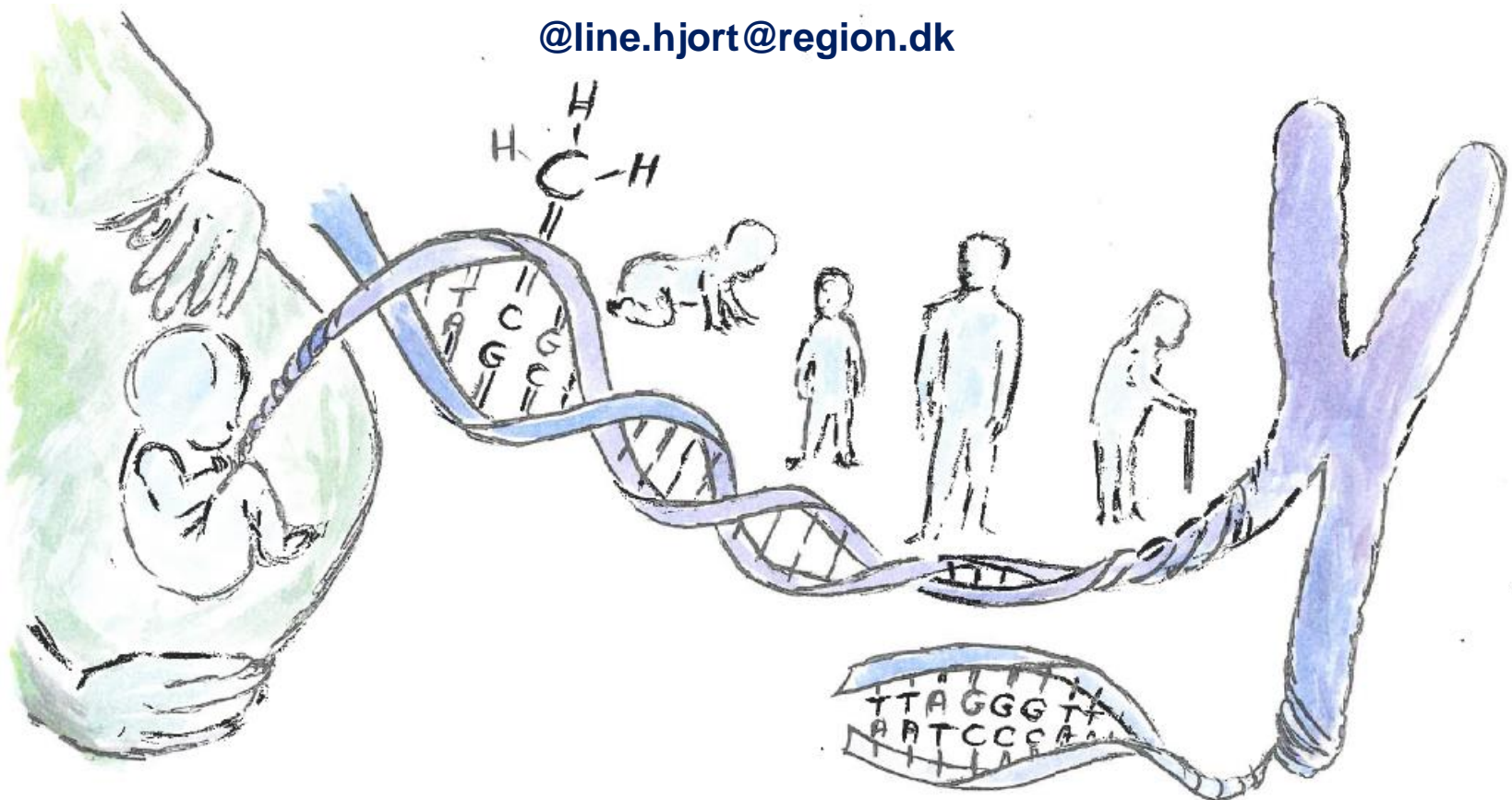
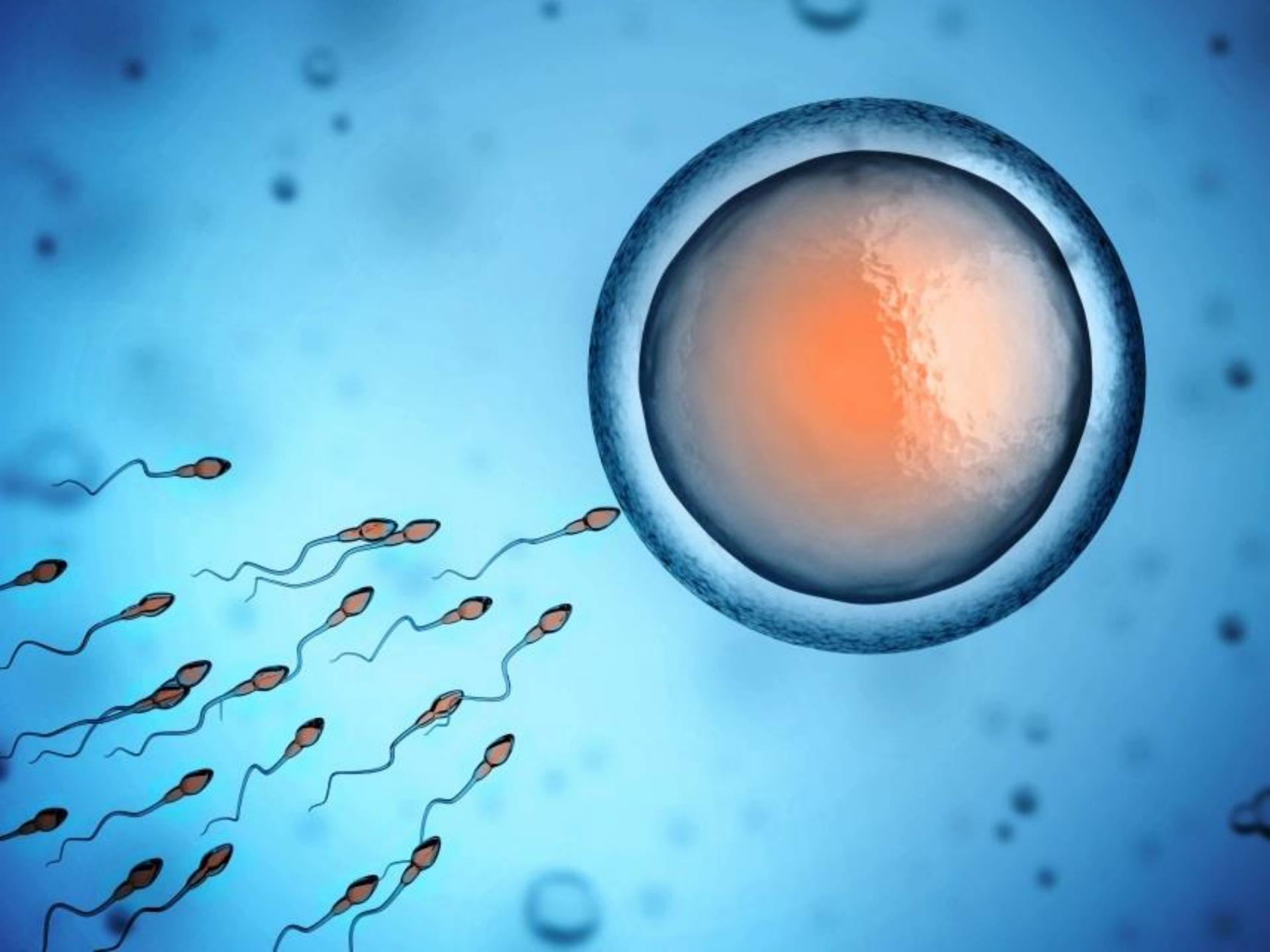


