Epigenetics

Heritable characteristics of the genome other than the DNA sequence

- Heritable during cell-division (mitosis)
- To a lesser extent also over generations (meiosis)

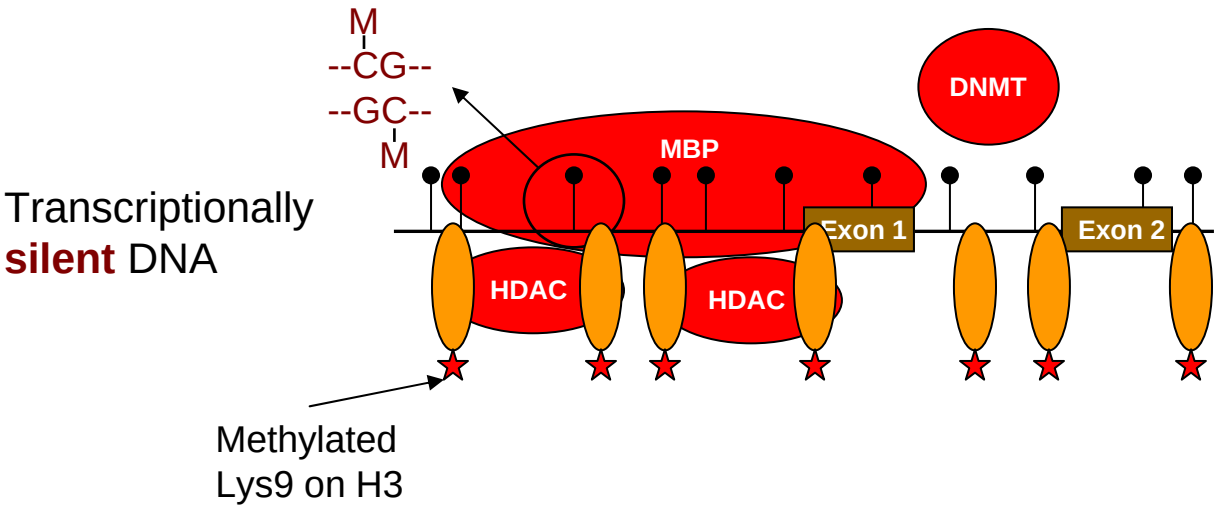
Presentation & scripts & data: \dorret\leuven2008

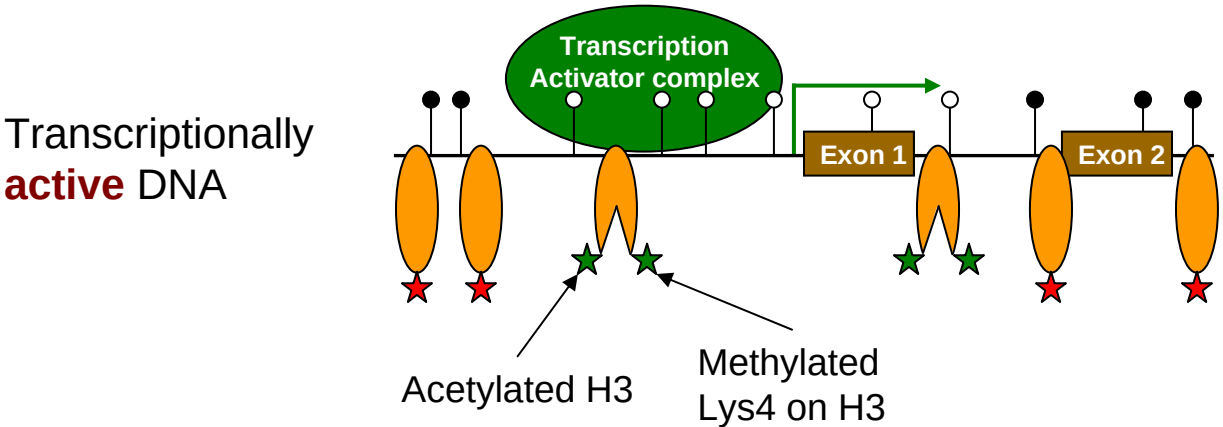
Roles for epigenetics

Processes requiring a stable control of gene expression

- Inactivation of ‘junk’ DNA, i.e. transposon-derived repeats (e.g. LINEs, SINEs).
- Selective activation and silencing of genes during cell differentiation.
- Imprinting, i.e. parent-of-origin specific silencing of gene expression.
- X Chromosome inactivation in female mammals.

How epigenetics mechanisms control transcription in mammals





→ *DNA methylation and histone modifications may be correlated*

Epigenetics mediates between environment and genetics

- A variety of ‘environmental’ stimuli can bring about epigenetic changes e.g. aging, diet, viral infection…
- Diets deficient in nutrients important for epigenetic metabolism like folate, choline and methionine are associated with genome-wide hypomethylation and with the development of cancer, Parkinson’s and Alzheimer’s diseases.
- The promoter region of the tumor suppressor gene p16 is frequently hypermethylated even in normal cells of smokers.
- Apart from cancer, other diseases caused by aberrant epigenetic functioning: e.g. atherosclerosis, osteoarthritis, neuropsychiatric disorders…

Epigenetics vs. genetics

Monozygotic twins are genetically identical but not phenotypically

A role for epigenetics?

