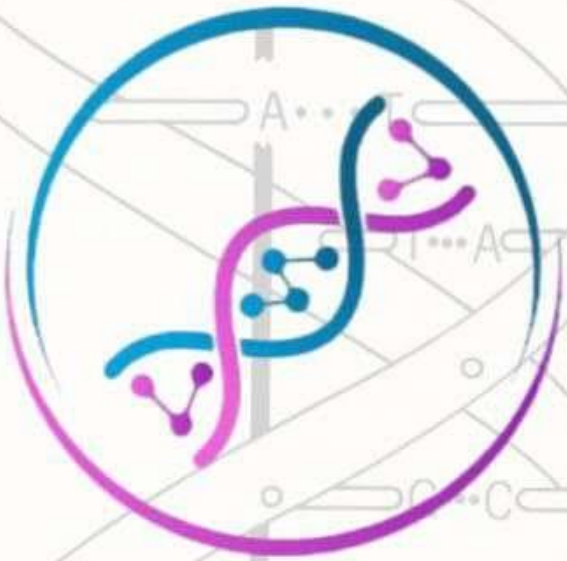
CLINICAL EPIGENETICS



GENETIC VISION TEAM

Clinical Epigenetics

Epigenetics means above the genetics, in other words changes around genetic material without altering genetic material, these changes affect the 3D structure of DNA to make it accessible or non-accessible to interact with proteins that are involved in gene transcription, Replication, Recombination and Repair System.

Epigenetics is a regulatory process because it does not interfere with the DNA itself also these changes transmit to daughter cells

Epigenetics :

- Change modifications to DNA (Chemical groups that bind with DNA such as methyl group)
- DNA packaging: changes proteins binding with DNA.
- Epigenetics doesn’t include changes in DNA sequence.
- The reflection in Gene's expression

In sickle cell anemia changes occur in the DNA itself with change from base pair to another base pair

Epigenetics marks

1- Post-translation modifications of histones: after the protein synthesis it undergoes Specific modifications to become functional we call them post translation modifications, some of these fundamental changes (methylation, acetylation, phosphorylation) occurring in histones usually, the methylation is associated with inhibition of the expression DNA, the acetylation turns on gene expression, and phosphorylation associated with metabolism.

The majority of DNA modifications to DNA is methylation, a methyl group is added to cytosine, which becomes 5 methyl-cytosine, usually led to lower expression of the DNA, it means we have a lesser expression of DNA when methylation occurs.

2- Modification to DNA: it has some proteins like histones (H2A, H2B, H3, H4) we have 4 proteins that become octamers forming nucleosomes, in some areas there are

proteins similar to histones but not histones, which give different modifications to protein, so that it becomes assessable or not assessable, and these kinds of modifications that happen, it’s the use of proteins different from histones to form nucleosomes we call it Non-canonical

canonical : its a standard proteins (H2A, H2B, H3, H4)

3- Noncoding RNA: plays an important role in the process of gene regulation, microRNA and non-coding RNA, play role in gene regulation.

4- X chromosome inactivation.

All these marks work in a particular way so that they change the 3D structure of a protein, which causes the DNA to open which then makes it more accessible for binding with proteins that are essential for gene Expression (Eu-chromatin) , or make the DNA close to make it non accessible for interaction with proteins that are essential for gene expression (heterochromatin.

Many of these epigenetic marks function in gene regulation by altering the structure of DNA and establishing heterochromatin vs. euchromatin
Usually, heterochromatin is associated with the repressed gene expression while Euchromatin is typically associated with turn on gene expression.

Epigenetics regulators: A set of enzymes with specific a function that controls the transcription process and 3D DNA structure, including histone post-translation modification and DNA methylation.

histone H3K9ac:
1- H3: it's a histone number
2- K: it is a single-letter abbreviation of the amino acid (Lysine)
3- 9: the order of the amino acid
4- ac: it is an abbreviation of acetylation
H3K9ac: Acetylation of Amino acid number 9 (lysine) in H3 protein.

Epigenetic regulators include:

1- Writer: add chemical marks on DNA or histone

→adds a certain group, for example, add methyl group or Acetyl group on histone or at DNA, methyl transferase add methyl group.

2- Eraser: remove chemical marks, which are opposite of Writer, by eliminating the chemical marks.

Demethylase: remove the methyl group

Deacetylase: remove the Acetyl group.

3- Readers: non enzymatic proteins that bind to specific chemical marks.

→Read Chemical signals and attach them.

