Epigenetics – Memories of Past Exposures and Predictors of Diseases

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School of Public Health

Where will I take you?

1. Motivation to study epigenetic epidemiology
2. Epigenetics
3. DNA methylation and exposures
4. DNA methylation and disease risks
5. Explanatory models
6. Ex: Gestational smoking & childhood obesity
7. Perspectives

Isle of Wight – 1989 Birth Cohort
2 and 3rd generation study

Funded by NIAID
1R01AI091905

Focus: Asthma, eczema, allergies, BMI, etc.

Dr. Hongmei Zhang, PhD

Xin Tong Genevieve Ray Lyons

Ali Ziyab

Caroline Mulatya

Nelis Soto-Ramirez

Melannie Alexander

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Failure of classical genetics

1. Gene effects explaining diseases are typically small (RR = 1.1-1.5) and not easily replicable
2. Risky genetic variants often detected in only small subgroups of the population
   → do not explain a large proportion of diseases
3. Gene × environment show much stronger risks – maintained over a long period
4. Genetic variants do not explain the heritability of common diseases ("missing heritability").
5. Changes in the genetic pool do not account for the temporal and spatial increases of diseases in the last 60 years.

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Genome:

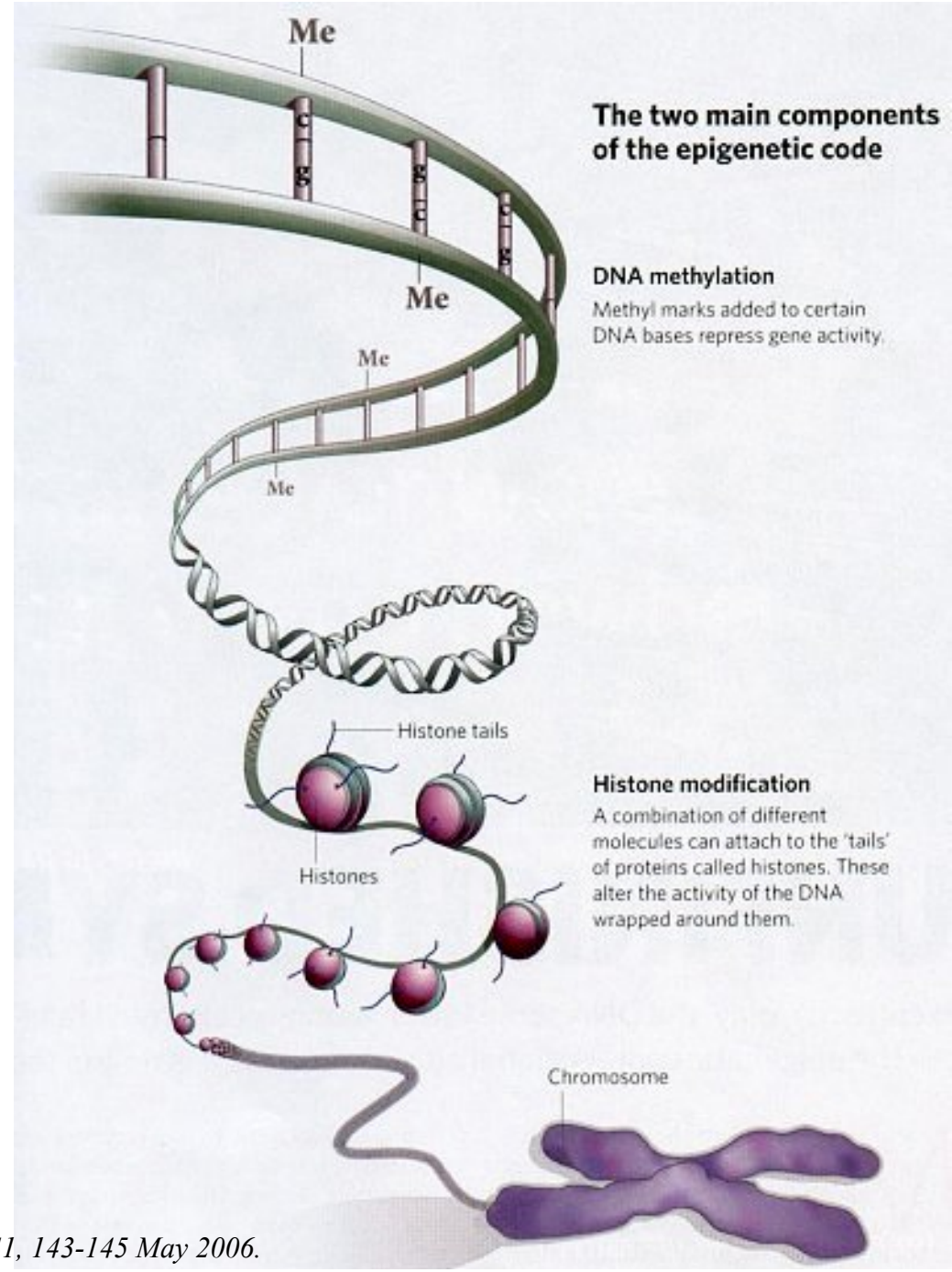
- Building blocks for the manufacture of proteins needed for the cell functional activity (however, ~25,000 genes and ~2,000,000 proteins)

Epigenome:

- Instruction on *how*, *when*, and *where* the information should be used
- Inheritance of variations above and beyond (epi) changes in the DNA sequence

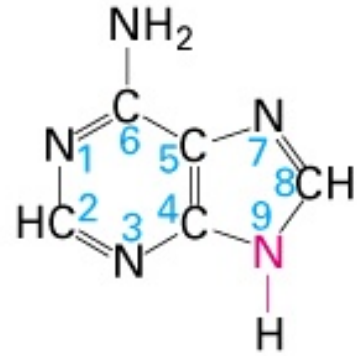
Epigenetics →

Science studying heritable changes of genes that regulate gene expression, but do not involve changes in DNA sequence.

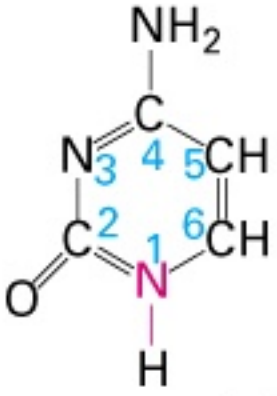


Nature 441, 143-145 May 2006.

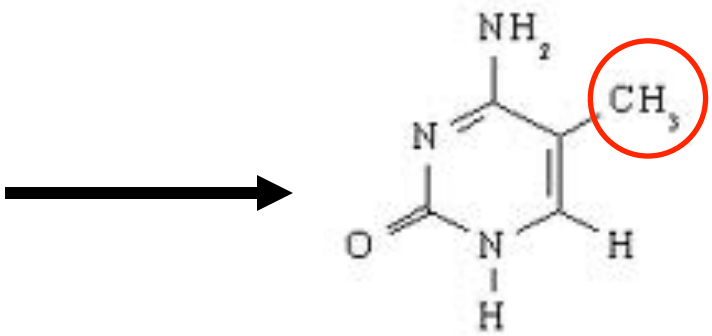
The five nucleotides that make up DNA



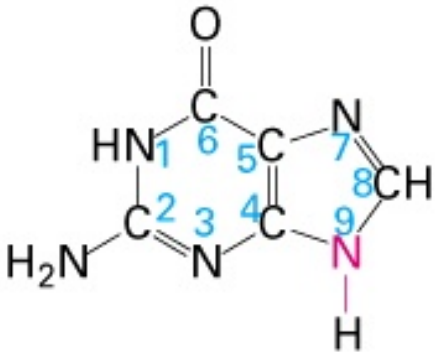
Adenine (A)



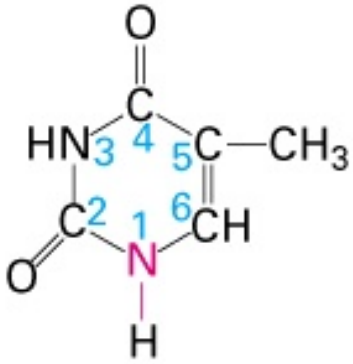
Cytosine (C)



Methylcytosine



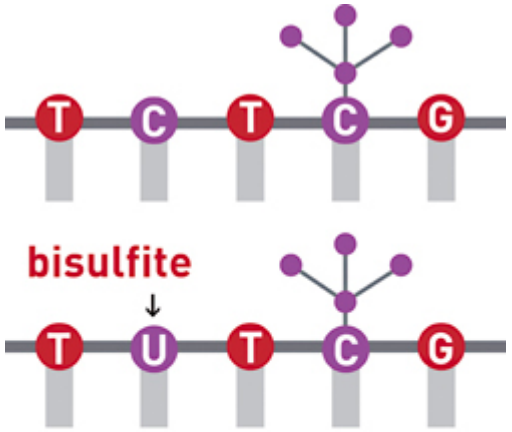
Guanine (G)



Thymine (T)

Detection of DNA methylation

Bisulfite conversion is the use of sodium bisulfite to convert cytosine residues to uracil,
but leaving 5-methylcytosine residues unaffected.