Epigenetic Mechanisms of MZ Discordance

- Epigenetic signals are dynamic

Developmental programs, Environment (external, internal), Stochastic events in the cell

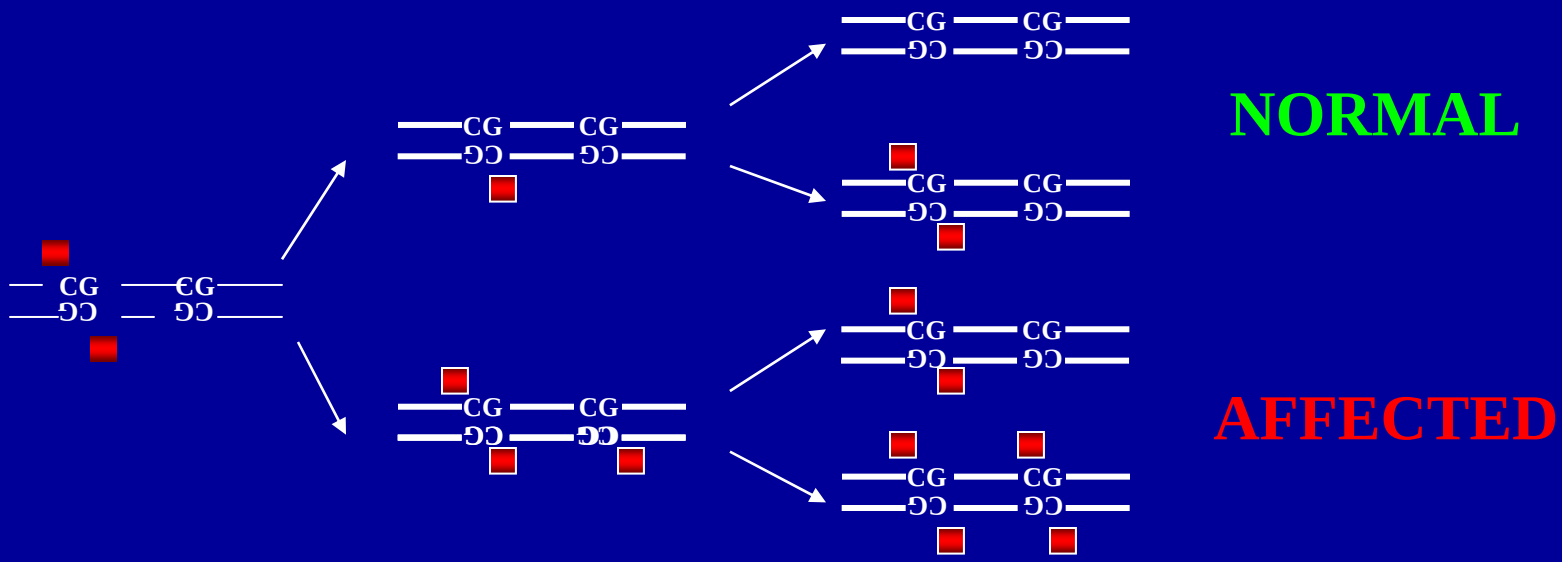


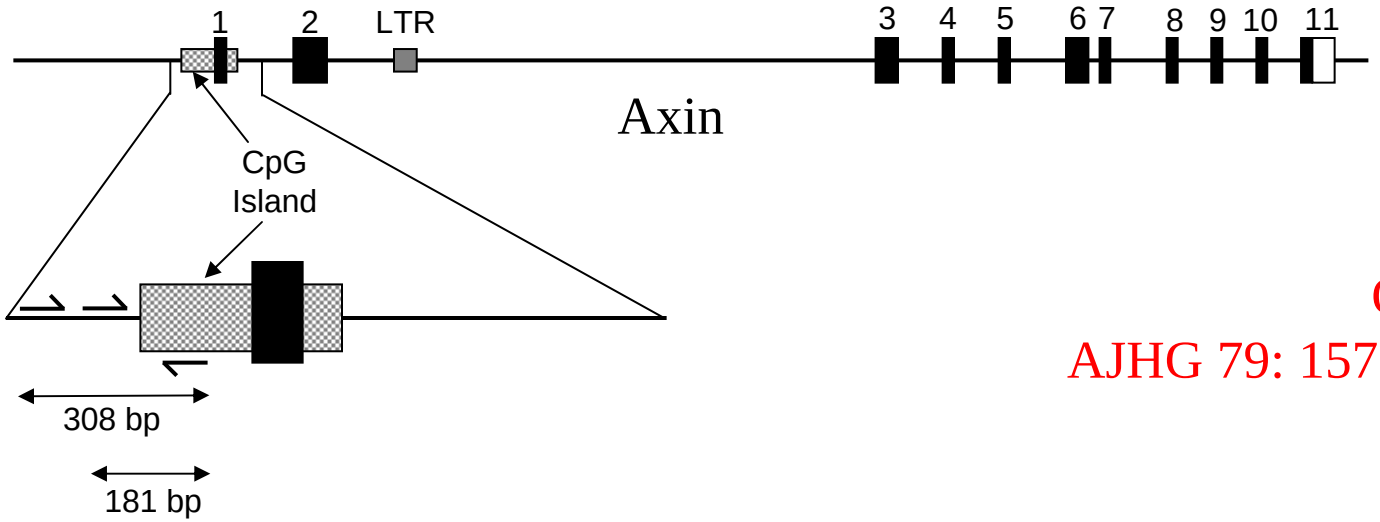
Fig. 1. Patient 1. Soft tumor and abnormal aspect in the lumbosacral area.