4- Remodeler: alter chromatin state (3D) structure according to Chemical modifications

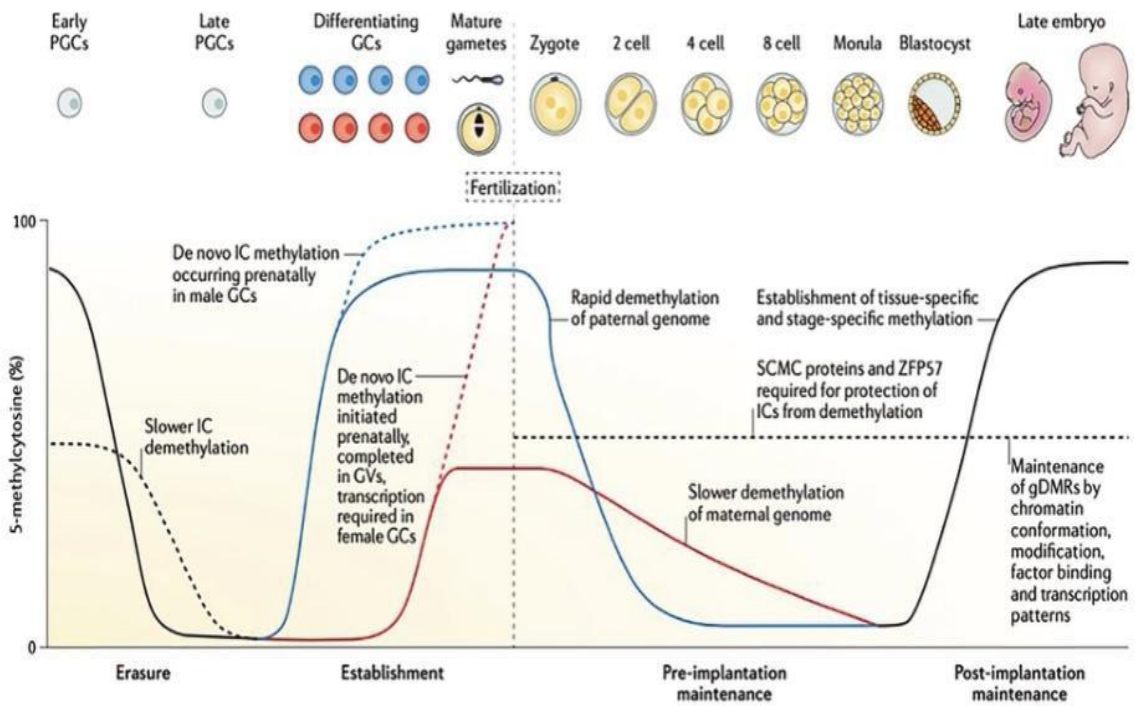


Figure 8.1 **The life cycle of imprints.** DNA methylation reprogramming during human development. Methylation of imprinting centers (ICs) *(dashed black line)* is erased more slowly than that of the rest of the genome *(black line)* in primordial germ cells (PGCs) and reestablished with different kinetics in male (paternal ICs, *dashed blue line*; whole genome, *blue line*) and female (maternal ICs, *dashed red line*; whole genome, *red line*) germ cells. After fertilization, the maternally and paternally derived genomes are widely demethylated, while differential methylation between maternal and paternal IC alleles (50% level) is maintained preimplantation and postimplantation. Factors and events involved in each stage, 5-methylcytosine level and approximate timing of imprint erasure, establishment and preimplantation and postimplantation maintenance are indicated. *gDMRs*, Germline differentially methylated regions; *GVs*, germinal vesicles; *SCMC*, subcortical maternal complex. (From Monk D, Mackay DJG, Eggermann T, et al: Genomic imprinting disorders: lessons on how genome, epigenome and environment interact, *Nat Rev Genet* 20:235–248, 2019. doi:10.1038/s41576-018-0092-0.)

During primordial germ cell specification in a fetus, at 5 week of gestation there is a global erasure (remove) methylation in imprinting centers and the rest of the genome in the Germ cells, for the imprinting center the process is slower.

During the development of germ cells, there is an erasure (remove) of Methyl groups then the re-establishment of methylation and imprint acquisitions in differentiating germ cell before maturing to sperm or oocyte.

Erasure methylation (de methylation) for imprinting centers is slower than the rest of the genome in germ cells.
Dotted or dash black line (- - - - -) : imprinting centres in Germ lines genome
Continuous black line: rest of germ cells genome
Dotted or dashed red lines: maternal imprinting centers
Continuous red line: rest of maternal genome
Dotted or dashed blue line: paternal imprinting centers.
Continuous blue line: rest of paternal genome.

Reform of the imprinting centers is faster in males than in females.

After fertilization the maternal and paternal derived genome is widely de-methylated, specific proteins protect imprinting centers from de-methylation (SCMC proteins and zfp57).