# Epigenetic mechanisms in developmental programming – analytical approaches and genome-wide study methods

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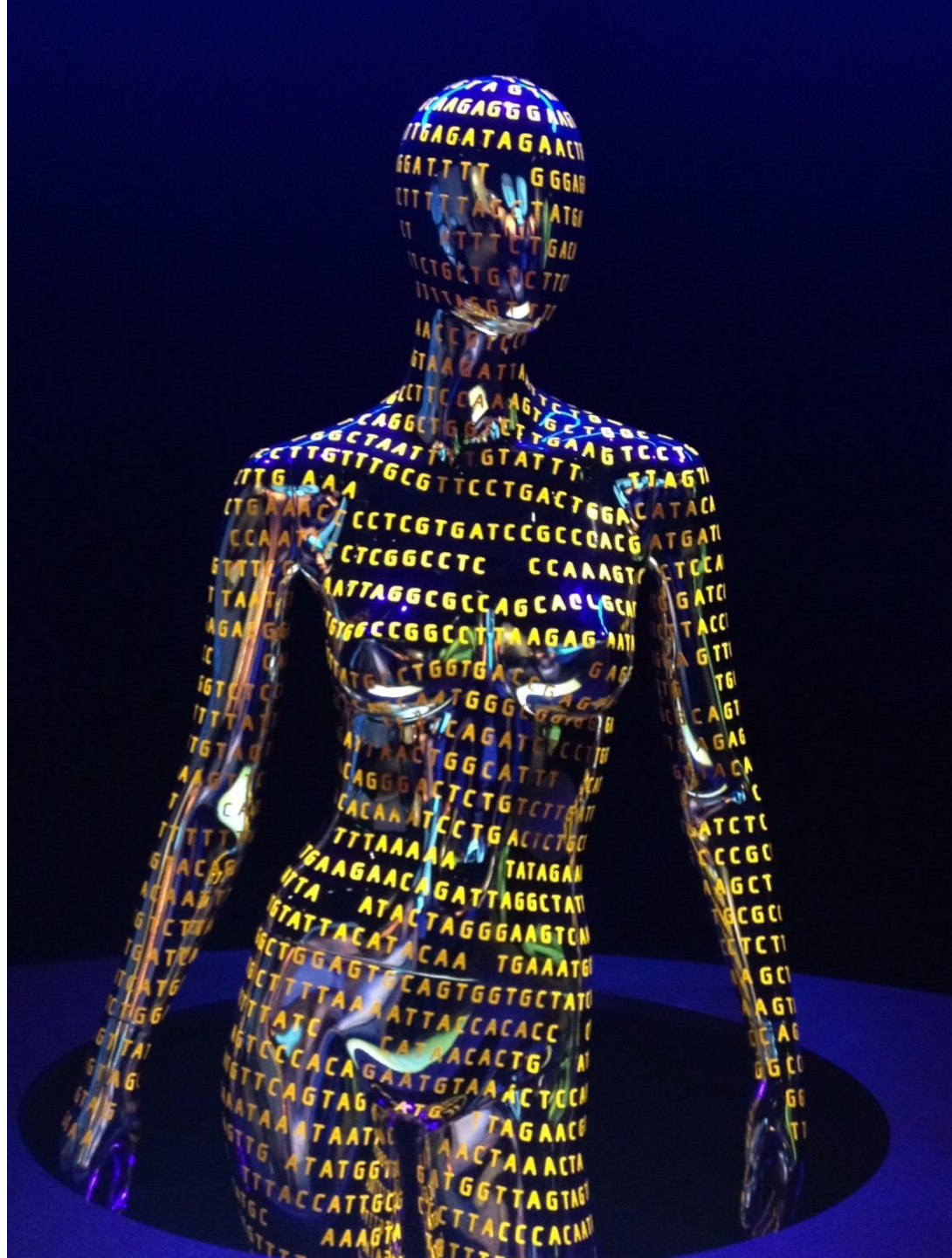






## Presentation outline

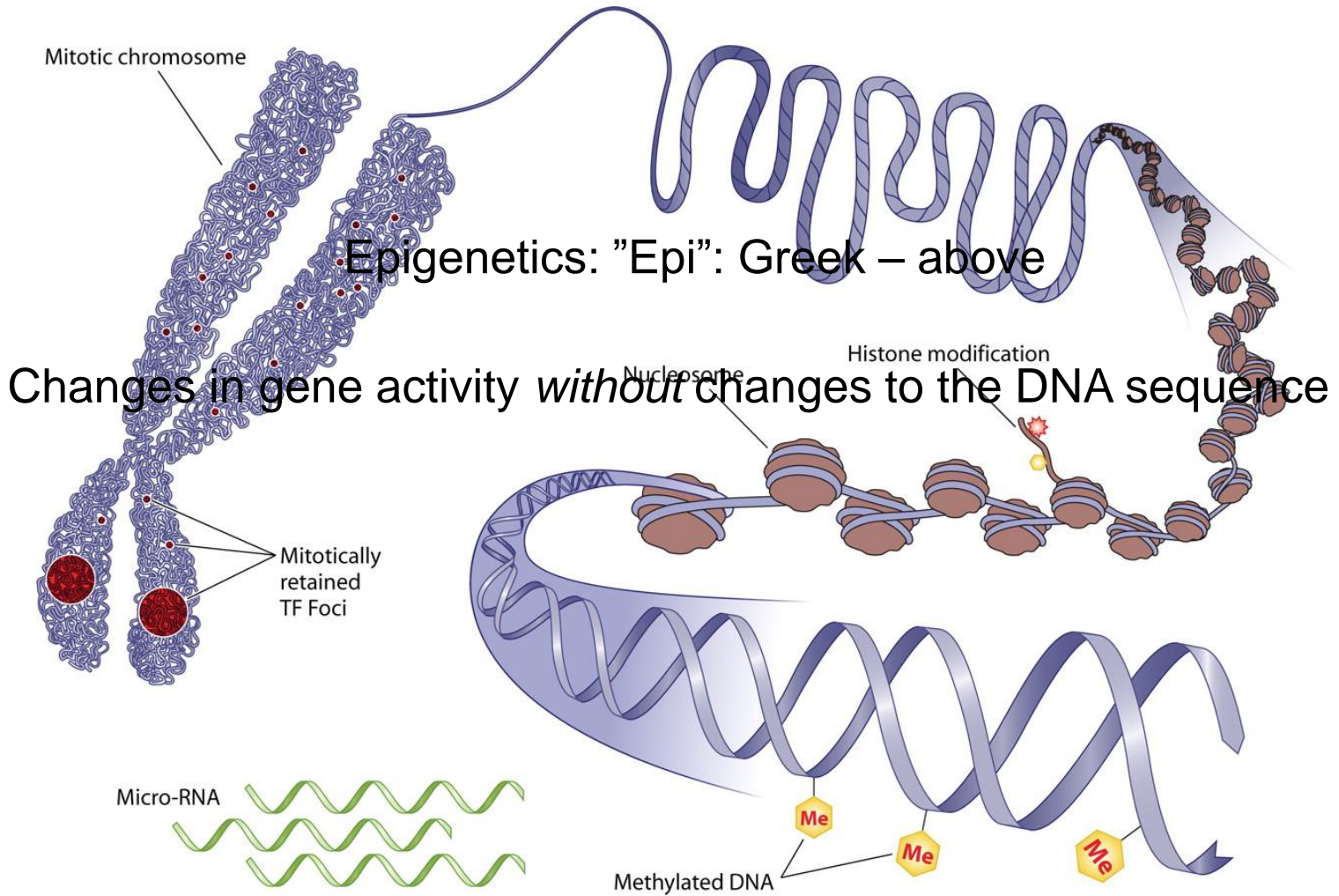
- What is epigenetics?
- Why do we study epigenetic mechanisms in relation to early life exposures?
- Analytical approaches and methods
- Genome-wide study examples