Fig. 2. Patient 1. Radiograph of the vertebral column shows complete duplication of the spine from L4 down.

urethra, a dilated pelvis of the right kidney, bilateral uterus unicornis with normal ovaries, hemivertebrae of thoracic vertebrae 6 and 10, and abnormal curvature of the sacrum. A persistent ductus arteriosus and secundum atrial septum defect was suspected, but results of cardiac investigations at 10 months were normal.

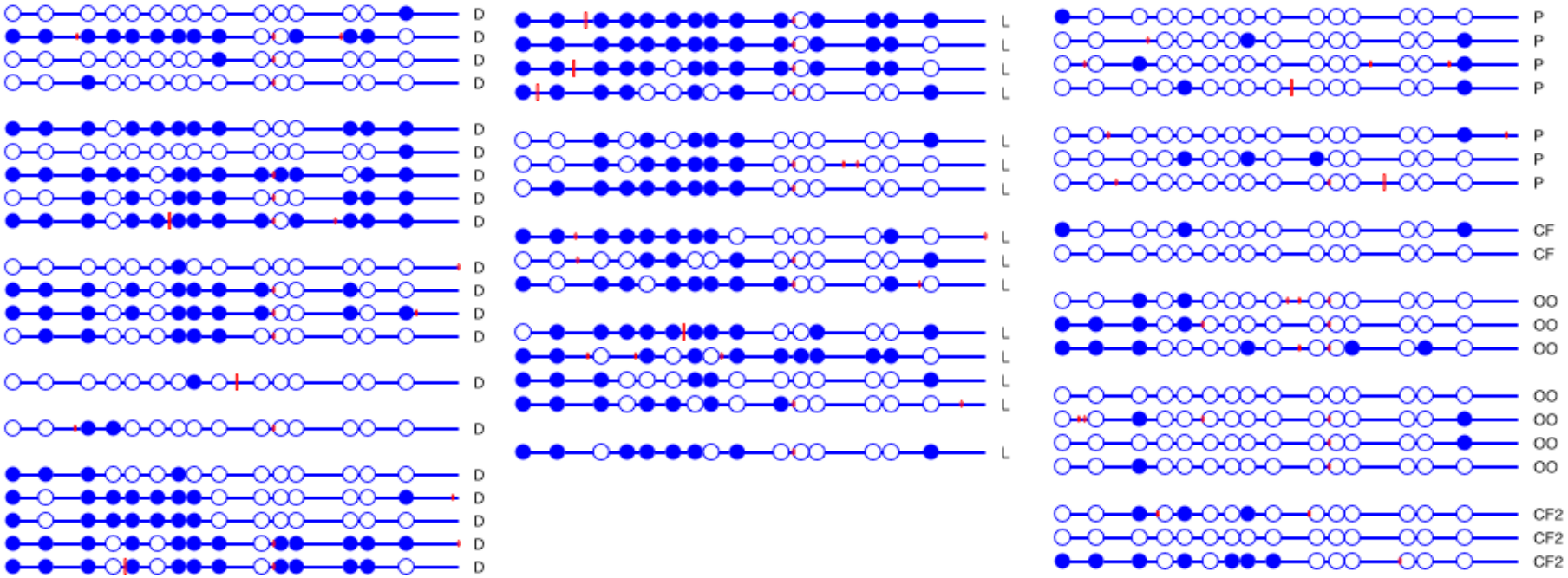
At physical examination for genetic evaluation at 4 months we saw a baby girl with epicanthal folds, but no other minor anomalies. She had a capillary nevus on her left buttock. In the anal region only a dimple was seen. The patient was operated on one day after birth, when a colostomy was made and a fistula connected to the colon

Discordant caudal duplication in MZ twins



Oates et al,
AJHG 79: 157-162, 2006

Twin 1- unaffected < Twin 2 - affected > Controls [e.g.]