→ Five nucleotides: - adenine, cytosine, uracil
- guanine, thymine

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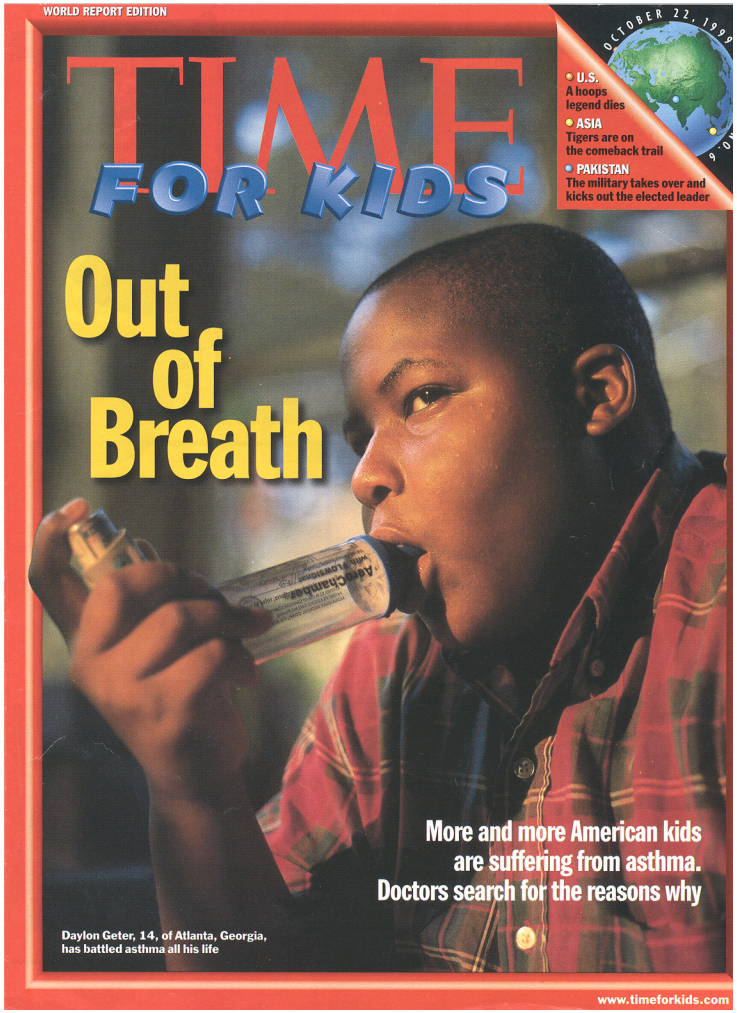
Exposures and epigenetics in the *course of generations and the course of life*

Accumulating evidence for the concept for ***fetal programming:***

Developmental conditions → long-term consequences in childhood and adulthood

- Not the current complex settings of neighborhoods, air pollution, and food, but the (grand-)maternal conditions (*fetal programming of offspring*)
- Not all exposures during all periods of life, but in *periods of change* (e.g., gestation, puberty, menopause)

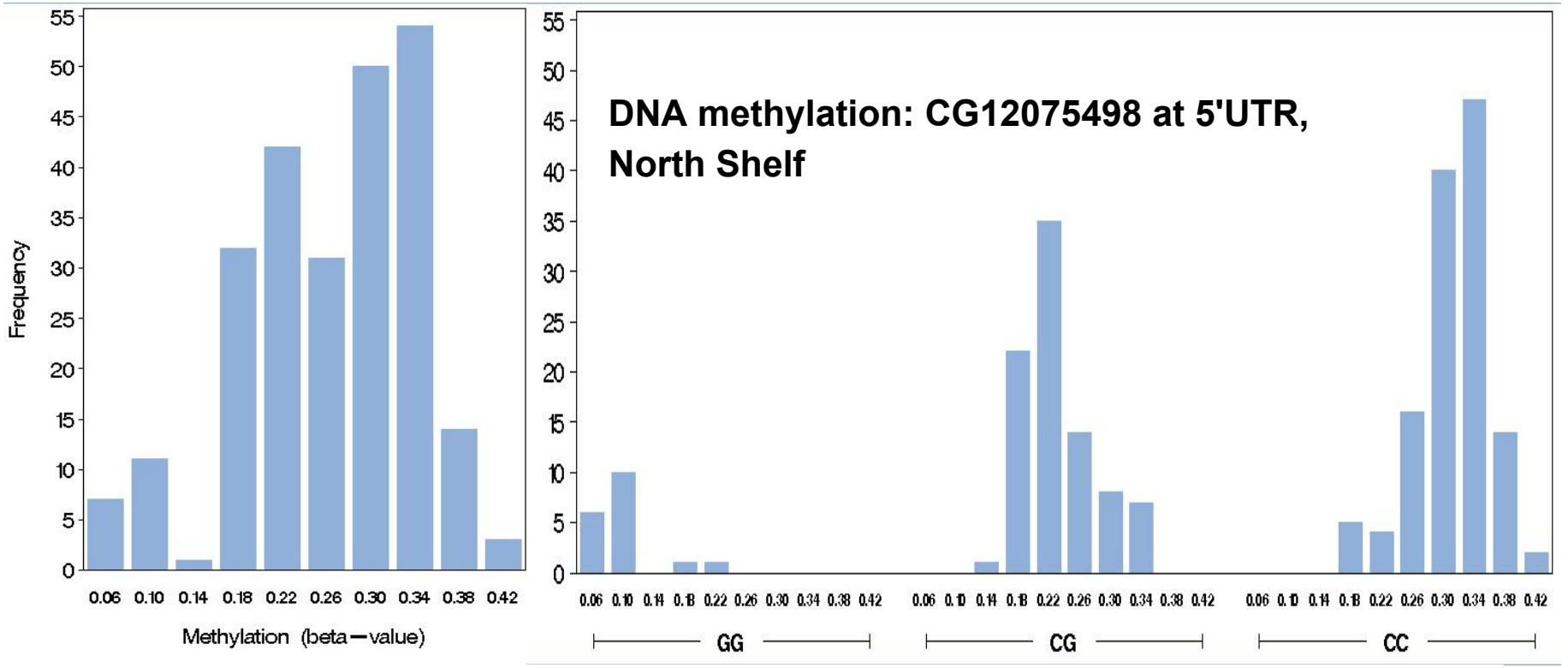
So what went wrong with ma & grandma? How can we reverse it?



GV variants affect the methylation: methQTLs

- Genetic variants in adjacent areas impact the modality of the DNA methylation distribution.

Figure: Thee distribution of DNA methylation is dependent on the genotype of *JAK1*

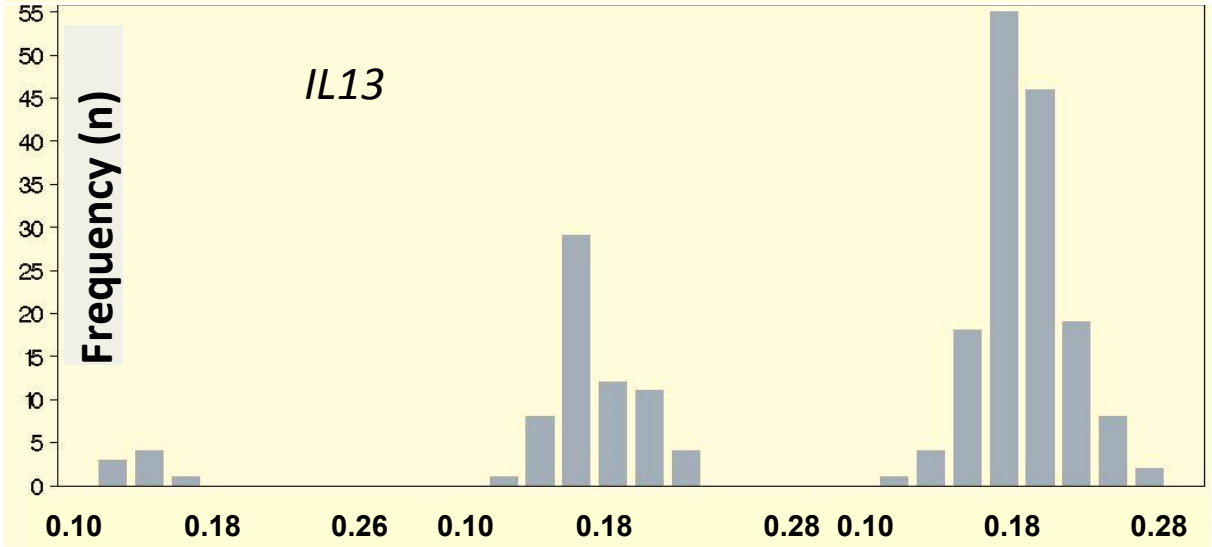


Crude *distribution*

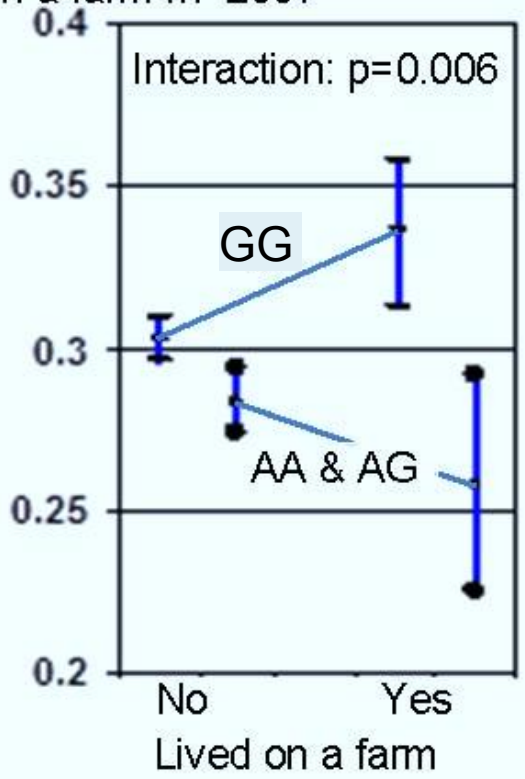
Stratified by *JAK1* rs11208559 flanking 5'UTR