Slower decline (De-methylation) in the rest of maternal genome than in the paternal genome.

THE ENVIRONMENT INTERACTS WITH THE EPIGENOME

1- Environmental factors during fetal development and infancy contributes to chronic disease susceptibility.

2- Geographic links between low birth weight in the UK associated with increased fetal mortality, as well as cardiovascular disease.

3- Regions with the highest rates of coronary heart disease Also had increased infant mortality rates in the decades

3-Regions with the highest rates of coronary heart disease

Also had increased infant mortality rates in the decades prior, which shows a correlation with low birth weight in the previous generation.

4-Poor prenatal nutrition is an environmental risk for both outcomes, which have a Negative repercussions on embryo which also differs if the continuous poor nutrition occur At an early stage or a late stage of pregnancy.

Dutch Hunger Winter during World War II

established prenatal starvation results in an increased risk of obesity, abnormal lipid profiles, cardiovascular disease, and neuropsychiatric disorders

Outcomes differ based on the timing of exposure

- Exposure during early gestations had normal birthweights (but increased risk of obesity).
- Exposure at later gestations reduced birthweights

Effects at different stages of fetal development, give different on the fetus, regardless of what these repercussions are.

Poor nutrition doesn't change DNA sequence but it changes things around the DNA, which Alters gene expression.

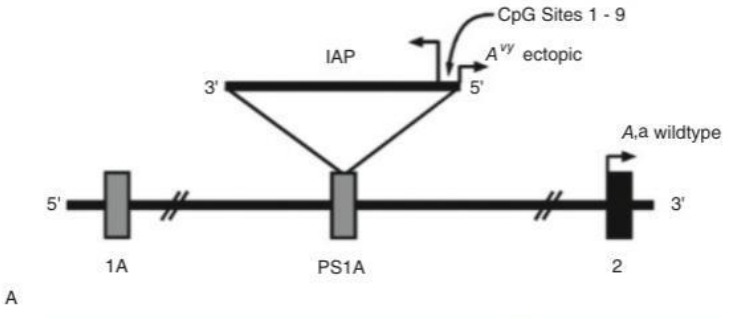
The fetus develops at different stages, if a disruption at the beginning of the pregnancy gives certain repercussions that differs from if the disturbance occurs at the late stage of pregnancy, this is evidence of an epigenetic role in the expression of phenotypes.

Contrast phenotypes are determined by (window of sensitivity) contrasting does the formality that gave us, gives at the beginning of pregnancy phenotype different from the end of pregnancy, gives contrasting phenotypes.

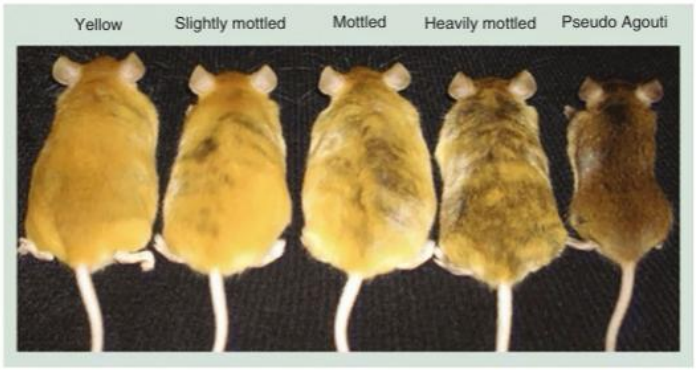
this (contrasting phenotype) given biological system where exist a window of sensitivity during which certain environmental exposure can cause lasting change.

Agouti mouse model

demonstrates how epigenetic mechanisms act as a temporal bridge between in-utero exposures and health outcomes in adulthood.



A



B

Figure 8.2 **Environmentally induced alterations in the epigenome *in* A^{vy} mouse.** (A) The A^{vy} allele contains a contraoriented intracisternal A-particle insertion within pseudoexon 1A (PS1A) of the Agouti gene. A cryptic promoter (*short arrowhead* labeled "A^{vy} ectopic") drives constitutive ectopic Agouti expression. Transcription of the Agouti gene normally initiates from a developmentally regulated hair cycle–specific promoter in exon 2 (*short arrowhead* labeled "*A,a* wild type"). (B) Genetically identical offspring heterozygous for the viable yellow allele (A^{vy}/a) in the Agouti gene representing the five coat color phenotypes, corresponding to different levels of DNA methylation and associated phenotypes, including obesity. Mice shown are the same sex and age. (A, From Dolinoy DC, Huang D, Jirtle RL: Maternal nutrient supplementation counteracts bisphenol A-induced DNA hypomethylation in early development, *Proc Nat Acad Sci* 104(32):13056–13061, 2007. doi:10.1073/pnas.0703739104; B, Jirtle RL: The Agouti mouse: a biosensor for environmental epigenomics studies investigating the developmental origins of health and disease, *Epigenomics* 6(5):447–450, 2014. doi:10.2217/epi.14.58.)