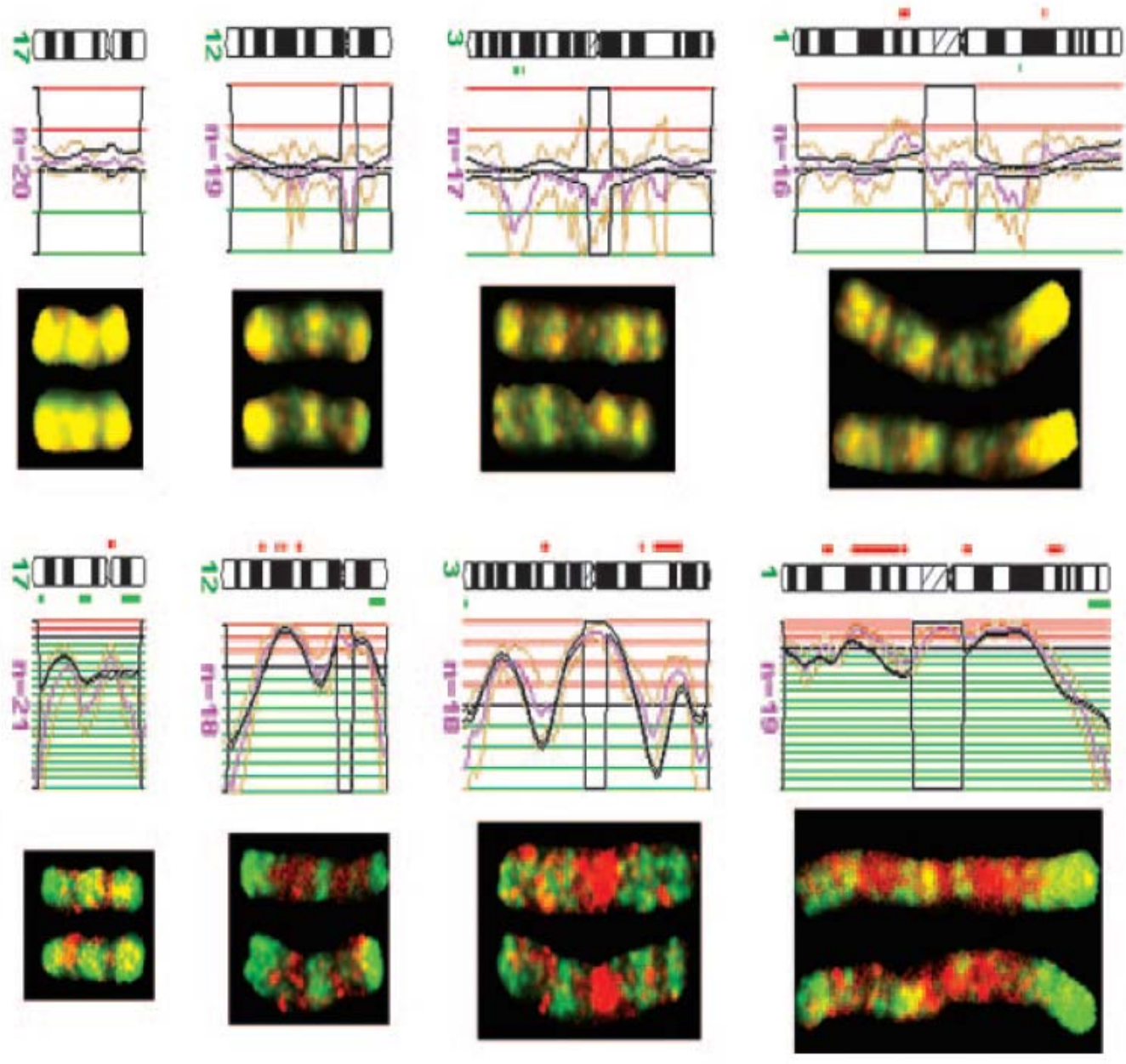
Age-related epigenetic changes

- Older MZ twin pairs have been reported to display larger epigenetic differences than younger twins

10604–10609 | PNAS | July 26, 2005 | vol. 102 | no. 30

Epigenetic differences arise during the lifetime of monozygotic twins

Mario F. Fraga*, Esteban Ballestar*, Maria F. Paz*, Santiago Ropero*, Fernando Setien*, Maria L. Ballestar†, Damia Heine-Suñer‡, Juan C. Cigudosa§, Miguel Urioste¶, Javier Benitez¶, Manuel Boix-Chornet†, Abel Sanchez-Aguilera†, Charlotte Ling‖, Emma Carlsson‖, Pernille Poulsen**, Allan Vaag**, Zarko Stephan††, Tim D. Spector††, Yue-Zhong Wu‡‡, Christoph Plass‡‡, and Manel Esteller*§§



Age-related epigenetic changes

- Is the genome that sloppy if it truly matters?

DNA methylation at the IGF2 locus
Heritability, age-effects and identification of responsible SNPs

Human Molecular Genetics, 2007, Vol. 16, No. 5 **547–554**
doi:10.1093/hmg/ddm010
Advance Access published on March 5, 2007

Heritable rather than age-related environmental and stochastic factors dominate variation in DNA methylation of the human *IGF2/H19* locus

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IGF2 locus

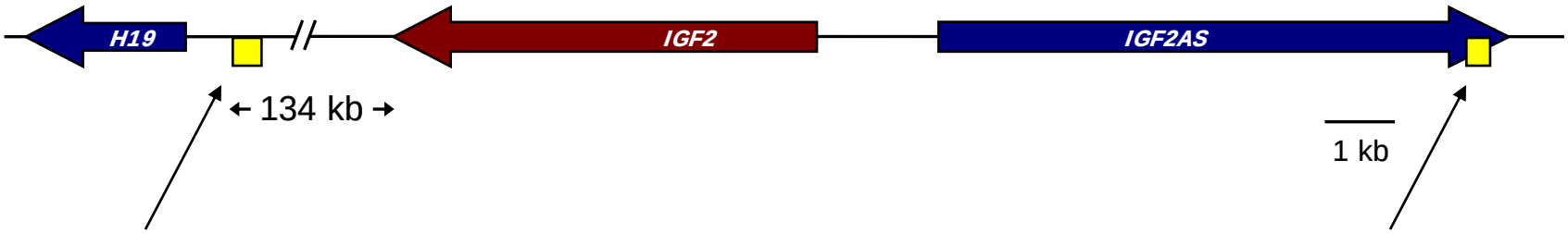
Insulin-like growth factor II locus (*IGF2*)

- One of the most in-depth characterised loci under epigenetic control.
- Imprinted: maternal allele silenced by DNA methylation (and other mechanisms).
- Implicated in body composition and cell proliferation in atherosclerotic plaques.
- Soma-wide loss-of-imprinting associated with ~10 fold increased risk of colorectal cancer.

