Erasmus MC
University Medical Center Rotterdam

# *EPIGENETICS AND GENOMIC IMPRINTING*

Hikke van Doorninck, PhD
Dpts Obstetrics & Gynaecology and Clinical Genetics
Erasmus Medical Centre,
Rotterdam, The Netherlands
j.vandoorninck@erasmusmc.nl

## Clinical clues to imprinting

- **Crossing different species**
  **horse X donkeys →mule or hinny**

♂ **lion x ♀ tiger = Liger**

(♂ **tiger x ♀lion = Tion)**

## Clinical clues to imprinting

- **Crossing different species**
- **Uniparental disomy/Deletions/Triploidy:**

- **Prader-Willi syndrome** absence **Paternal 15 q11-13**
- **Angelman syndrome** absence **Maternal 15 q11-13**

Erasmus MC

## Clinical clues to imprinting, Prader-Willi

- **Prader-Willi syndrome** absence **Paternal 15 q11-13**
- **Angelman syndrome** absence **Maternal 15 q11-13**

Erasmus MC

## Clinical clues to imprinting, Angelman

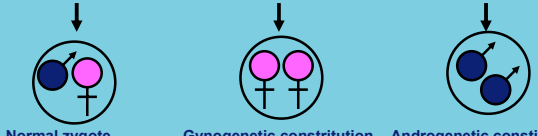
- **Prader-Willi syndrome** absence **Paternal 15 q11-13**
- **Angelman syndrome** absence **Maternal 15 q11-13**

Erasmus MC

## Human pathology suggesting parental specific contribution

*Wake, 1978*

*Pedigree analysis of chromosomes*

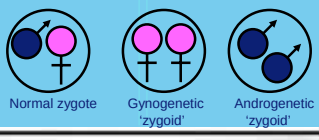


**Normal zygote** | **Gynogenetic constitution** | **Androgenetic constitution**

= Hydatidiform mole

## Mouse experiments proving sex-specific contribution

*Manipulate germ cells:*




pathologic interpretation


Surani, McGrath and Solter, 1984-1987

Erasmus MC

## Conclusion

### Imprinting is

Epigenetic marking in a sex-specific manner resulting in monoallelic expression of imprinted genes:

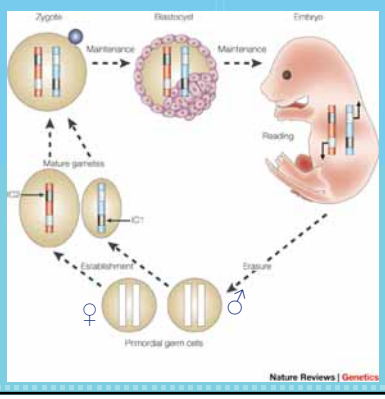
- Embryonic growth
- Placental function
- Behavioral processes

**~ Paternal genes important in placentation**

**~ Maternal genes important in embryogenesis**

Erasmus MC

## Imprints are set in gametes




Wolf Reik & Jörn Walter
*Nature Reviews Genetics, 2001*

Erasmus MC

**Imprinted genes have role in evolution of mother-offspring interaction**

**~ Parental conflict theory** (Haig 1993)

Most imprinted genes (also) expressed in placenta:

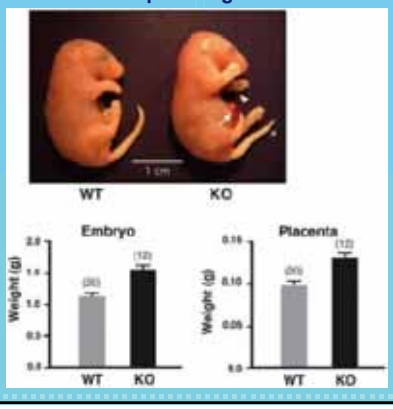
-Many affect growth (paternal genes stimulate but maternal genes reduce growth)

-Many have role in nutrient transfer (Constância et al., 2004)
- o active amino-acid transport system A
- o solute carrier family
- o organic cation transporter family

-Regulate interactions between different cell types with feto-maternal interfaces in the placenta