Exposure and methQTLs DNA-M

- methQTLs predict DNA-M



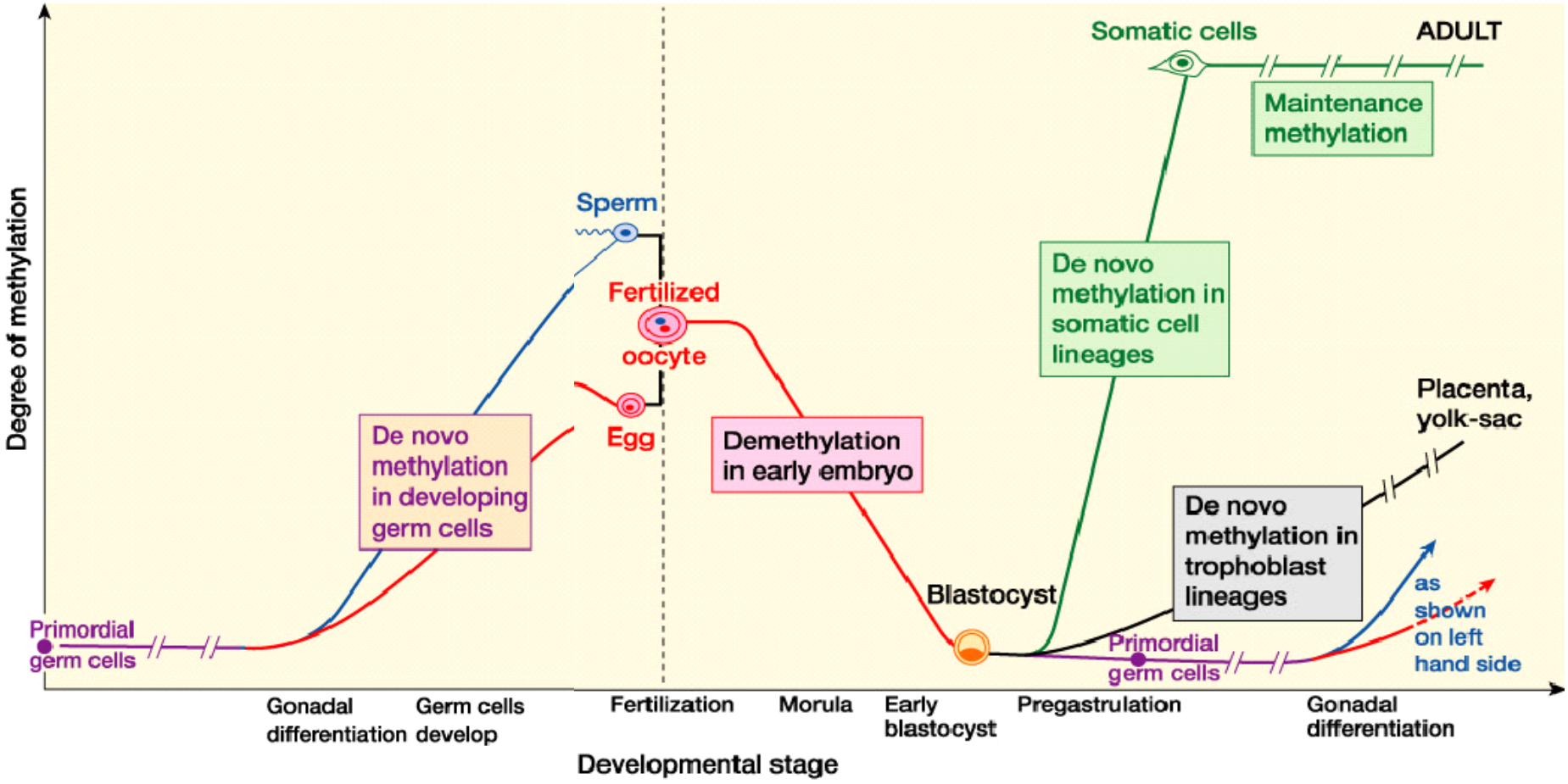
DNA methylation of *IL13* TSS1500 (cg04303330) as a result of *IL13* promoter rs1800925 and grown up on a farm (n=233)



- methQTLs interact with exposure to alter the DNA-M

Holloway, Karmaus, et al. manuscript in prep.

Changes in DNA methylation during mammalian development



Associations between exposures and DNA methylation – large variety of exposures

- Diet (~650 articles)
- Smoking (~400 articles)
- Maternal stress and offspring DNA-M (~60 articles)
- PCB, DDE, persistent organic compounds (~60 publications, animals and humans)
- Air pollution (~30 articles)
- Maternal BMI (Hoyo et al. 2012, Gemma et al. 2009)
- Disadvantaged socio-economic position (SEP) in childhood (Borghol et al., 2011)

Norwegian cohort (cord blood) and Isle of Wight birth cohort (blood at age 18)

Joubert et al. (2012) identified 26 methylated CpG sites in newborn cord blood samples that were potentially associated with maternal plasma cotinine (an objective biomarker of smoking) measured during pregnancy.

We found 38 CpG sites differentially methylated in girls at 18 years of age when the mother smoked during gestation.

Of 406,855 CpG sites tested, we found agreement between the two studies for 14 of 26 of the CpG sites identified in five of 10 genes.

Both studies identified

- *AHRR*
- *MYO1G*
- CYP1A1
- *CNTNAP2*
- *GFI1*

- "Fingerprint" or a less specific "footprint" in the DNA-M profile?

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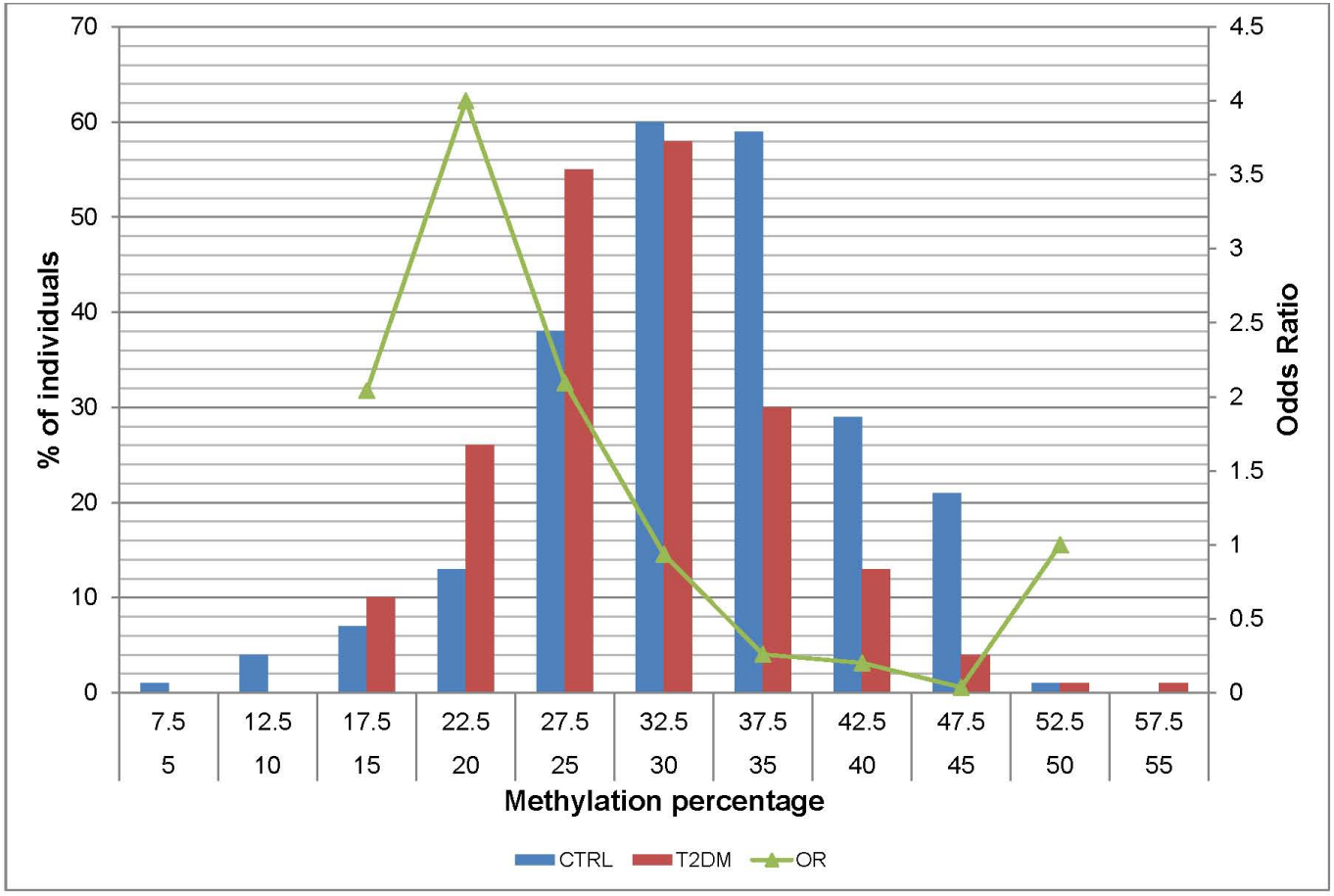
Different concepts for DNA-M as risk factors

1. DNA methylation as independent variable
2. DNA methylation solely as intervening variable between exposure and disease (the magic bullet)
3. DNA methylation, conditional on genetic variants, as intervening variable between exposure and disease