LUMC

IGF2 locus

IGF2 on chromosome 11p15.5



CpG island *H19* 5' region
- 413 bp
- 13 CpGs

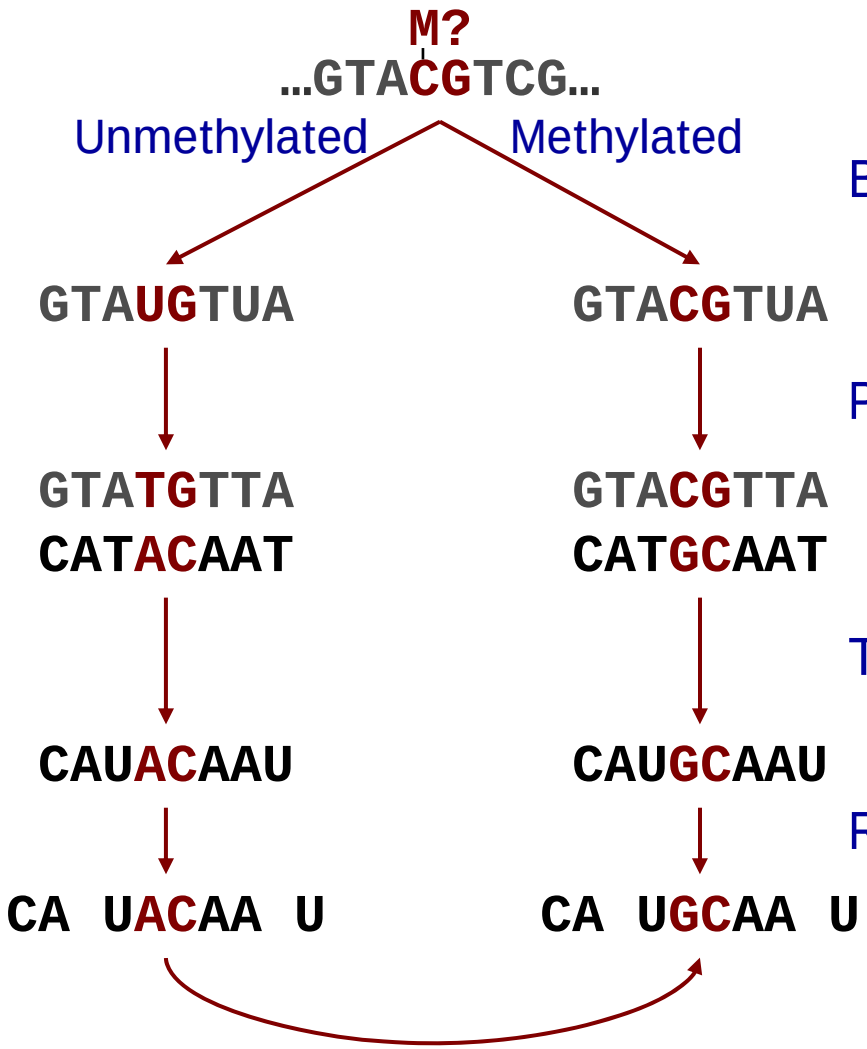
IGF2 DMR
- 338 bp.
- 7 CpGs
- LOI associated with colorectal cancer (Cui, Science 2003)

Note – maximising accuracy
- Triplicate measurement, SD<0.1
- Success rate CpG >80%
- Mean success rate 91%

Measuring DNA methylation

- Using mass-spectrometry.
- Quantitative measurement of DNA methylation of individual CpG sites in 400 bp sequences.

Molecular biology



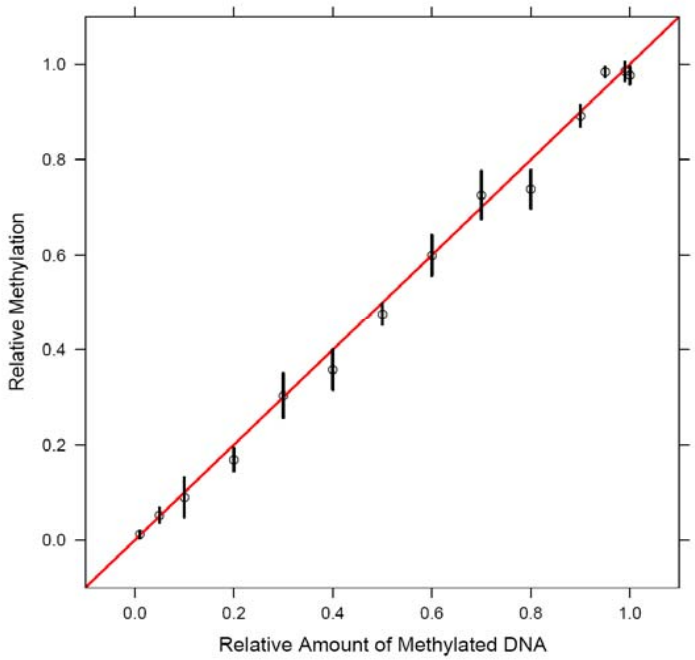
Bisulfite treatment

PCR with T7 tagged primers

Transcription reverse strand

RNAse cleavage at U

Quantification of methylation



Twins from The Netherlands Twin Register

'Young' - Adolescent twins

- Mean age 17 years
- 108 MZ (54 pairs)
- 88 DZ (44 pairs)