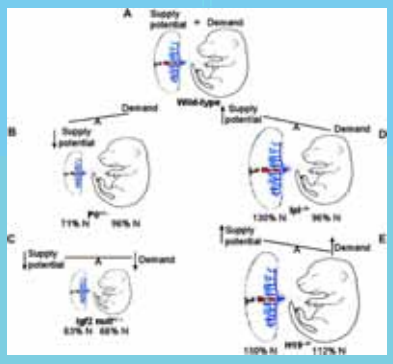
Erasmus MC

**First imprinted gene: IGF2**




Erasmus MC

**Supply and demand mutants**




Erasmus MC

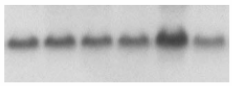
## *Ipl* KO mice

Genotype: +/+ +/- mat +/- pat +/+ -/- +/+

18S

Ipl

*Ipl* (imprinted gene): cDNA probe



Actin probe

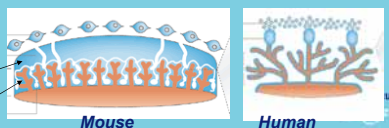
Erasmus MC

## Ipl-positive cells disappear at mid to late mouse gestation

sp

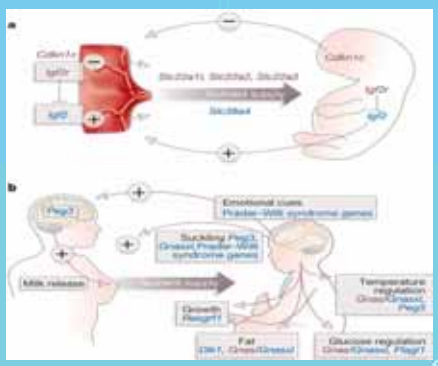
la


la


Erasmus MC

## Genetic control of supply and demand for nutrients



Resourceful imprinting, Constância, et al 2004

Erasmus MC

More then 100 imprinted genes known
(www.geneimprint.com/site/genes-by-species)

## Imprinted genes that affect growth

| Gene | Loss of function in mice |
|---|---|
| *Ipl* | ↑ placental growth |
| *Mash2* | placental differentiation - lethal |
| *Igf2r* | ↑ fetal & placental growth - lethal |
| *Grb10* | ↑ fetal growth |
| *Gnas/GnasXl* | ↑↓ growth & post-natal behaviour; energy metabolism |
| *Cdkn1c* | ↑ placental growth; proliferation defects - lethal |
| *Igf2* | ↓ fetal & placental growth |
| *Peg1* | ↓ fetal growth; nurturing |
| *Peg3* | ↓ fetal growth; nurturing |
| *Rasgrf1* | ↓ postnatal growth; long term memory |

## Human placenta imprinting

-Human and mice both similar genes imprinted but some exceptions:

*Limited evolutionary conservation of imprinting in the human placenta.*
Monk et al., 2006

- Intra uterine growth retardation humans:

*Unbalanced placental expression of imprinted genes in human intrauterine growth restriction.* McMinn et al., 2006

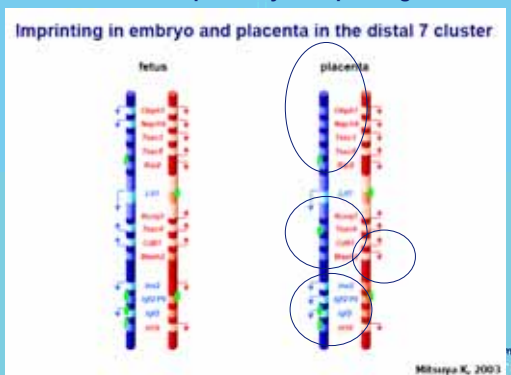
***RNA expression microarray on human IUGR:***

- *Increased* ***PHLDA2***
- *Decreased* ***MEST, MEG3, GATM, GNAS*** **and** ***PLAGL1***

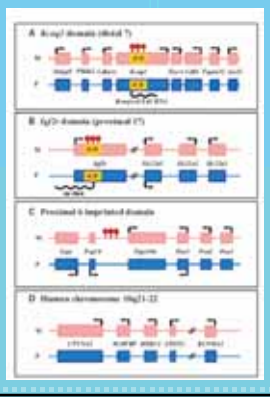
Erasmus MC

## Tissue specificity of imprinting

Imprinting in embryo and placenta in the distal 7 cluster

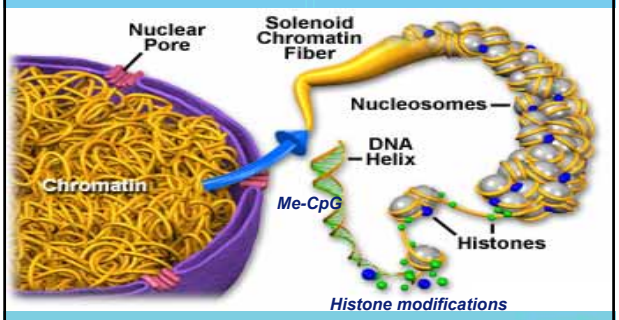


## Methylation of imprinting control regions



Erasmus MC

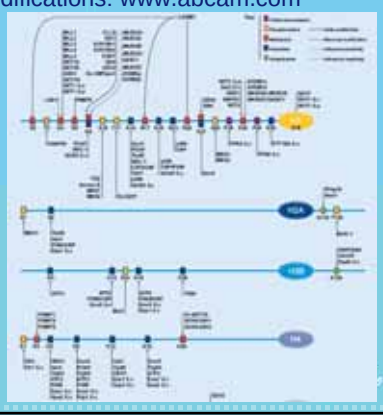
## Epigenetics is second regulatory genome