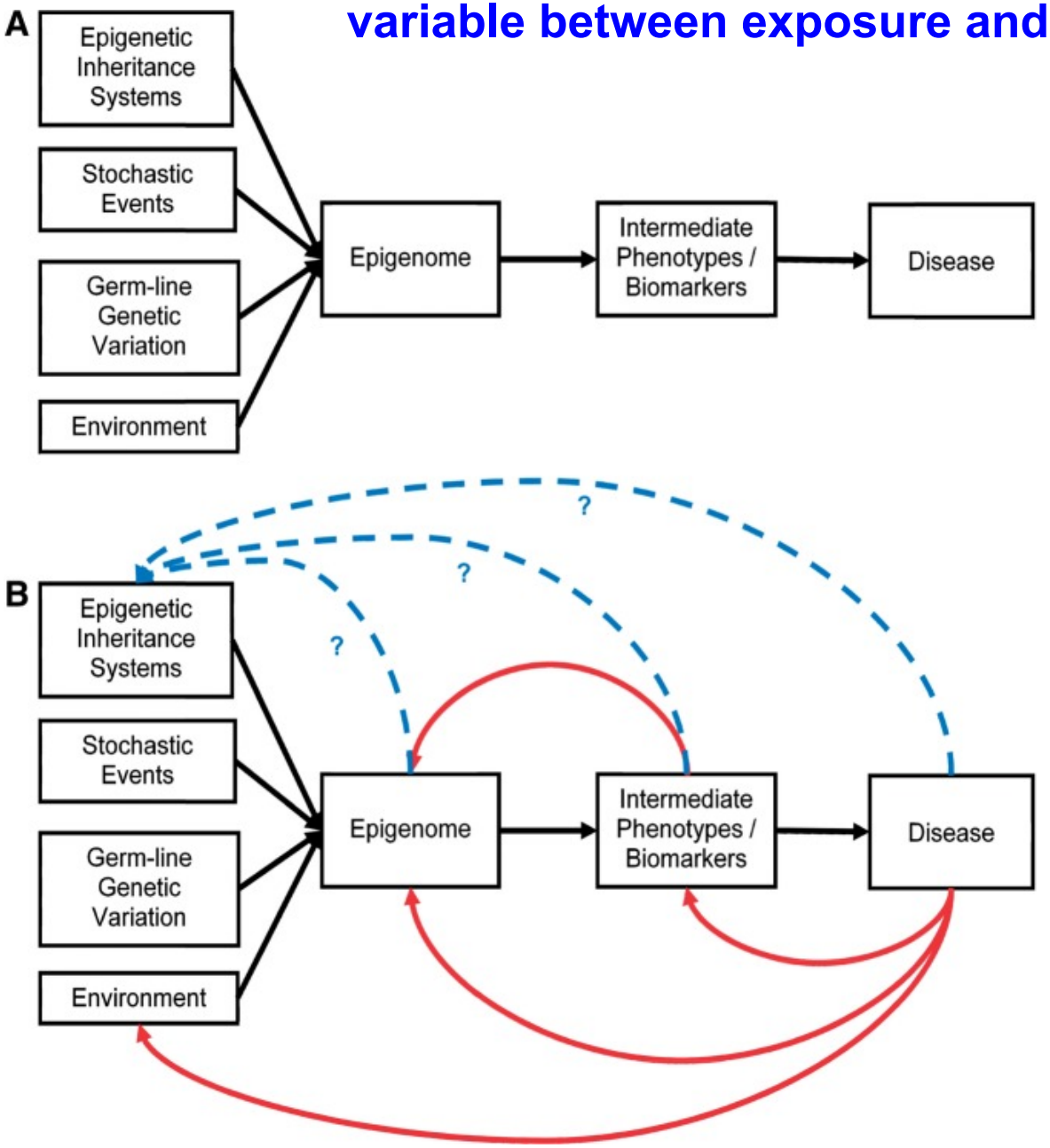
1. The independent effect of DNA-M (T2DM)



Data provided by: Toperoff et al., Publication in: ”Human Molecular Genetics, 2012, Vol. 21: 371–383 (peripheral white blood cells)

2. DNA methylation solely as intervening variable between exposure and disease

Int J Epidemiol. 2012;41:5-9. **Is epidemiology ready for epigenetics?** Relton CL, Davey Smith G.



The magic bullet concept.

General findings

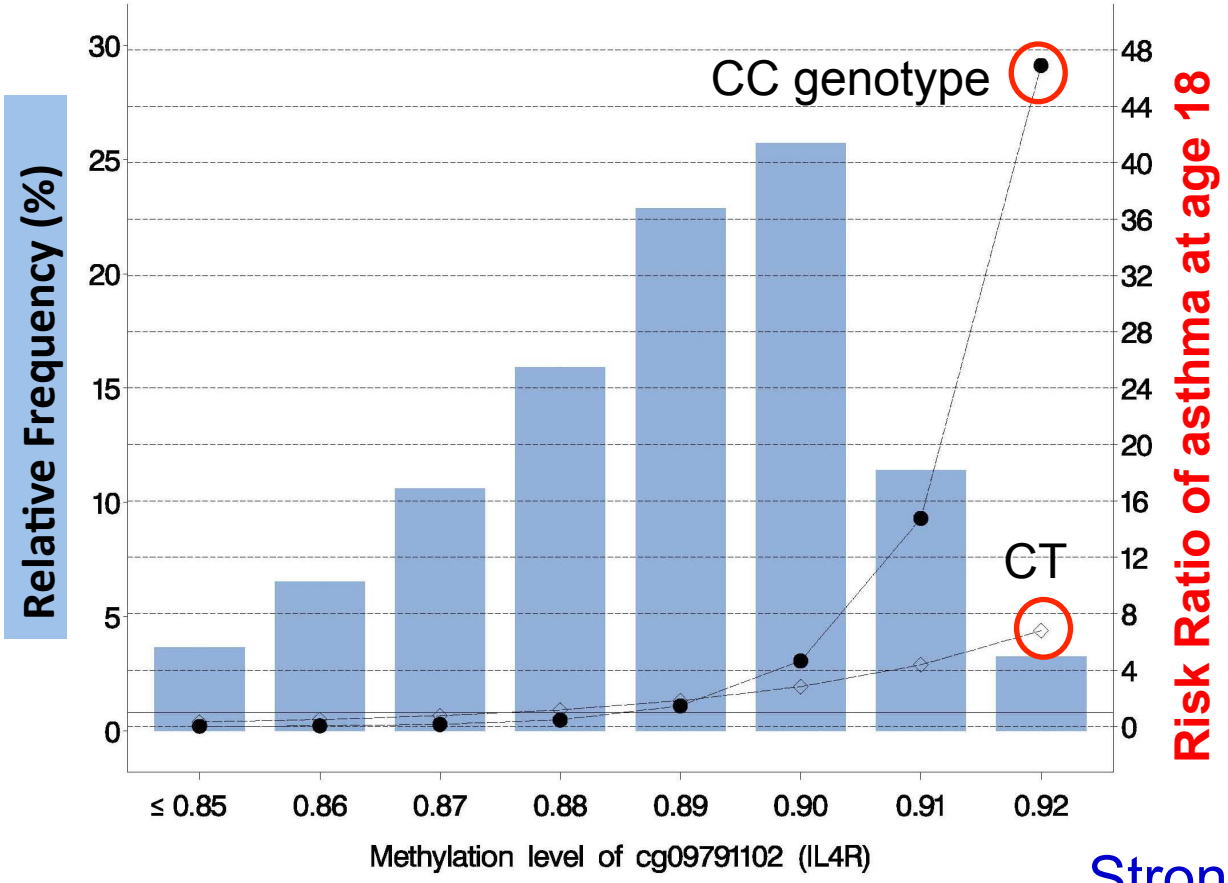
- Genetic variants: relative risk between 1.1 to 1.5 (-3)
- DNA methylation: relative risk between 1.5 to 4

Novel concept: What happens if we look at the interactionof genetic variants and methylation?

1. Karmaus et al., Curr Opin Allergy Clin Immunol 2013, 13:63–69
2. Soto-Ramírez et al. Clinical Epigenetics 2013, 5:1
3. Ziyab et al, Journal of the European Academy of Dermatology and Venereology a 2012

Large effects of DNA-M × modGV interactions

In all explored 13 genes, we found strong modifying effects of DNA-M on genetic variants (modGV)