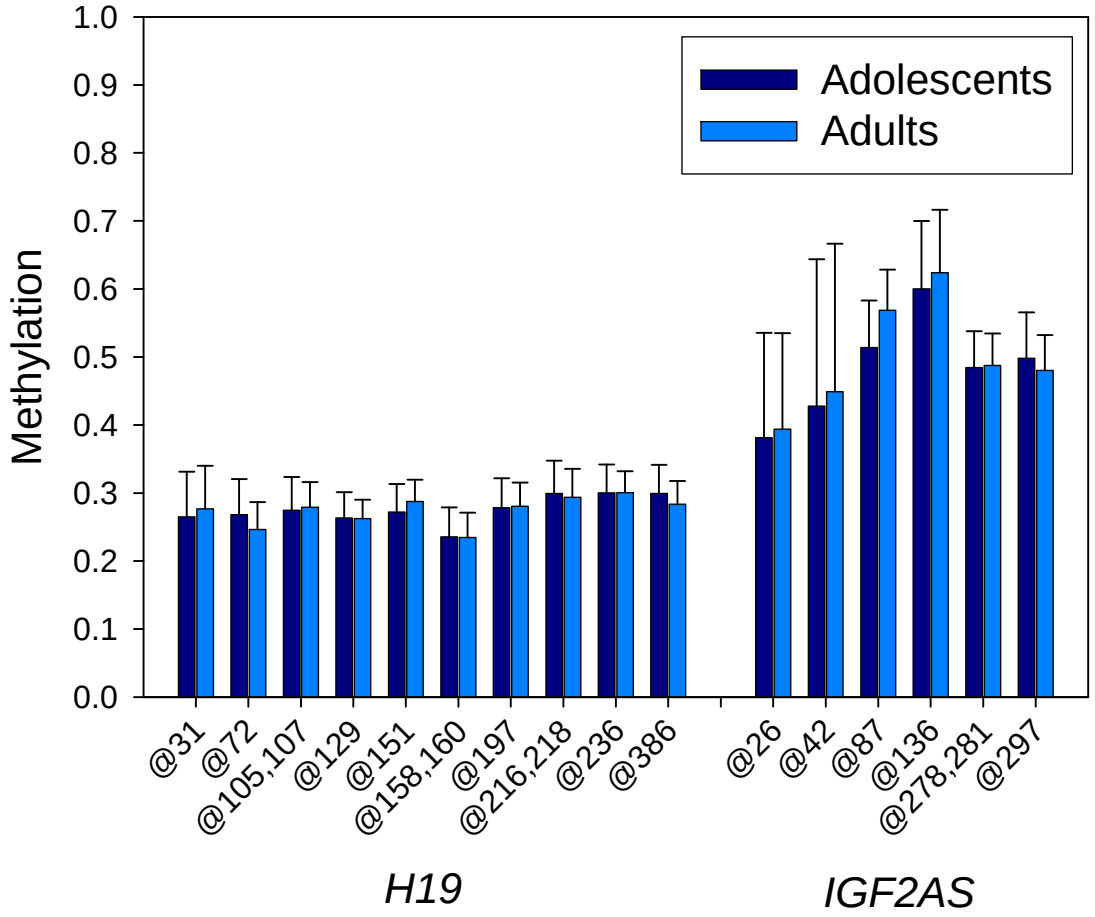
'Old' - Adult (middle-aged) twins

- Mean age 45 years
- 96 MZ (48 pairs)
- 80 DZ (40 pairs)

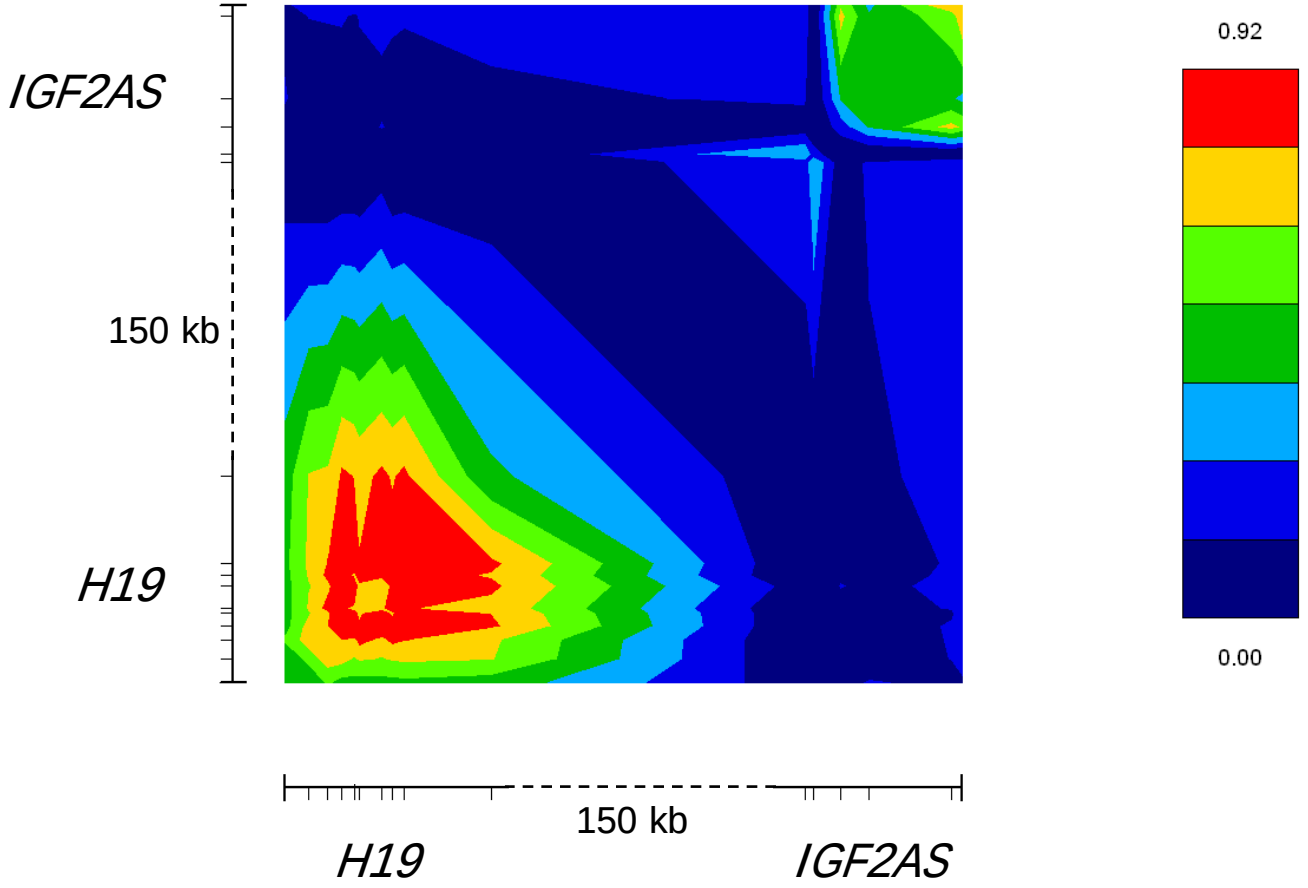
- In total 372 individuals
- DNA from leukocytes