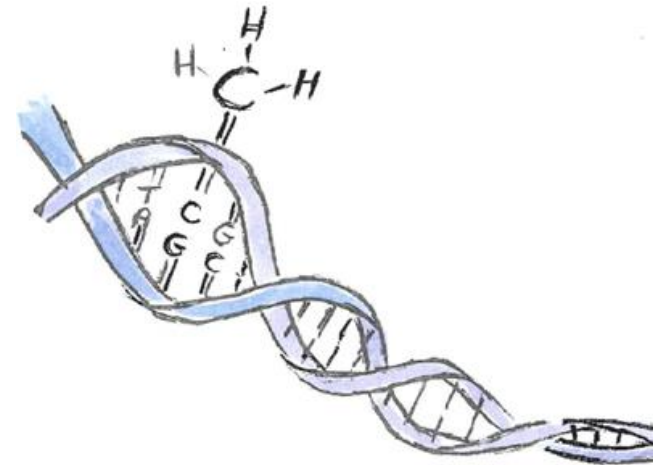
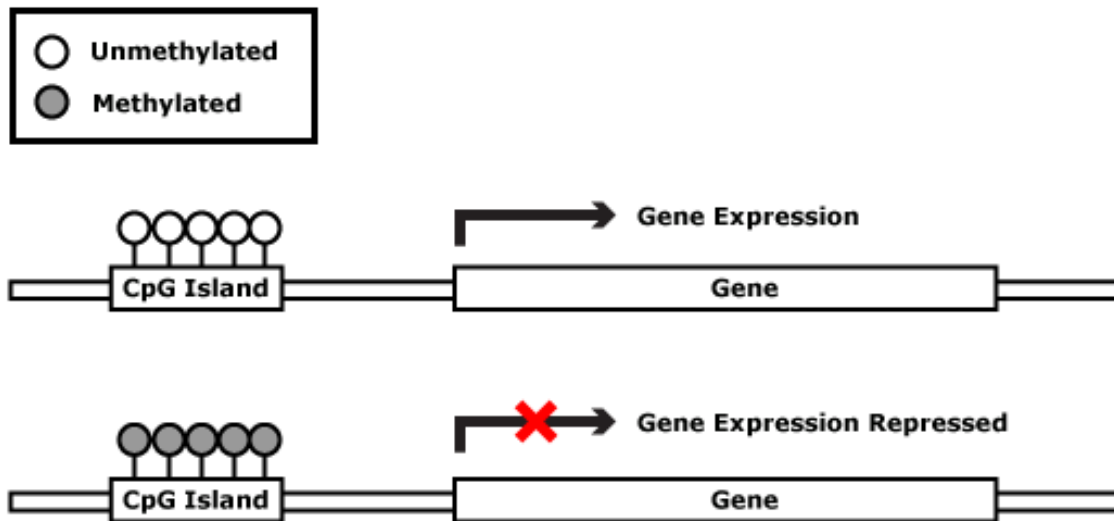


# Epigenetic mechanisms



# DNA methylation

- DNA methylation occurs at cytosine residues and is mostly studied at CpG sites (*Ehrlich et al, 1982*)
- CpG sites are found in high density in gene promoters, called CpG islands, where methylation may repress transcription (*Bird, 1986, Ling and Groop, 2009*)

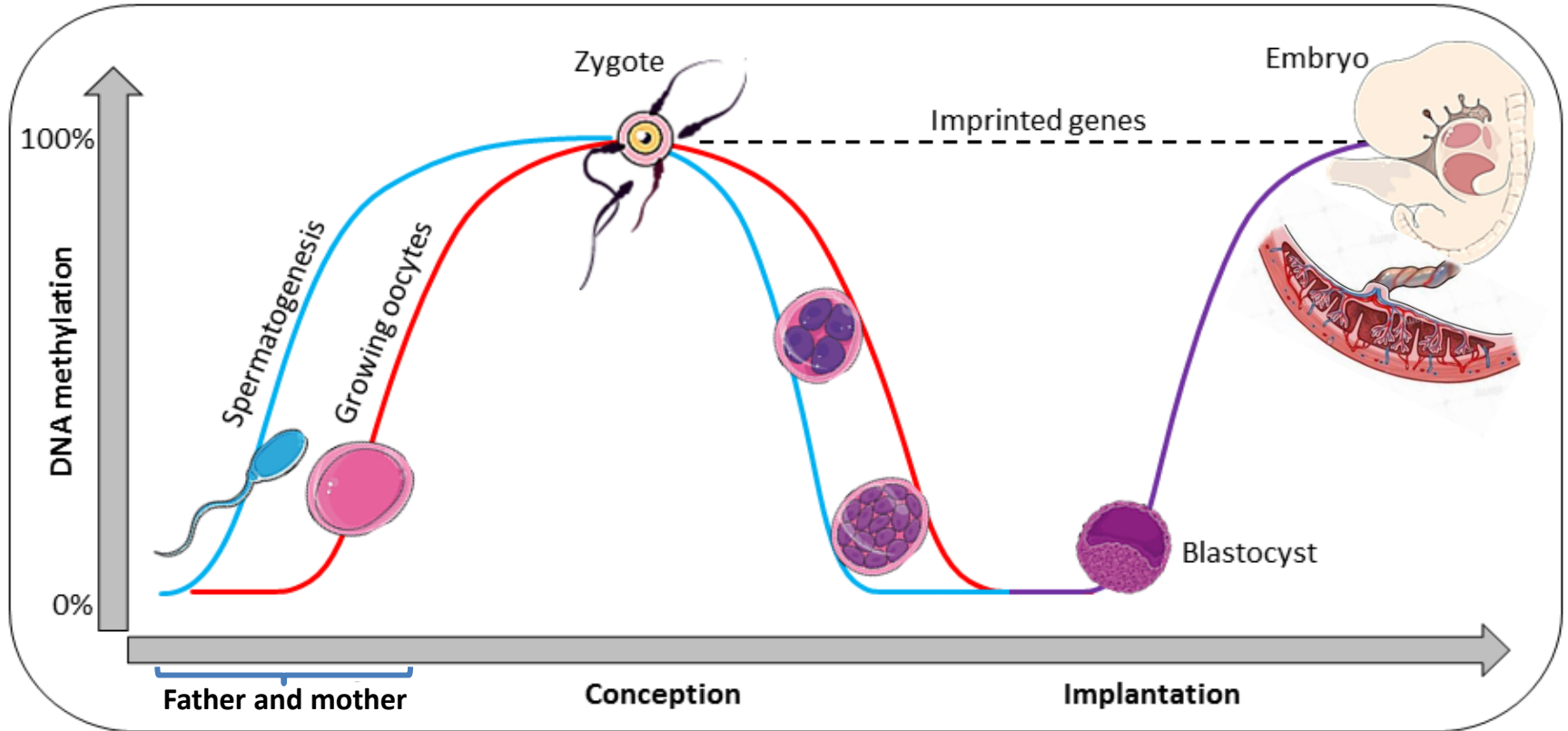




How does it look like in “real life”

CTCGATTCTCCGCGTGCCAGAGAAGGGGTGGGACTTCAGAACCCCCAACCCCG  
GCAATCTGGGTGCGGGAGCCTGGCGCACTGCGGGCCTCCCTCTAACCCTG  
GGCTTCCCTGGCGTCCAGGGGCCTCGGGGCAGTCCCGATTCTCCACCC  
CCGAAGCCGCGCCAGGACCAACGAGGGCGCAGCCGTATGCCCCAGCCCGCTC  
CGCGGAGCCCCCTCACAGCCACCCCCCGCCCAGACCGCGCCCCTCGCGCGCTCGA  
AGCACCTTCCCAAGGGGCTGGTCCTTGCGCCATAGTCGCGCGCGGAGCCTCTG  
GAGGGACATCAAGGATTTCTCGCTCCTACCAGCCACCCCCAAATTTTGGGA  
GGTACCCAAGGGTGCGCGCGTGGCTCCTGGCGCGCCGAGGCCCTCCCTCGAG  
GCCCCGCGAGGTGCACACTGCGGGGCCAGGGCTAGCAGCCGCCCGGCACGTC  
GCTACCCTGAGGGGCGGGGCGGAGCTGGCGCTAGAAATGCGCGGGGCCT  
GCGGGGCA GTTGCGCAAGTTGTGATCGGGCCGCTATAAGAGGGGCGGGCAG  
GCATGGAGCCCCTAGGAATCGCAGCGCCAGCGGTTGCAAGGTAAGGCCCC  
GGCGCGCTCCTTCCTTCTCTGCTGGTCTTTCTTGGCAGGCCACAGGGCCC  
CACACAACTCTGGATCC

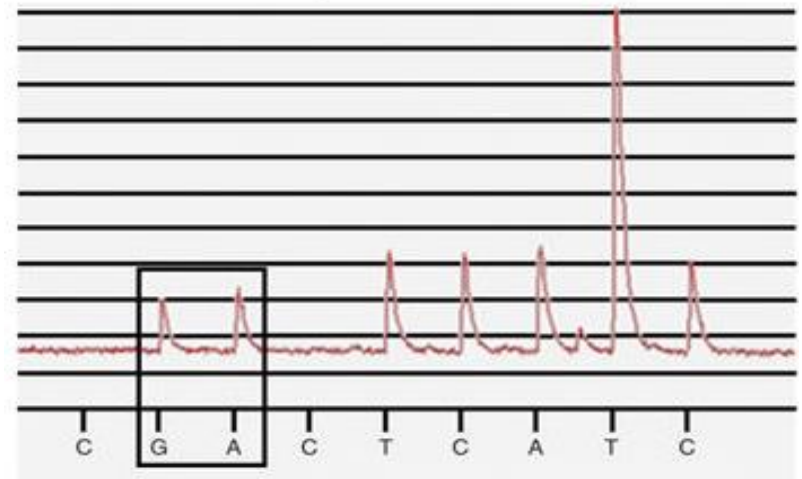
# DNA methylation and fetal/placental development