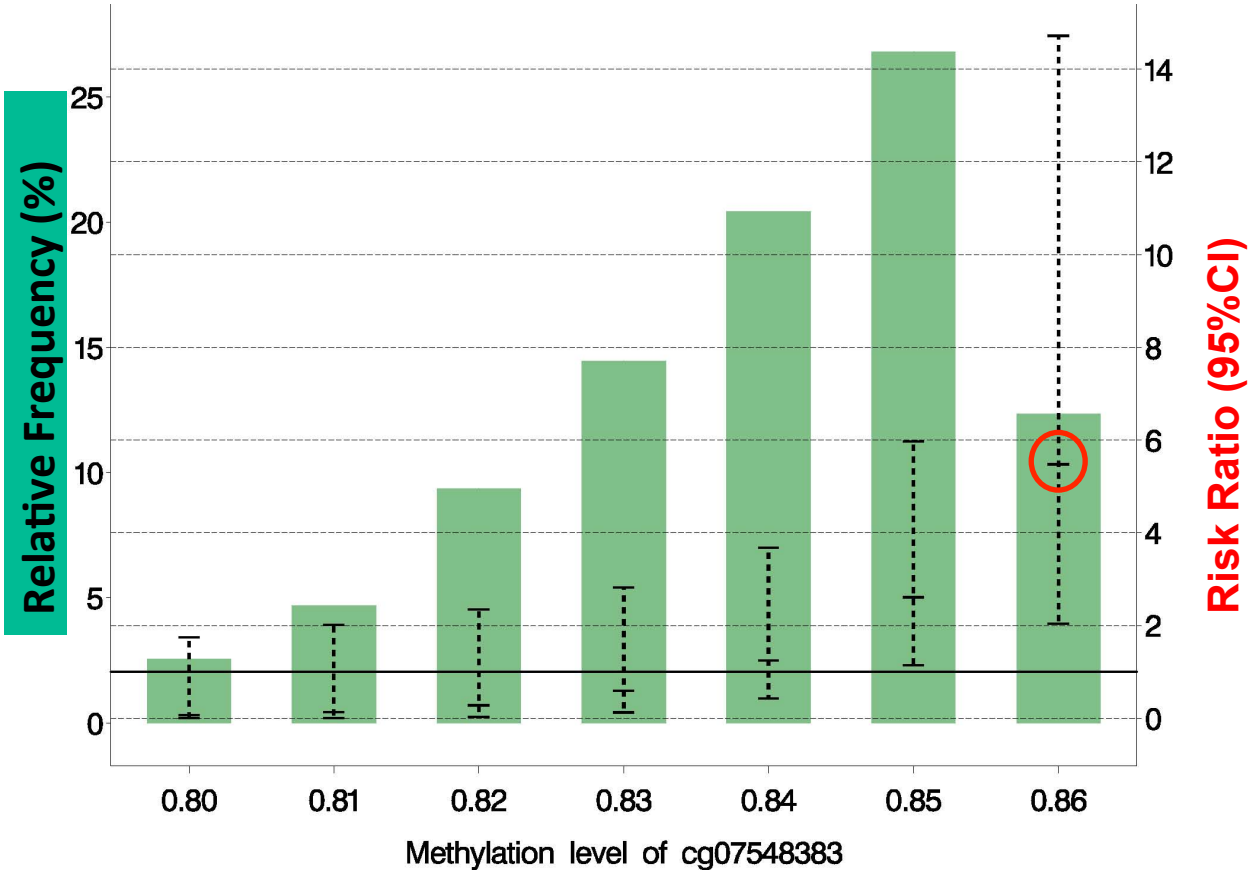
Interaction between the *IL4R* gene (rs3024685: combined genotypes AA and AG vs. GG) and cg09791102 (p-value adjusted for multiple testing =0.002) on the relative risk of asthma at age 18 (n=244, F1 women).

Strong effects → small sample sizes

Breakthrough in the relative risk

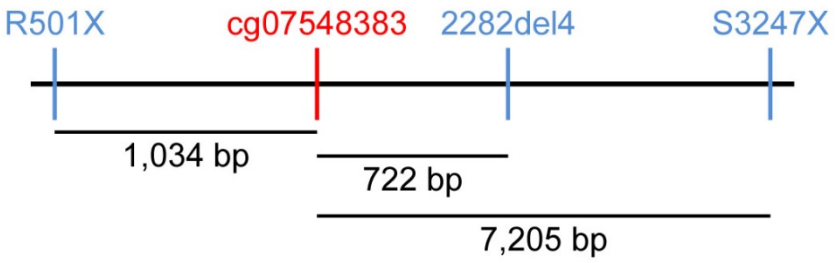
Soto-Ramírez et al. Clinical Epigenetics 2013, 5:1

FLG gene × DNA-M interaction for eczema



Interaction between the *FLG* gene (loss of function: R501X, 2282del, or S3247X) and methylation of cg07548383 on the risk ratio of eczema at age 18.

Ziyab et al, Journal of the European Academy of Dermatology and Venereology a 2012



Genetic variants × DNA-M interaction

- Genetic variants × DNA methylation
  → quantum leap in the estimation of relative risks:
  10-fold *plus* (for asthma, eczema, and allergy)

- A genetic polymorphism cannot have full penetrance if it is methylated. The strength of the modifying effect of DNA-M is stunning.

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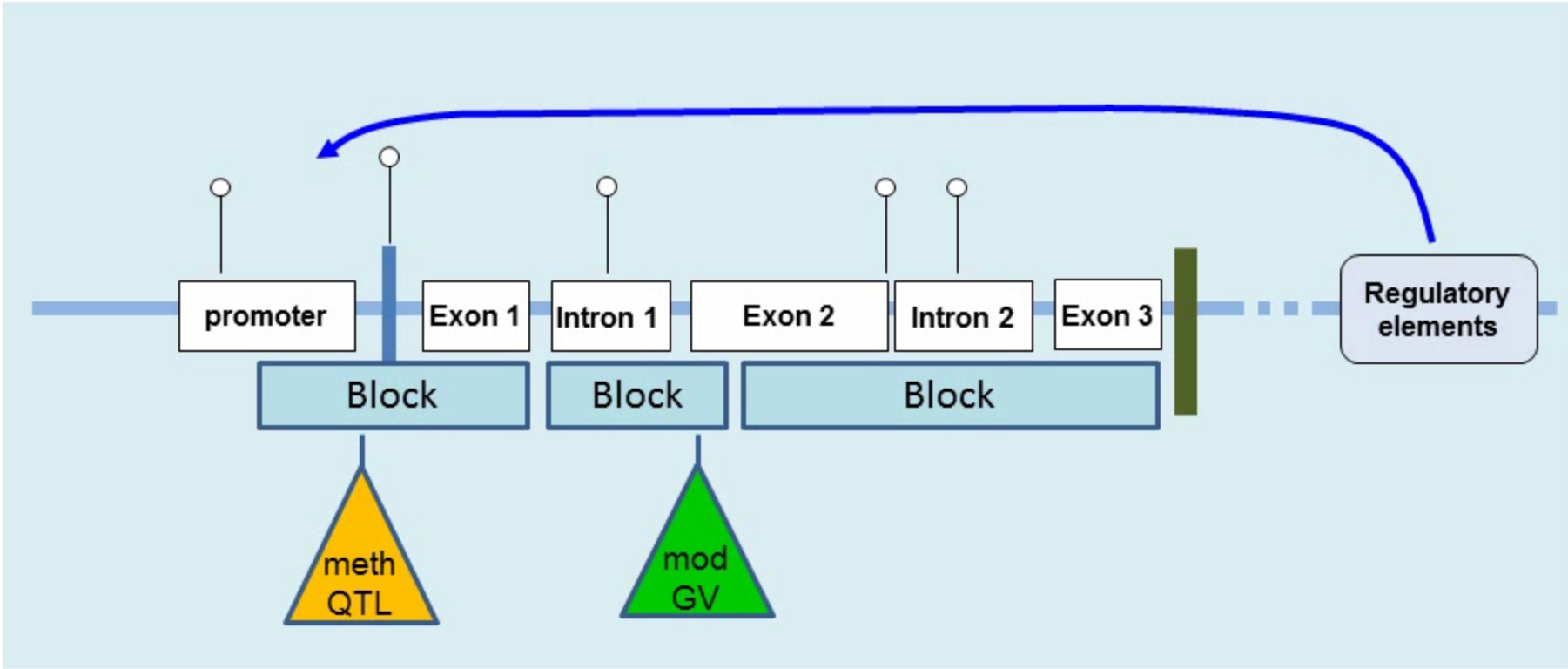
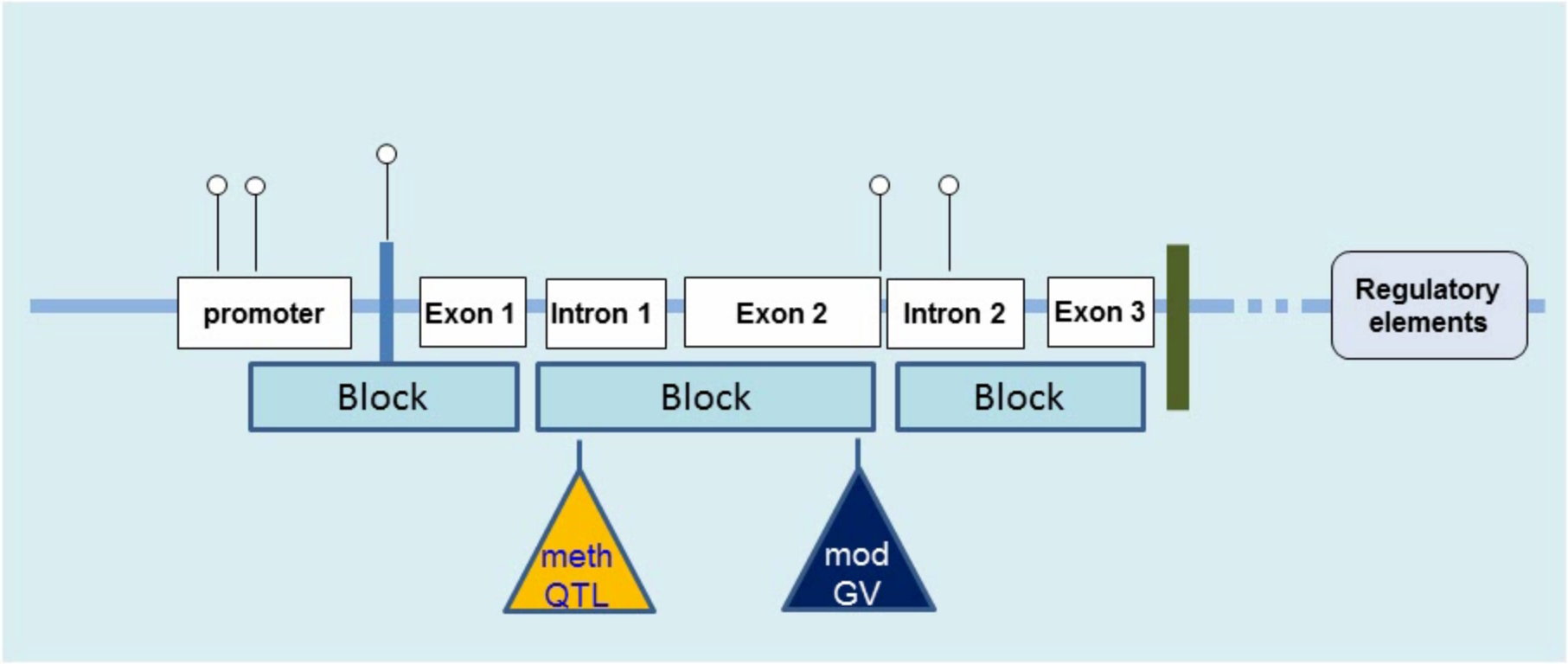
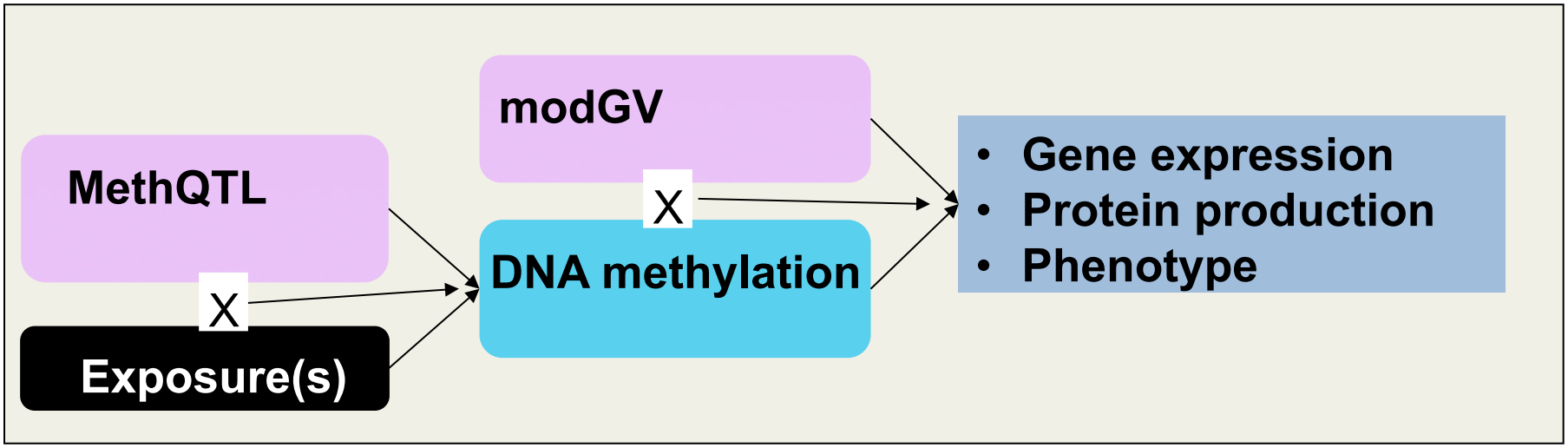
Mechanistic model using the promoter