LUMC

Average methylation

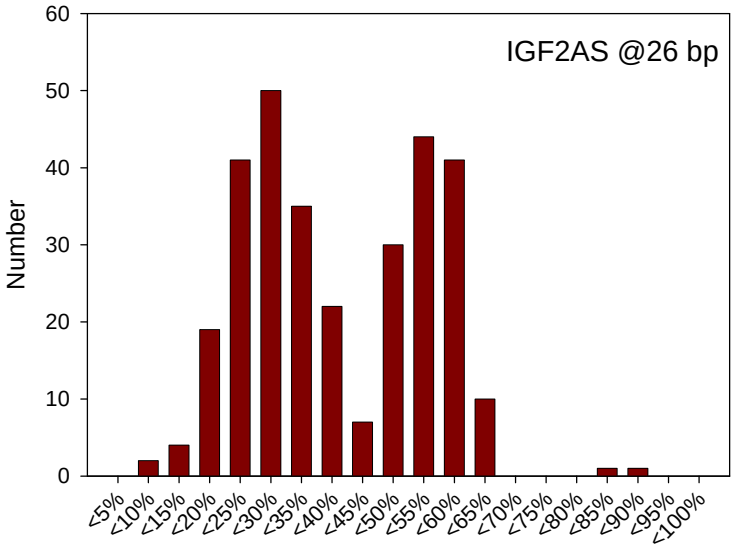


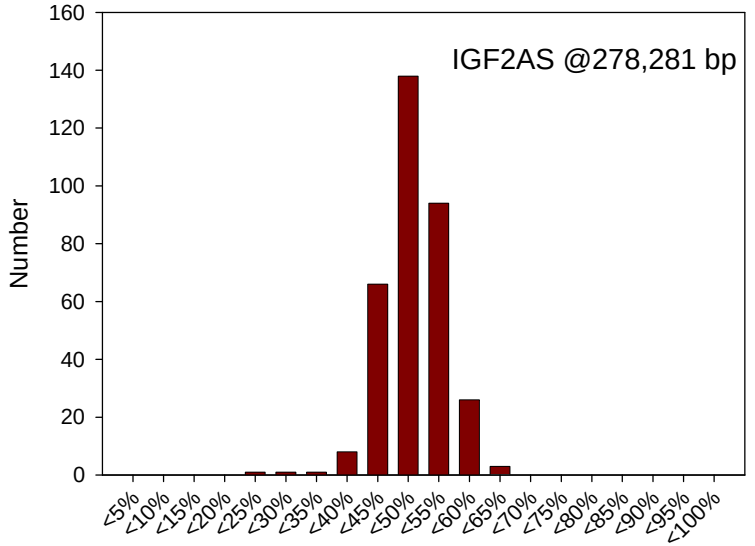
Methylation is correlated: patterns

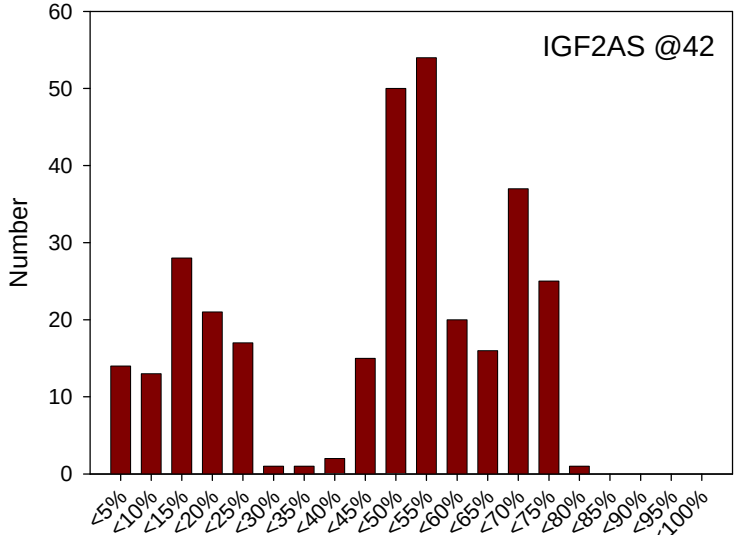


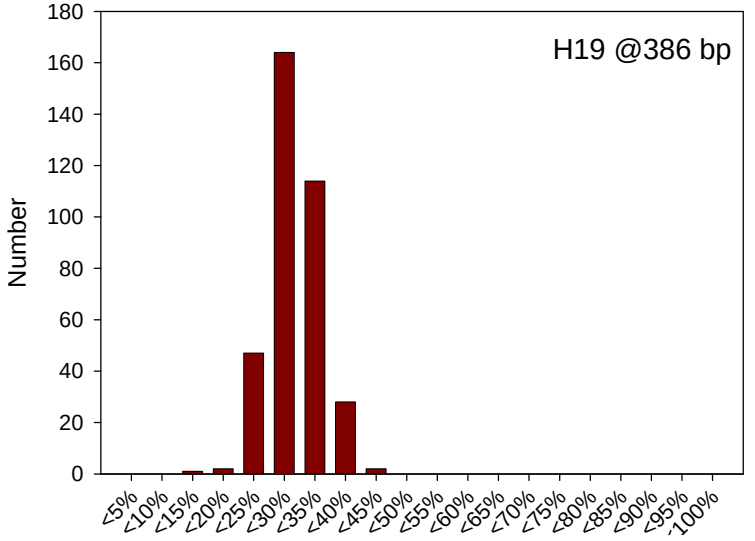
- Correlation between *H19* and *IGF2AS* up to .25

Inter-individual variation