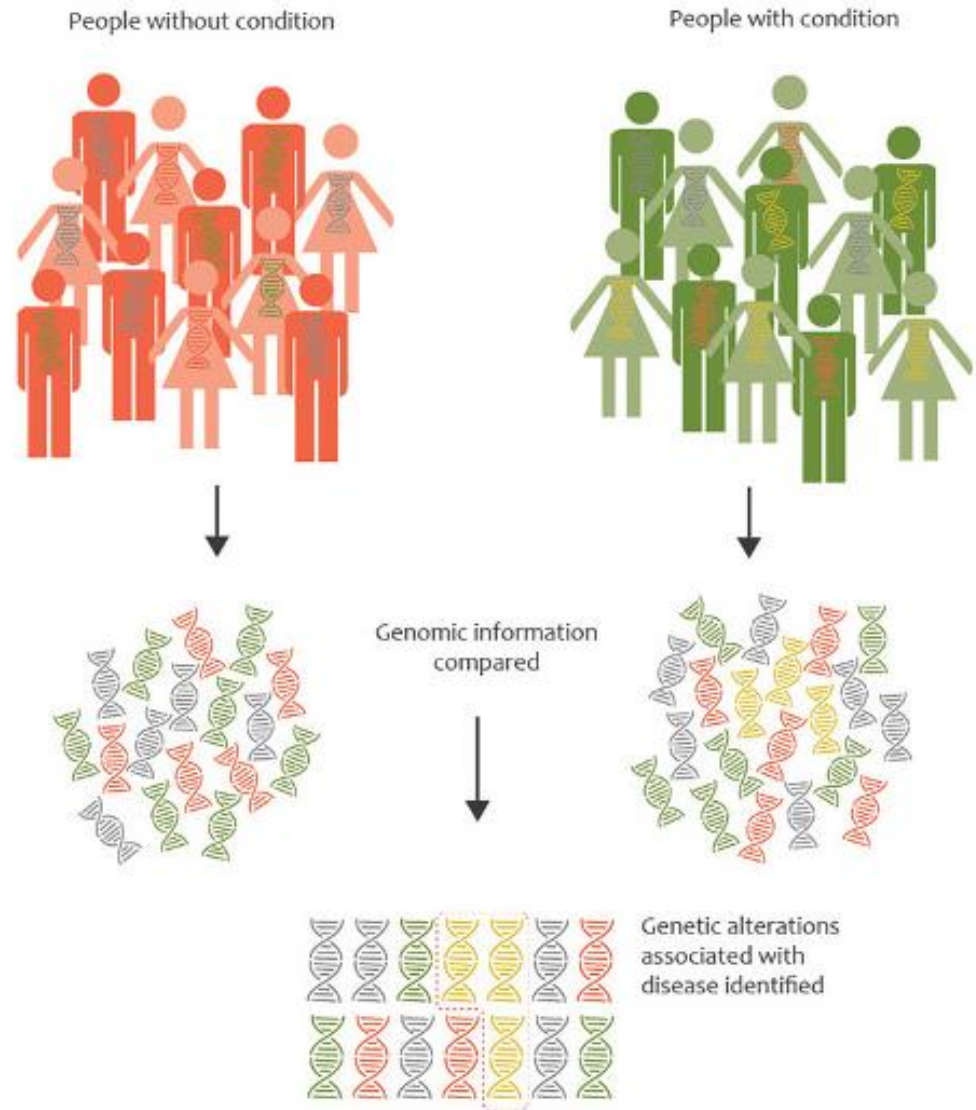
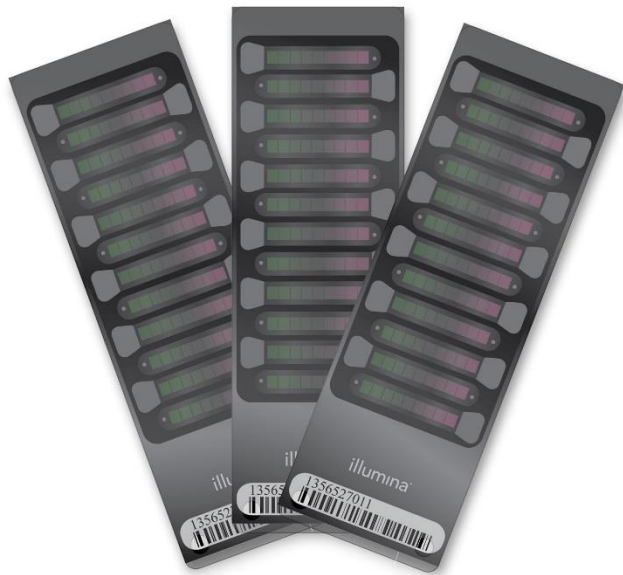
## How to study epigenetics?

- Look at a single gene region – candidate gene studies  
(when you have an idea of what you are looking for)

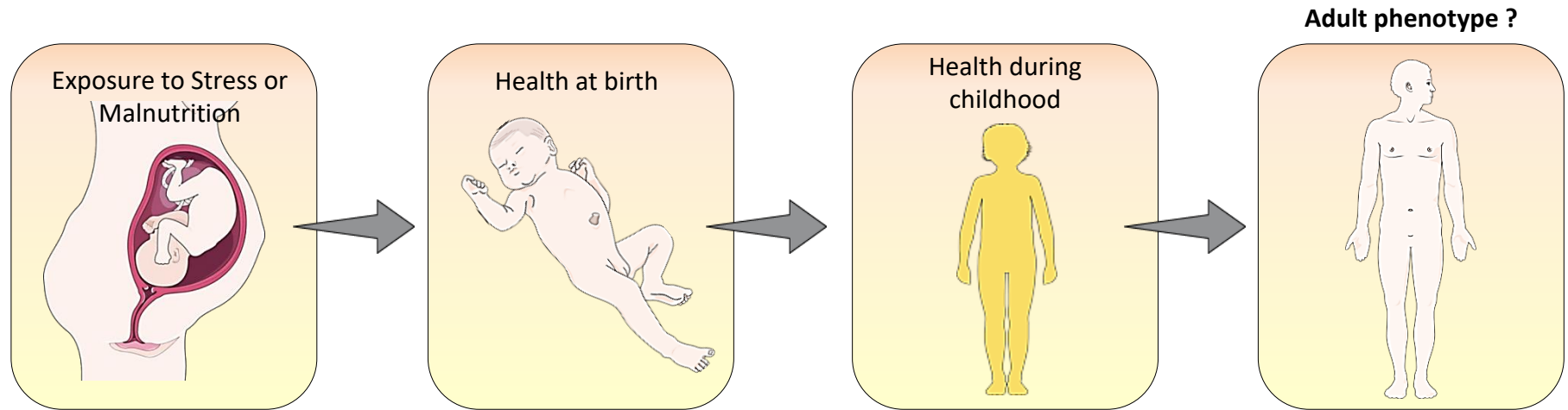


# Genome-wide epigenetics

## Epigenome-wide association studies (EWAS)



# Epigenetic studies of the Developmental Origins of Health and Disease hypothesis (DOHaD)



Where do epigenetic changes fit in???