Mechanistic model – intragenic DNA methylation



Two-stage model



Two-stage model:

1. Genetic variants in interaction with exposure change DNA methylation during developmental time windows.
2. DNA methylation interacts with the genetic variants (masking or knockout genes – OR – strengthening their penetrance)
3. Gene expression is altered – OR – different proteins will be produced.

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Example:
Gestational smoking and obesity in offspring

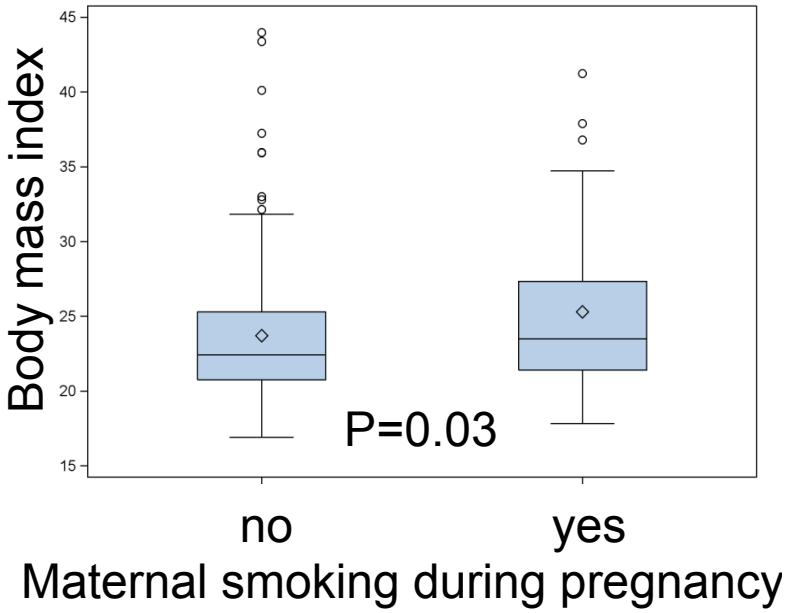
(based on work by Mitra Yousefi)

- > 90 studies: Maternal smoking → risk factor for offspring obesity
- In the US, at least 12 % of women continue smoking during pregnancy

  → >400,000 smoking exposed newborns

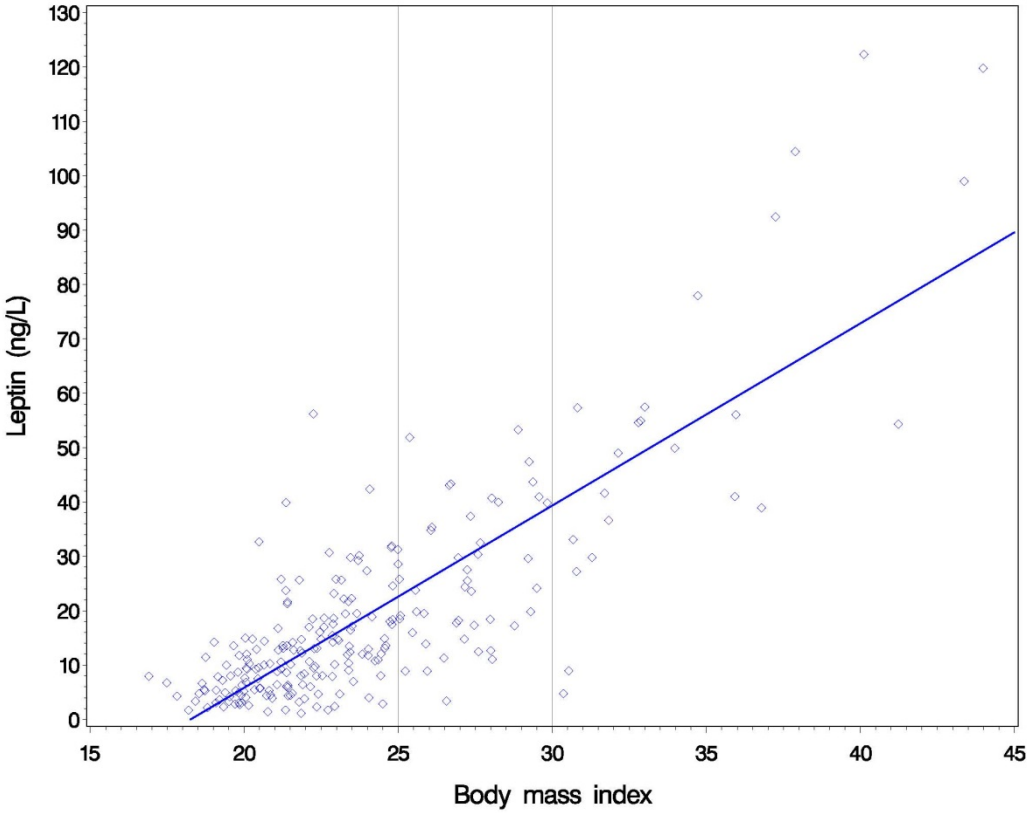
- US: >17 % of all children are obese,
  Worldwide: 150 million children – overweight
  50 million - obese
- Is this epidemic inherited epigenetically?
- How can we reset it?

Gestational smoke exposure, BMI and leptin at age 18 in girls (n=235)



- Leptin → energy intake and energy expenditure

- Manufactured primarily in the adipocytes of white adipose tissue
- Binds to the leptin receptor which is encoded by *LEPR* gene



How can gestational smoke exposure have a long-term effect on child's obesity?

- Investigate
  - genetic variants of the LEPR gene
  - DNA methylation of the LEPR gene

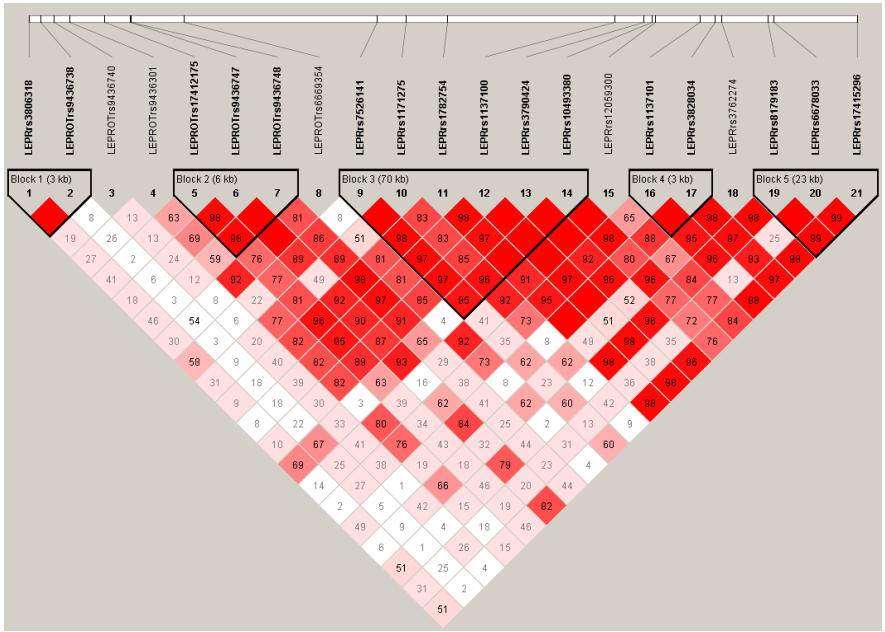
  as step between gestational smoking and BMI in girls at 18