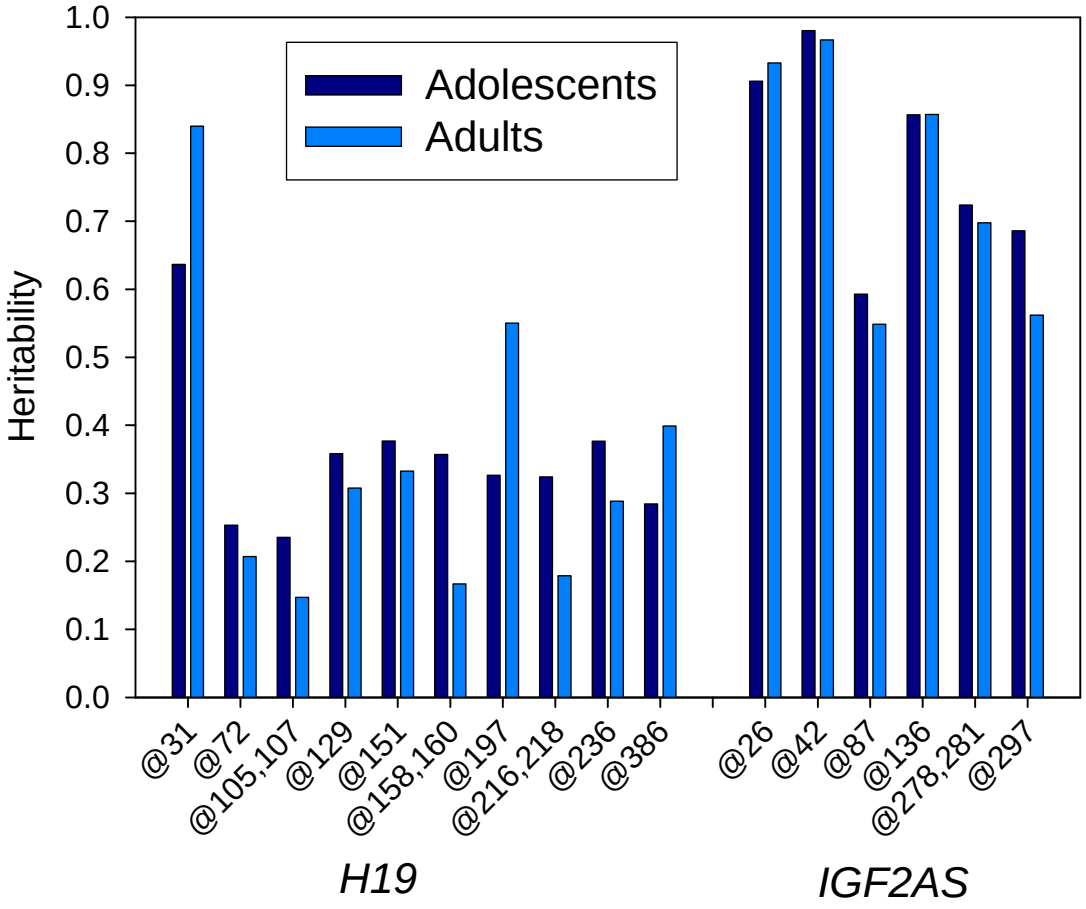


Heritability

Statistical analysis

- Variance components analysis
- Software Mx

Heritability



- No significant difference between adolescent and adults.
- No influence environment between adolescence and middle age?
- No influence of common environmental factors.

Heritability