## Gestational diabetes and maternal obesity are associated with epigenome-wide methylation changes in children

Line Hjort,<sup>1,2,3</sup> David Martino,<sup>4,5</sup> Louise Groth Grunnet,<sup>1,3</sup> Haroon Naeem,<sup>6,7,8</sup> Jovana Maksimovic,<sup>5,6</sup> Anders Henrik Olsson,<sup>1</sup> Cuilin Zhang,<sup>9</sup> Charlotte Ling,<sup>10</sup> Sjurdur Frodi Olsen,<sup>11</sup> Richard Saffery,<sup>5,12</sup> and Allan Arthur Vaag<sup>1,13</sup>

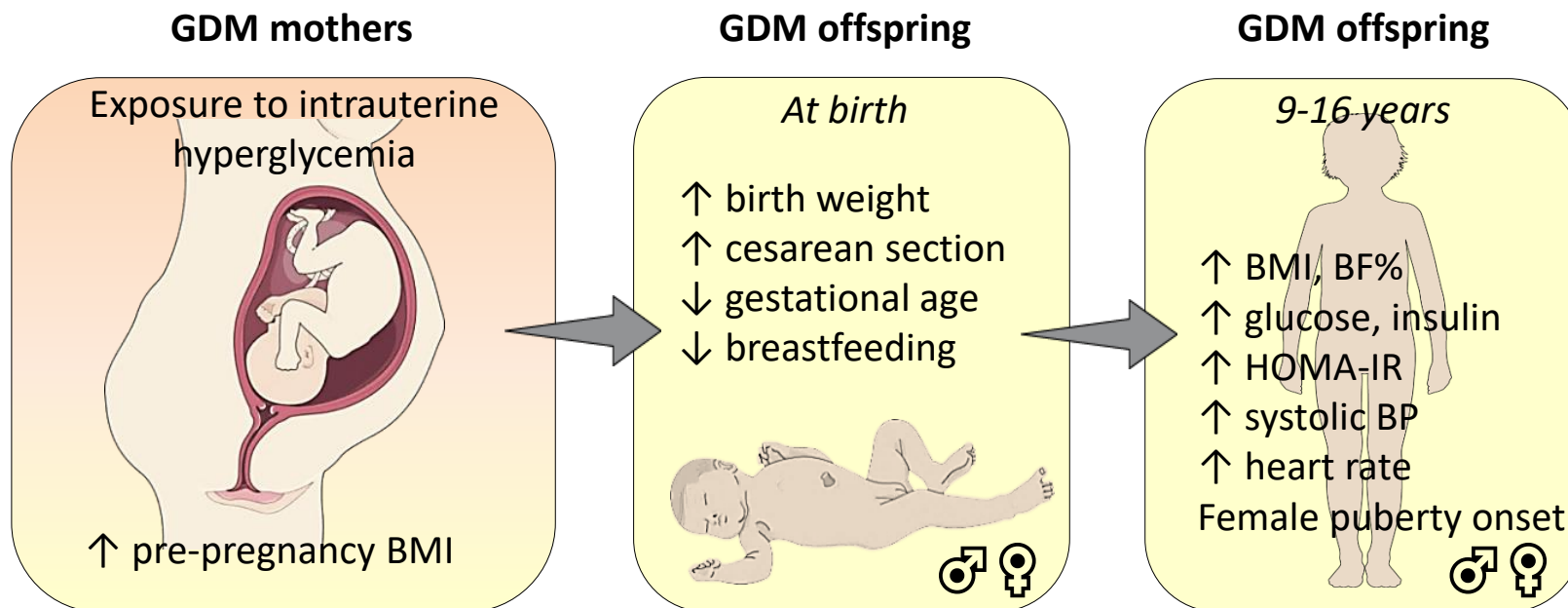
<sup>1</sup>Department of Endocrinology (Diabetes and Metabolism), Rigshospitalet, Copenhagen, Denmark. <sup>2</sup>Faculty of Health and

### Aim:

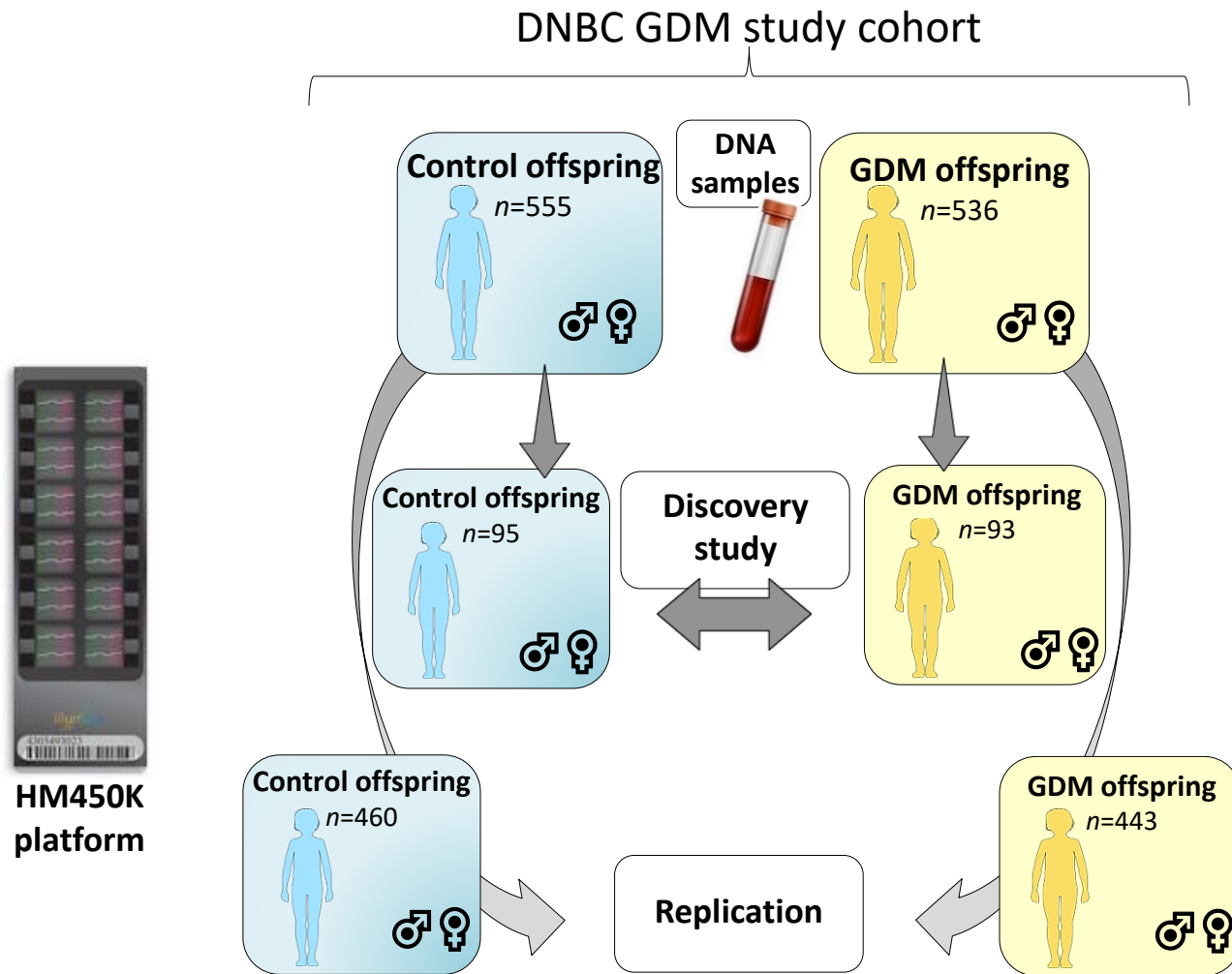
To explore genome-wide DNA methylation changes in blood in 9- to 16-year-old offspring exposed to GDM *in utero*, initially using a genome-wide-based discovery approach followed by replication

Diabetes Centre, LRL, Scania University Hospital, Malmö, Sweden. <sup>10</sup>Centre for Fetal Programming, Statens Serum Institut, Copenhagen, Denmark. <sup>12</sup>Cancer and Disease Epigenetics, Murdoch Children's Research Institute, Melbourne, Victoria, Australia. <sup>13</sup>Cardiovascular and Metabolic Disease (CVMD) Translational Medicine Unit, Early Clinical Development, IMED