How do we find relevant information? Filtering: 1-2

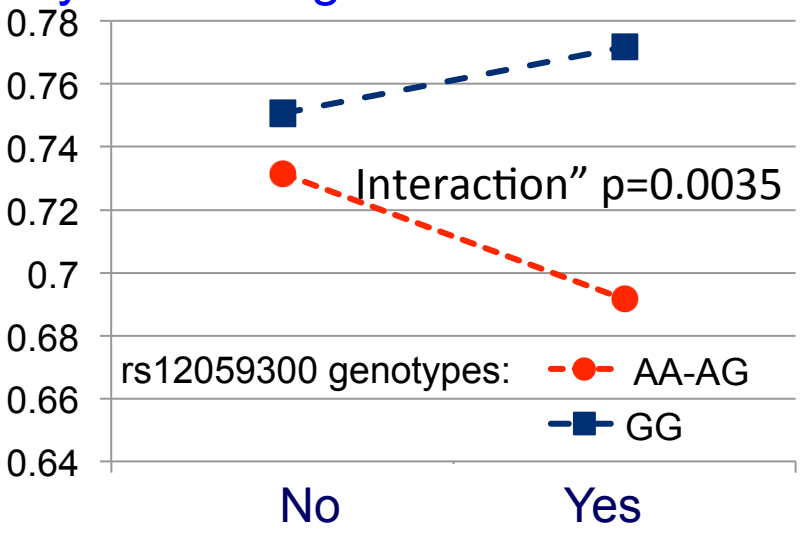
1. Focus on a limited number of SNPs: from 21 to 10

2. Test at which CpG sites DNA-m is affected by SNPS and gestational smoking: of 31 CpG sites → 16 affected by an interaction of methQTLs and gestational smoking

Linkage disequilibrium analysis of 14 *LEPR* SNPs and 7 *LEPROT* SNPs

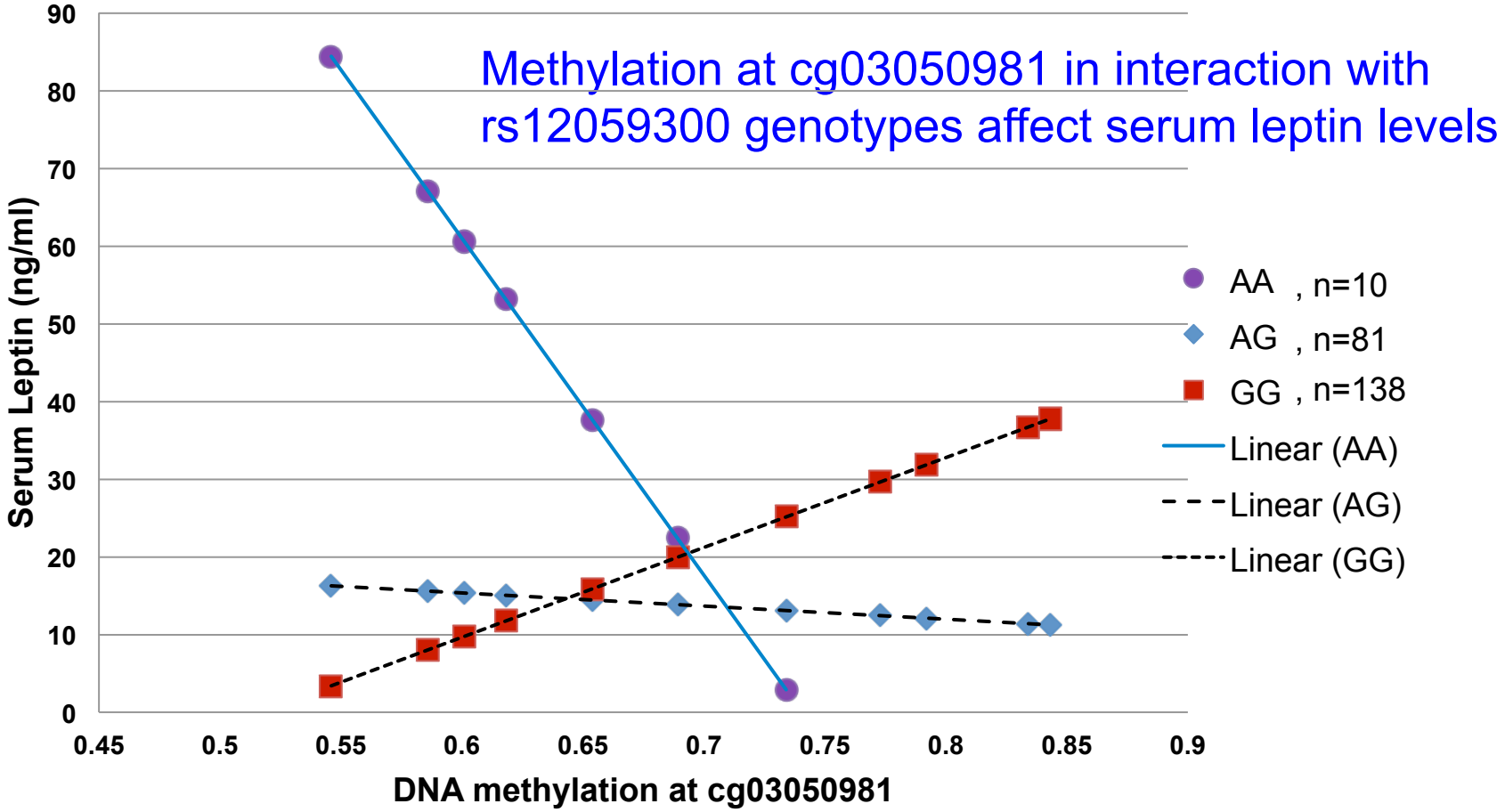


Methylation at cg03050981

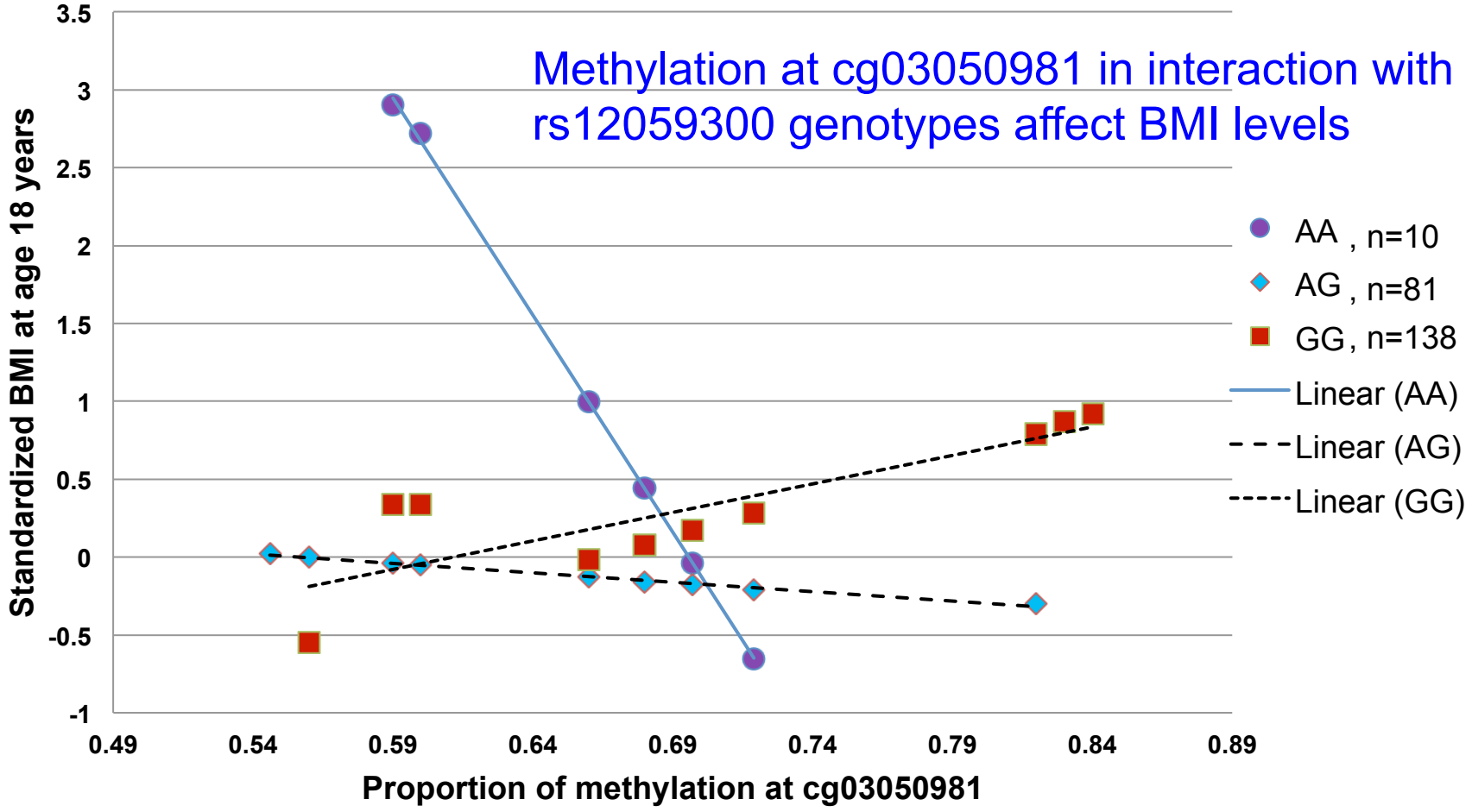


How do we find relevant information? Filtering: 3

3. Test at which CpG sites interact with affected by SNPs resulting in altered serum leptin levels:
   16 CpG sites affected by methQTLs and gestational smoking → 2 interacted with modGV (SNPs) → altered leptin levels



4. Corroborate whether the identified CpG × modGV interaction for serum leptin also affects BMI at age 18 years.



Summary LEPR

- DNA methylation of LEPR is affected by maternal smoking and in turn may increase serum leptin levels and BMI.
- Limitation: other genes such as FTO
- Is this effect inherited epigenetically?
- If and how can we reset it?
- If we can reset the DNA-methylation, we may eliminate obesity not only in this but also in future generations.