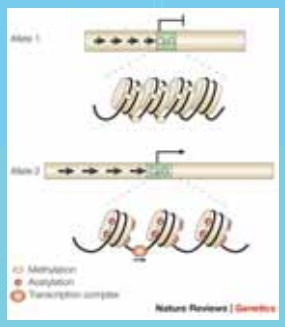

- Chromatin = Histones + DNA and their modifications

Erasmus MC

## Histone modifications: www.abcam.com

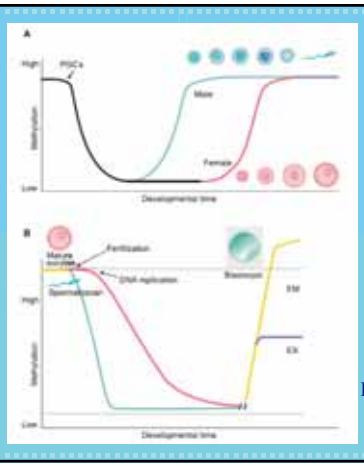



## Effect of histone acetylation on gene expression



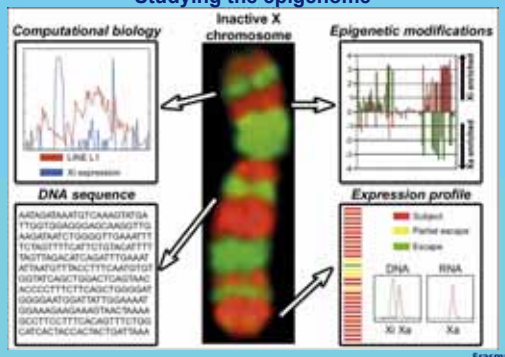
Wolf Reik & Jörn Walter, ***Nature Reviews Genetics, 2001***

| DNA Methylation | Epigenetics | Imprinting |
|---|---|---|
| Methyl group on CpG | Methyl group on CpG **AND/OR** | Methyl group on CpG |
| Repressive mark | Histon modifications, histon code | + Secondary histone modifications |
| Less in placenta then in embryonic tissues | **Heritable, reversible + metastable (diet and environment)** | **Gamete specific, influenced by endocrine disruptors and methyl supplements** |
| | Bi-allelic | Mono-allelic |
| | Adaptation~ Barker hypothesis Developmental plasticity | Haigs conflict theory Developmental plasticity |



Reik 2001

## Studying the epigenome




Chadwick & willard 2004

Erasmus MC

## Epigenome study techniques

RNA Expression:

- •RNA micro arrays
- •Candidate gene Q-RT-PCR
- •Illumina Golden Gate assay

DNA methylation:

Bisulphite pyro-sequencing
Genome wide Luminometric methylation assay (LUMA)
Differential methylation hybridization (DMH)
DNA adenine methyltransferase-Identification (Dam-ID)
*(see epigenomics company site)*

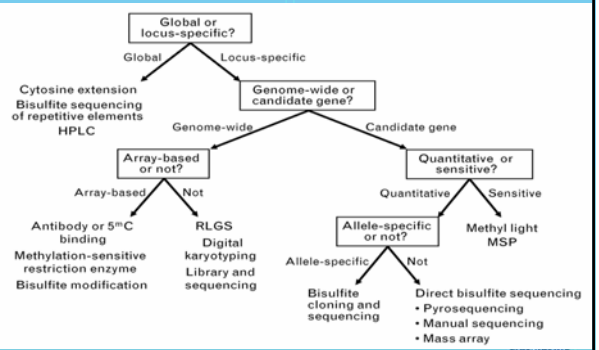
*(Techniques used in studies of epigenome dysregulation due to aberrant DNA methylation: An emphasis on fetal-based adult diseases. Ho and Tanga, 2007)*

Histone modifications:

Chromatin immune precipitation with PCR or with microarray= ChIP on chip

Erasmus MC

## Decision tree to guide the selection of appropriate DNA methylation analysis methods




*From: Shen: Curr Opin Clin Nutr Metab Care, Volume 10(5).September 2007.576–581*

## Study histone modifications

- ChIP-Seq combines chromatin immunoprecipitation with massively parallel sequencing for genome wide identification of binding sites of DNA associated factors and characterization of epigenetic modifications.

- Chip
- Special adapters solexa system
- Amplification
- Sequencing of $10^8$ bases
- Software analysis of gigabase

Erasmus MC

## Future:

- Identification of novel placental-specific imprinted genes and epigenetically regyualetd genes

- Study of expression patterns of imprinted genes in extra-embryonic tissues; functional analysis

- Role of epigenetic “mutations” in placental dysfunction

- Causes for epimutations

Erasmus MC