## Clinical characteristics of the DNBC GDM subcohort ( $n = 1234$ )



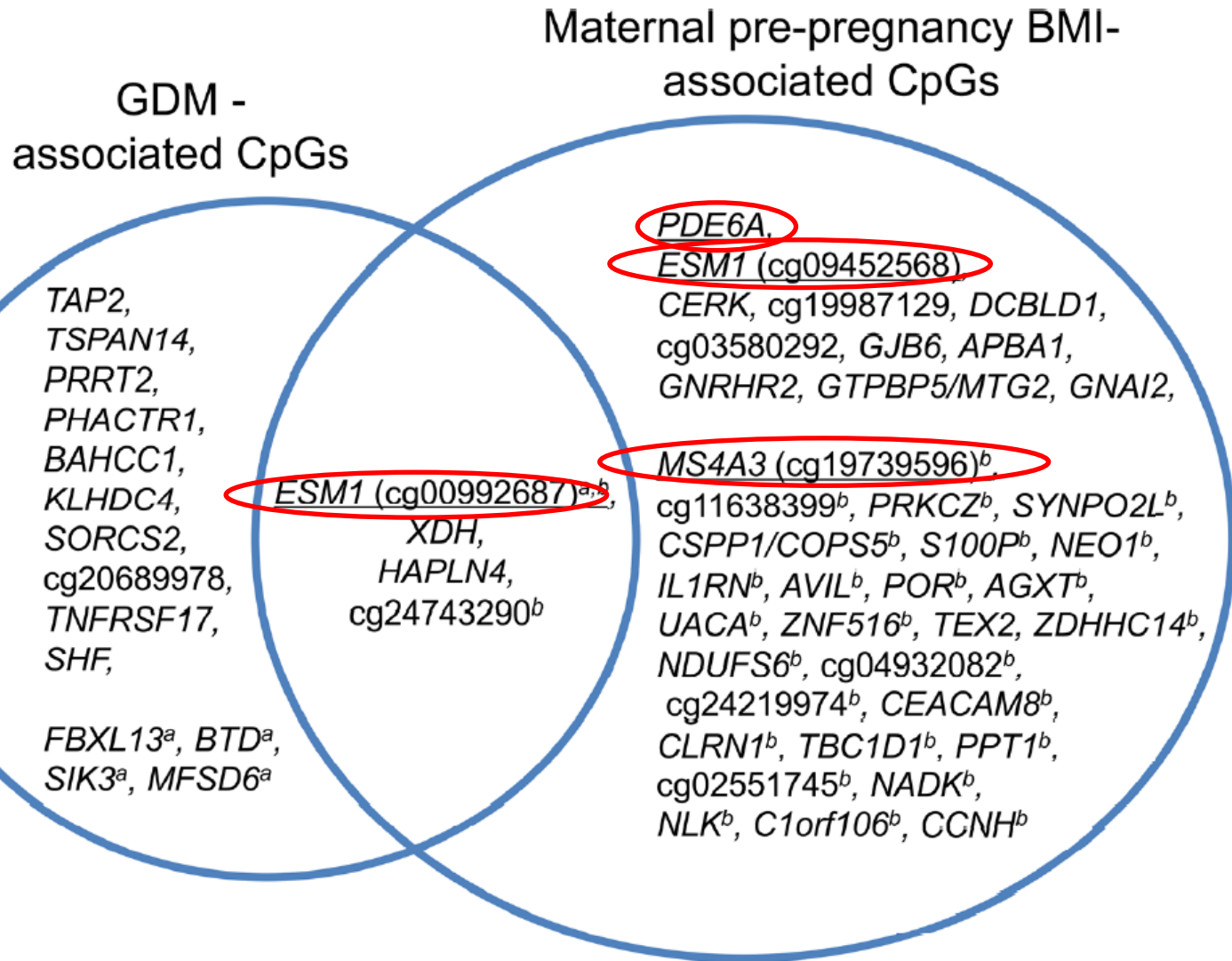
# DNA methylation study





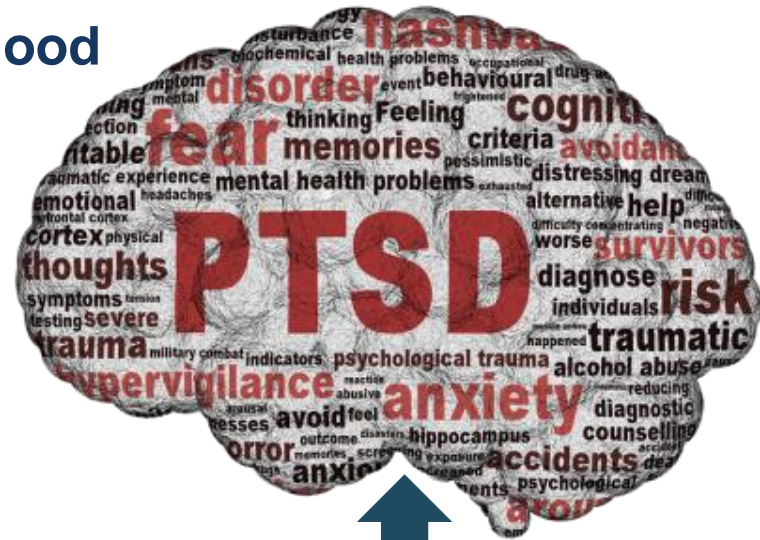
# DNA methylation discovery study ( $n = 188$ )

Adjustment for maternal BMI, and offspring BMI, age and sex



# Maternal PTSD in pregnancy and childhood

- PTSD is a severe psychiatric condition, resulting as a direct consequence of exposure to a traumatic event or from chronic trauma exposure.
- Children born to traumatized women are more likely to have behavioral, developmental and clinical problems, including PTSD symptoms.



## Aim:

What are the possible mechanisms for generational transmission of trauma derived from PTSD in pregnancy?

## Study population and methodology

- Interview of 130 women who experienced sexual violence/torture during the war in Kosovo:
- Inclusion of 117 women:
  - 84 of the women had PTSD during pregnancy, 33 had not.
  - The rest developed PTSD after giving birth
- All of the women gave birth to at least one child born after the war ended (today aged 1-18 years old, not related to the sexual assault).

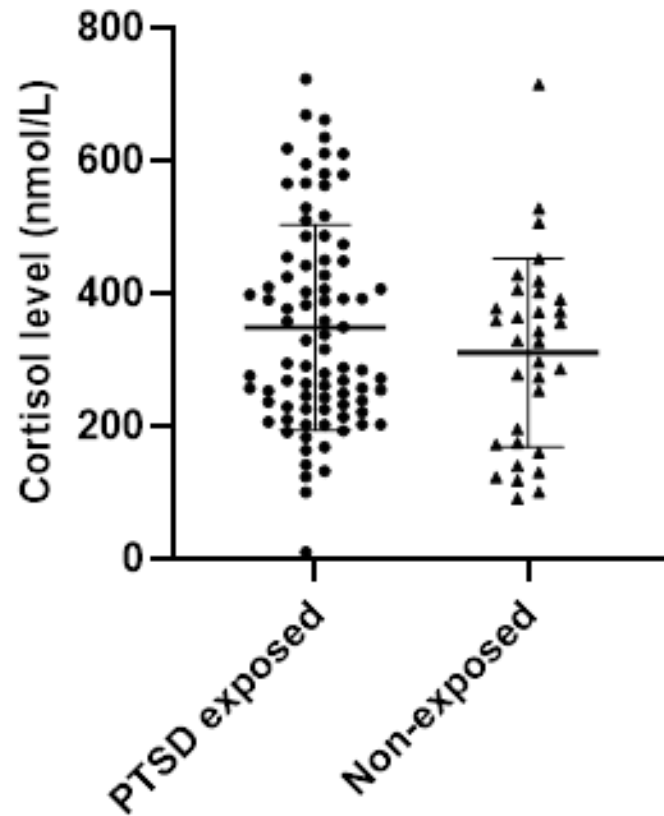
We examined:

1. The children's stress levels (**cortisol concentration**)
2. If PTSD exposure was associated with DNA methylation differences