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Central role of the environment (maternal and grand-maternal)

The biological game of life is adaptation to the conditions of existence (adapt or parish).

Environment

↓ (evolution = slow)

Genome

↓ (stable, inheritable)

Genetic disease→**rare**

Environment

↓ (adaptation = quick)

Epigenome

↓ (labile, inheritable)

Acquired disease→**common**

Dream-come-true settings and new perspectives

1. Depending on genetic variants, DNA methylation = archive of past exposures (finger- or footprint)
2. Genetic variants × DNA methylation → diseases

➔ DNA methylation links exposures with diseases

➔ Quantum leap in the estimation of relative risks

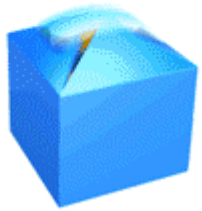
➔ 90-95% of the cases of asthma, eczema, allergy, and obesity can be explained

Dream-come-true settings and new perspectives

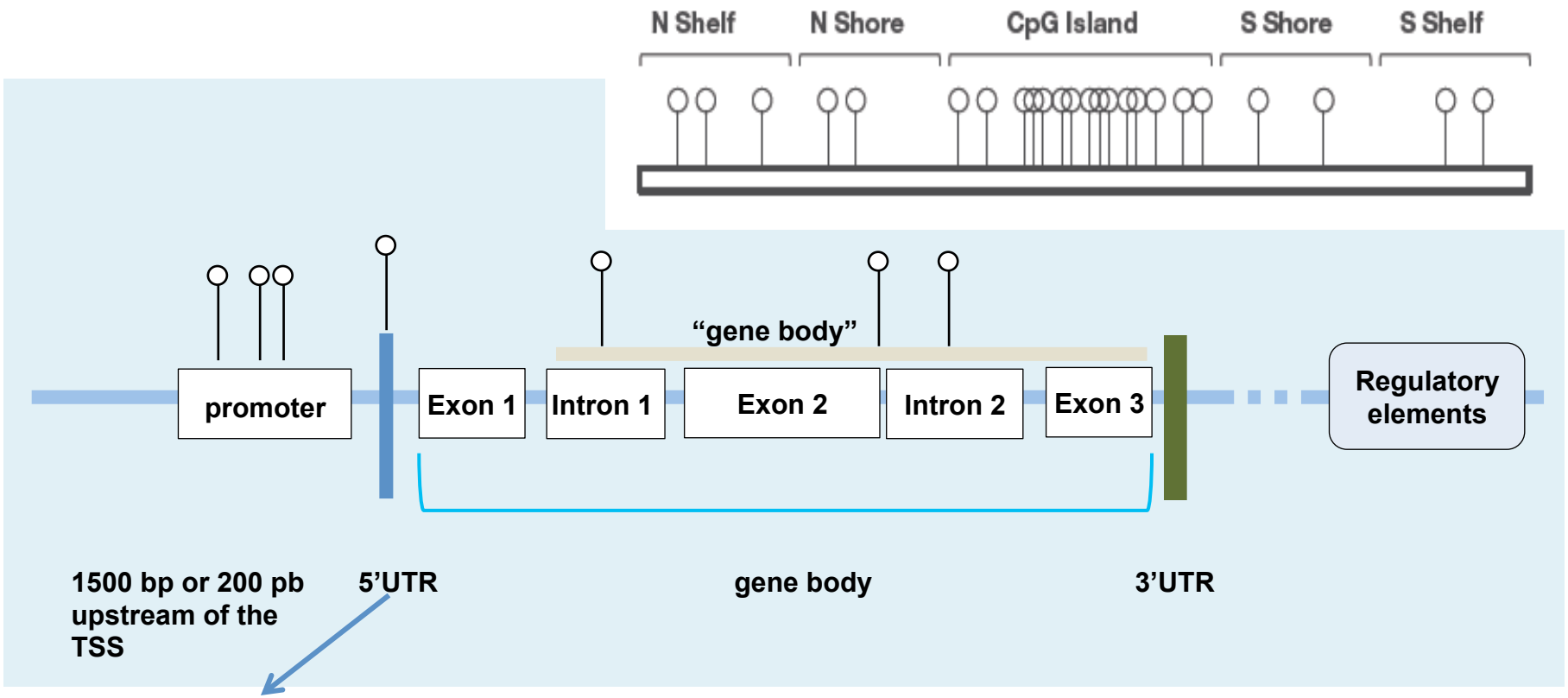
3. Epigenetic research integrates
   - Diverse dimensions of exposures
   - Different scientific fields (cross-disciplinary)
   - Social, psychosocial, chemical, and physical factors affect epigenetic marks
4. Birth cohort studies are essential to understand whether methylation and of which CpG is inherited, acquired during gestation, and is sustained to result in diseases in child- and adulthood.

Thank you

Questions



Target sequences for DNA methylation sites



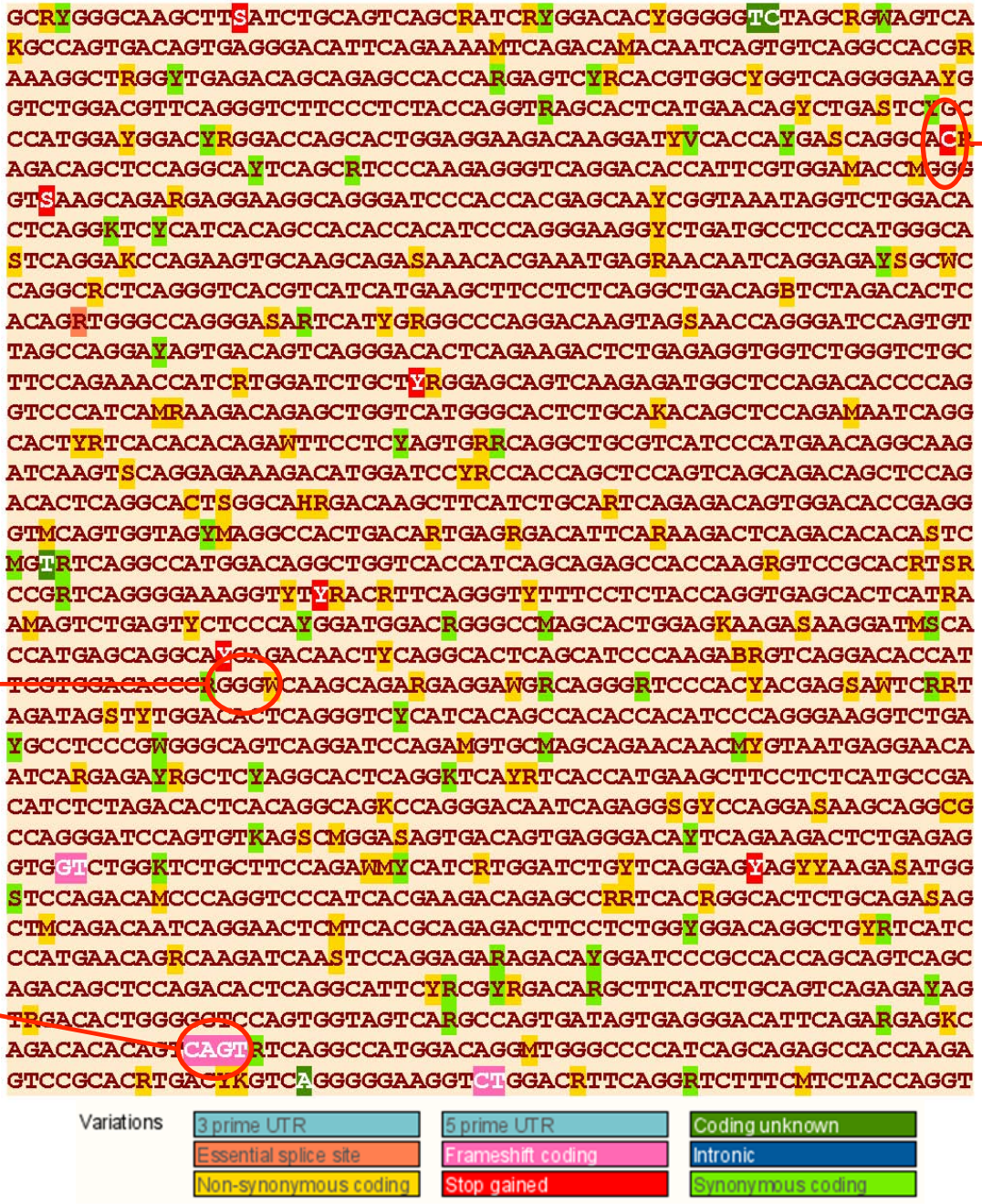
The **5' UTR region:**

Begin: transcription start site (TSS)

Includes: regulatory sequences

End: 1 nucleotide before the start codon (usually AUG) of the coding region

Partial view of exon 3 – *FLG* gene



Provided by Ali Ziyab