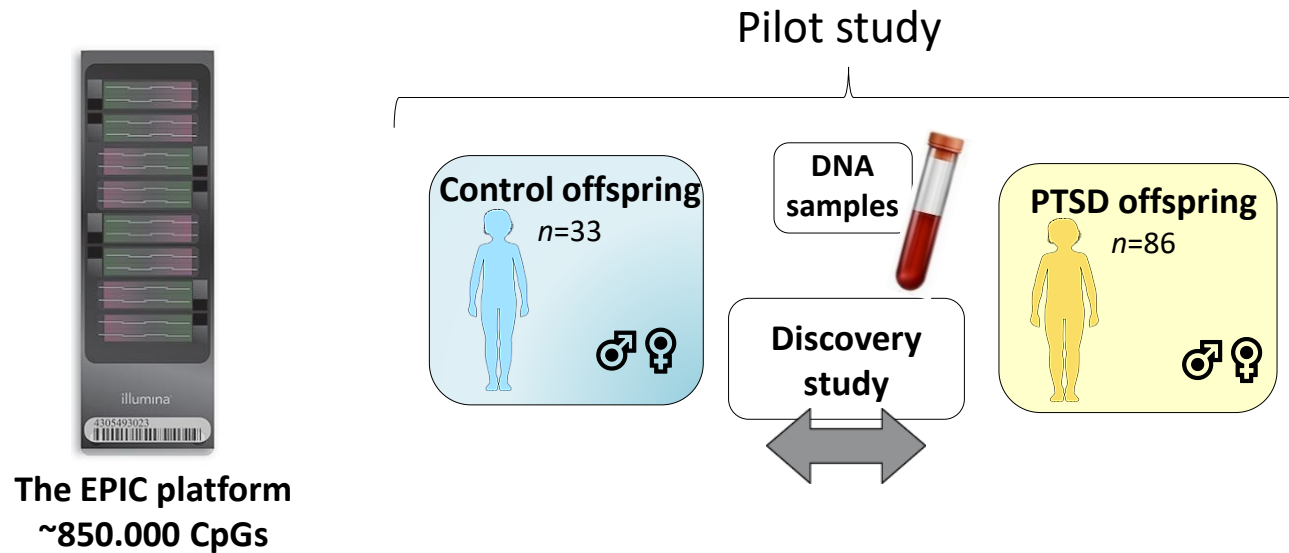
**B.**

Cortisol levels in PTSD-  
vs. non-exposed offspring



- Children born to mothers with pregnancy PTSD were more likely to have high cortisol levels (15.5%) compared to children of mothers without pregnancy PTSD (3.0%),  $P=0.04$

# Epigenome-wide association study (EWAS) of DNA methylation



## EWAS analysis:

- To identify differentially methylated CpGs we performed multiple regression modelling adjusting for mothers age, the sex and age of the child
- After FDR: no CpGs were significantly associated with PTSD in pregnancy.....

## Candidate gene look-up in the EWAS data

- Literature search to identify stress related genes: (*NR3C1*, *NR3C2*, *HTR3A*, *SLC6A4*, *OXTR*, *FKBP5*, *BDNF*).
- Differential methylation of all seven genes were replicated in the discovery study between offspring exposed to maternal PTSD during pregnancy, and the controls.

**Table 6:**

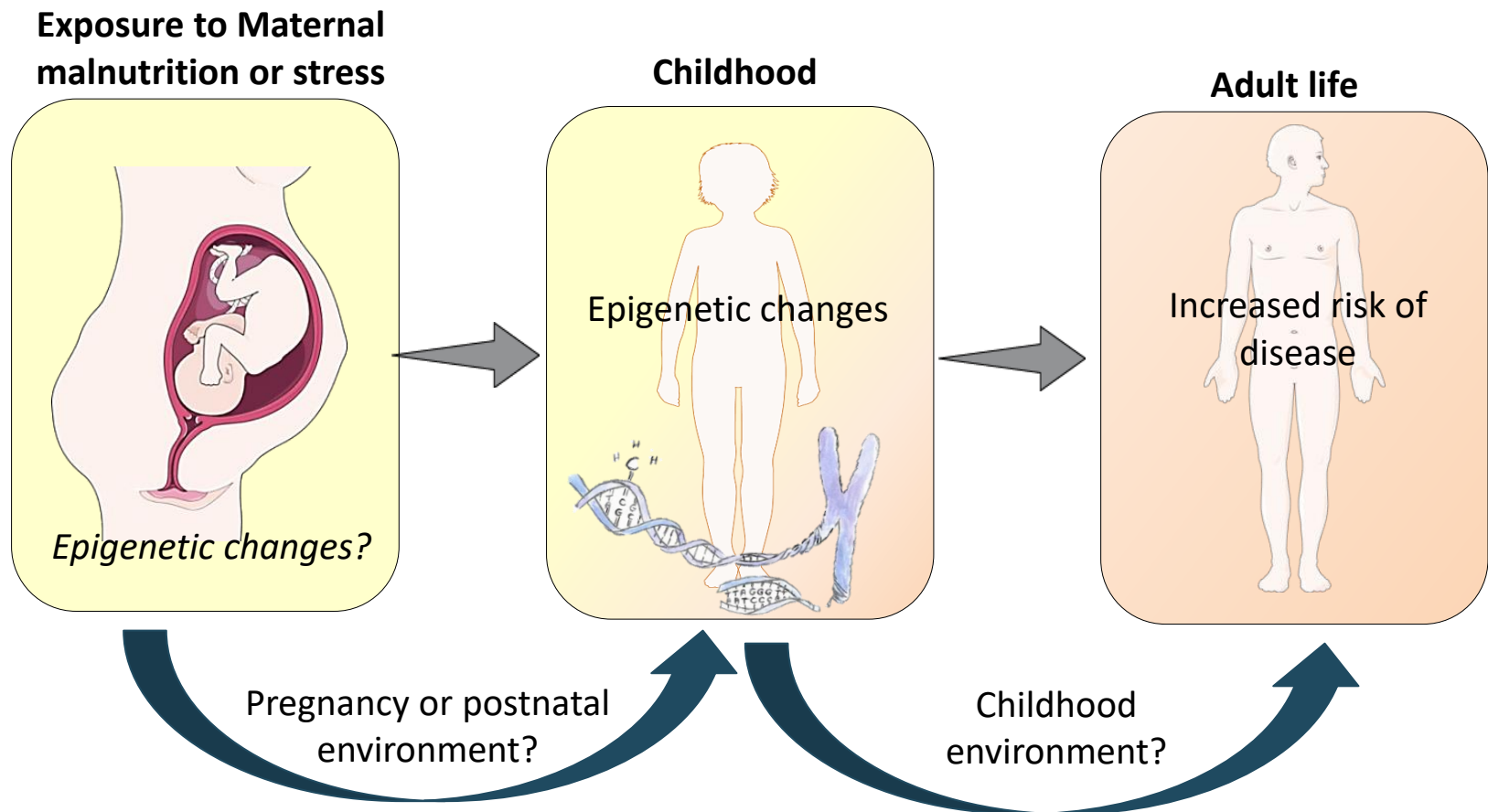
Gene	Probe	$\Delta\text{beta, \%}$	chr nb.	position	strand	UCSC Reference gene	P-value (Ass. with PTSD)	P-value (Ass. with Cortisol)
<i>HTR3A</i>	cg12612985	-0.78	chr11	113847121	-	TSS1500; Body	0.008	0.01
<i>SLC6A4</i>	cg0199110	-0.55	chr17	28555935	-	5'UTR	0.049	-
<i>OXTR</i>	cg17036624	-2.00	chr3	8811601	-	TSS1500	0.020	-
<i>OXTR</i>	cg03710862	-0.73	chr3	8811728	-	TSS1500	0.029	-
<i>OXTR</i>	cg14483142	-0.98	chr3	8811758	+	TSS1500	0.036	-
<i>NR3C1</i>	cg07715663	-0.79	chr5	142721796	+	Body	0.017	-
<i>NR3C1</i>	cg21209684	-1.88	chr5	142783848	-	5'UTR; TSS1500	0.036	0.019
<i>NR3C1</i>	cg26464411	-3.42	chr5	142784222	-	TSS1500; 5'UTR	0.048	-
<i>NR3C2</i>	cg1315799	-2.11	chr4	149191991	-	Body	0.008	-
<i>NR3C2</i>	cg13373360	1.33	chr4	149364881	+	TSS1500	0.032	-
<i>FKBP5</i>	cg09268536	1.21	chr6	35611576	+	5'UTR	0.049	-
<i>BDNF</i>	cg18595174	-1.69	chr11	27701991	+	Body; 5'UTR	0.039	-
<i>BDNF</i>	cg20340655	0.68	chr11	27721661	+	TSS1500	0.002	-
<i>BDNF</i>	cg15688670	1.86	chr11	27723190	+	TSS1500; Body	0.014	0.035
<i>BDNF</i>	cg04481212	0.69	chr11	27740495	+	Body; 5'UTR	0.029	0.025
<i>BDNF</i>	cg04106006	2.31	chr11	27742454	-	Body; TSS1500; TSS200	0.033	-
<i>BDNF</i>	cg10022526	0.83	chr11	27744557	-	TSS1500	0.049	-



## Conclusions

The effects of malnutrition and maternal stress during pregnancy may become biologically embedded in the child by epigenetic mechanisms, with potential for both short and long-term effects on their development and risk of disease in later life.

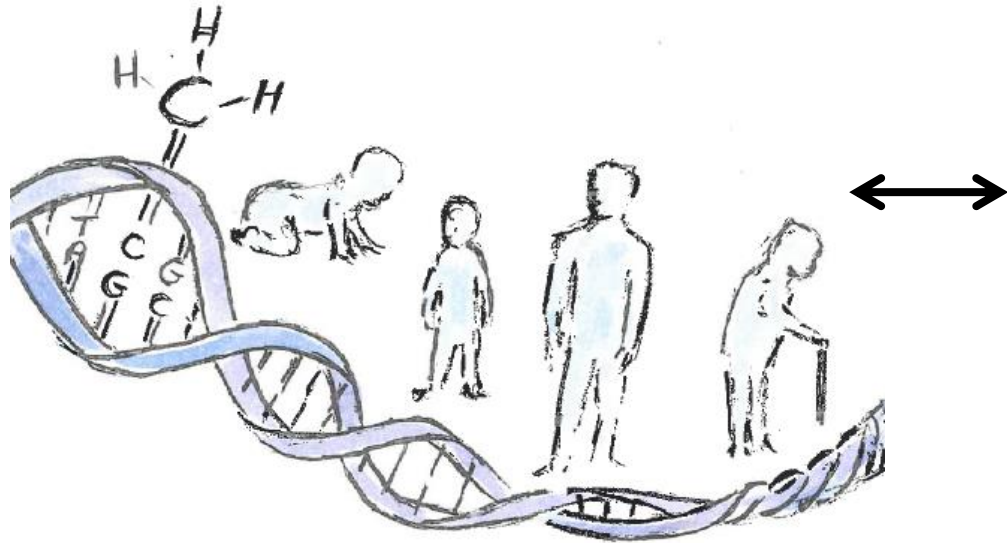
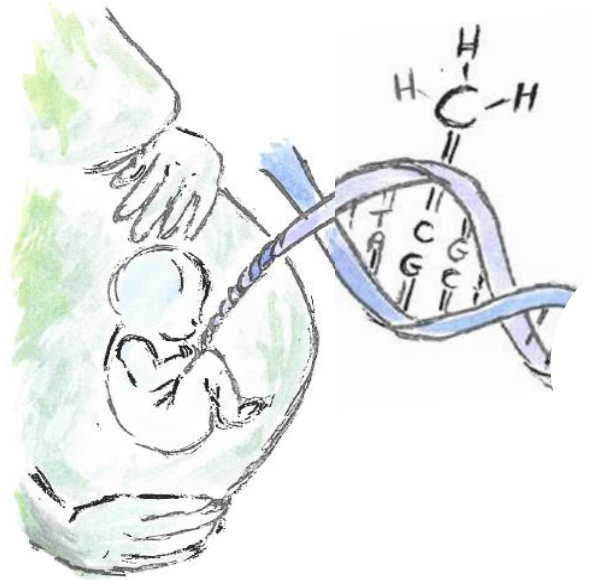
*– However, some unknowns on timing of exposure and other confounders, that require careful future study designs:*



# Thanks for your attention

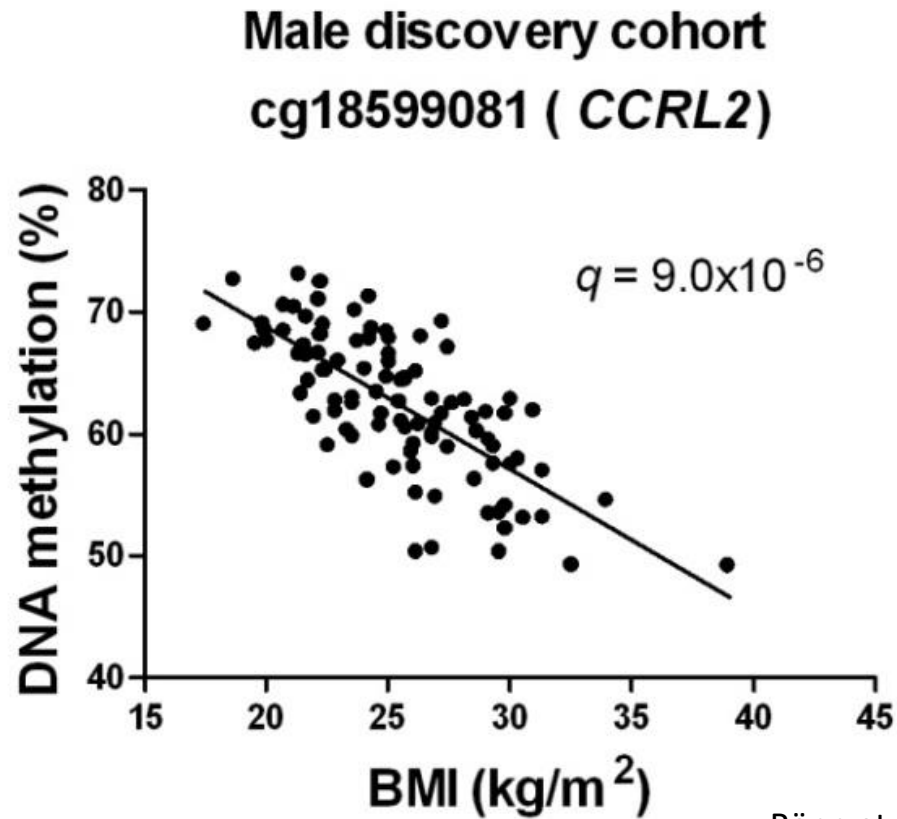


## Stability vs. flexibility



## Epigenetic flexibility

### BMI

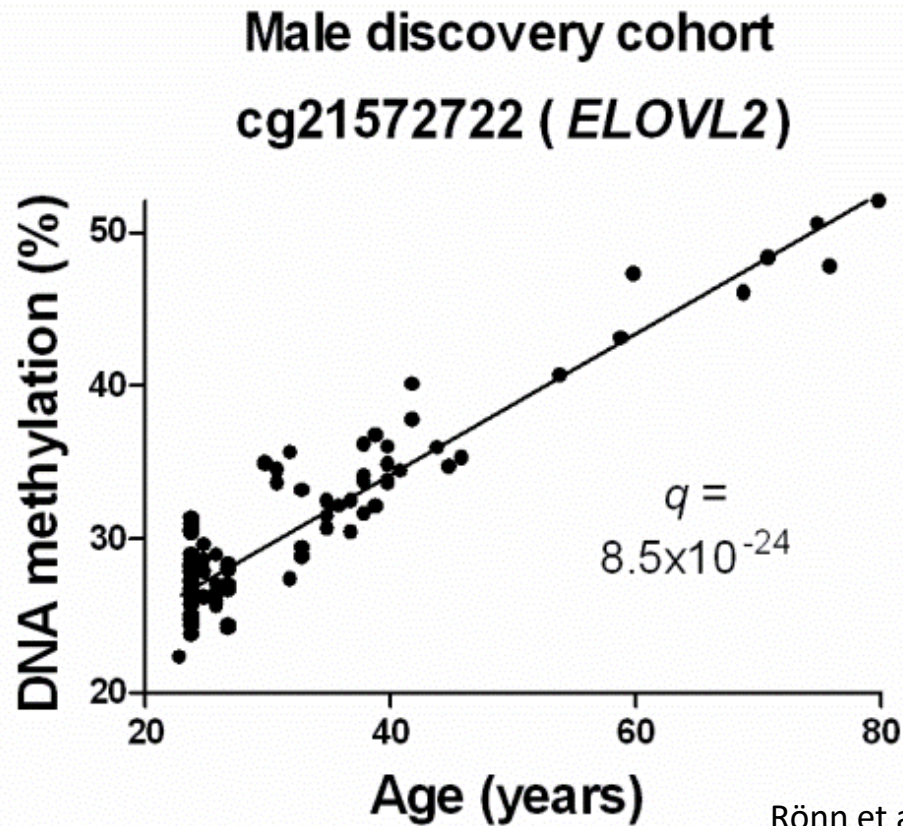


Rönn et al. *HMG* 2015



## Epigenetic flexibility

# AGE



Rönn et al. *HMG* 2015