Avy is a viable yellow allele, phenotype includes:
yellow fur, obesity, type II diabetes, and predisposition to tumors.
Associated phenotypes are dependent on levels of DNA methylation at the IAP (intracisternal A-particle retrotransposon)
A diet rich in methyl donors fed to pregnant Agouti mice alters the expression of the Agouti gene in offspring, which impacts long term health.

They found when pregnant Agouti mice fed with diets rich in methyl group there is an increase in methylation which lead to a change in phenotype from yellow color to pseudo agouti offspring which is so close to a normal phenotype

All mice in this figure are inbred which means they have the same genetic material, and each of them has different fur color because of differences in the methylation process.

Assisted reproductive technologies (ART)

It helps people who suffer from reproductive problems by Assisting them to conceive.

ART can disrupt two critical periods of developmental epigenetic
reprogramming:

1- Oocyte maturation, injection of female with substances that enhance ovulation.

2- Retention of gametic imprints following fertilization, because in ART you work with Petri dishes.

And if we Remember after fertilization, the imprinting sites protected from De-methylation by specific proteins, but in ART this production is absent.

ART affect methylation

it can cause damage or certain changes resulting from ovarian follicular stimulation

Aspects of ART:

1- in vitro fertilization

2- Intra-cytoplasmic sperm injection

3- Freezing of embryos

These aspects May lead to dysregulation of preimplantation epigenetic reprogramming.

Risks of adverse pregnancy outcomes:

1- Low birthweight for gestation age (lower than normal)

2- Preterm birth (born before the determined time).

3- Congenital malformations (this is a lower percentage but it is more than normal fertilization).

4- Increased rate of imprinting disorders

During the lifespan, DNA methylation patterns continue to change in both predictable and seemingly random ways
When you compare the methylation of a young person with older person we find a difference in methylation.

In the twins when they grow up you find that there are difference in the methylation process so they are identical in their genetic material, but different in methylation pattern as they age they will diverge, so that is the discordant phenotypes.
Discordant: Mono-Zygote twins one of them has the disease and the
other is normal.

DNA methylation differences in monozygotic twins is associated with many discordant phenotypes:
1- Psychiatric disorders
2- Schizophrenia

3- Bipolar disorder

4- Autoimmune diseases

5- Lupus erythematosus

6- Multiple sclerosis

THE ROLE OF EPIGENETICS IN HUMAN DISEASE

Nuclear transfer experiments in mouse embryos showed that mammalian maternal and paternal genomic contributions to the fertilized egg have different effects on the developing embryo

Zygotes created carrying either two nuclei of maternal or paternal origin

generating exclusively embryonic or placental tissue, respectively, but no

viable embryos

chemical groups are attached to DNA, are attached to histones are different and they are accumulating trans of gene expression that you have different results to combine

Hydatidiform moles : are androgenetic in origin

paternal genome (no maternal genome

Ovarian teratomas : are gynogenetic (two maternal genomes (no paternal genome).

the genetic material is different in the paternal and maternal, even though the sequence of the DNA is the same, but the chemical groups are attached to the DNA, although there are chemical groups are attached to that histones are different.

you have a nucleus with the two paternal genomes or nucleus with two maternal genomes, we have finds with developments

Ovarian teratoma : reveals well-differentiated fetal structures of all three germ layers (ectoderm, mesoderm, endoderm)

Hydatidiform mole : contains only extraembryonic trophoblast elements

Maternally and paternally transmitted genomes are not functionally.

Although the maternal genome and paternal genome are the same (number of genes), but there is a difference in the Chemical groups that are attached to DNA And histones (methylation, Acetylation also in imprinting regions).
Functional differences are attributed to genomic imprinting.

Generally, for each gene, we have two alleles within our cells, by Default both alleles are expressed, but sometimes we have exceptions from the role in which one allele only express that is what happens in genetic imprinting only one allele is expressed while the other is not, and this is a normal process, but this process is regulated, if any change in regulation of this process it lead to Abnormalities.

In imprinting genes: only one allele will express (mono allelesexpression)

Mono allelic: is a natural process and different between types of cells and between the developmental stages. Also, some genes imprinted at maternal chromosomes, will other imprinted at the paternal chromosome, in other words some genes only express in paternal chromosome while others only express at the maternal chromosome.

CATEGORIES OF EPIGENETIC DISORDERS

Genomic imprinting

More than 120 Imprinted genes

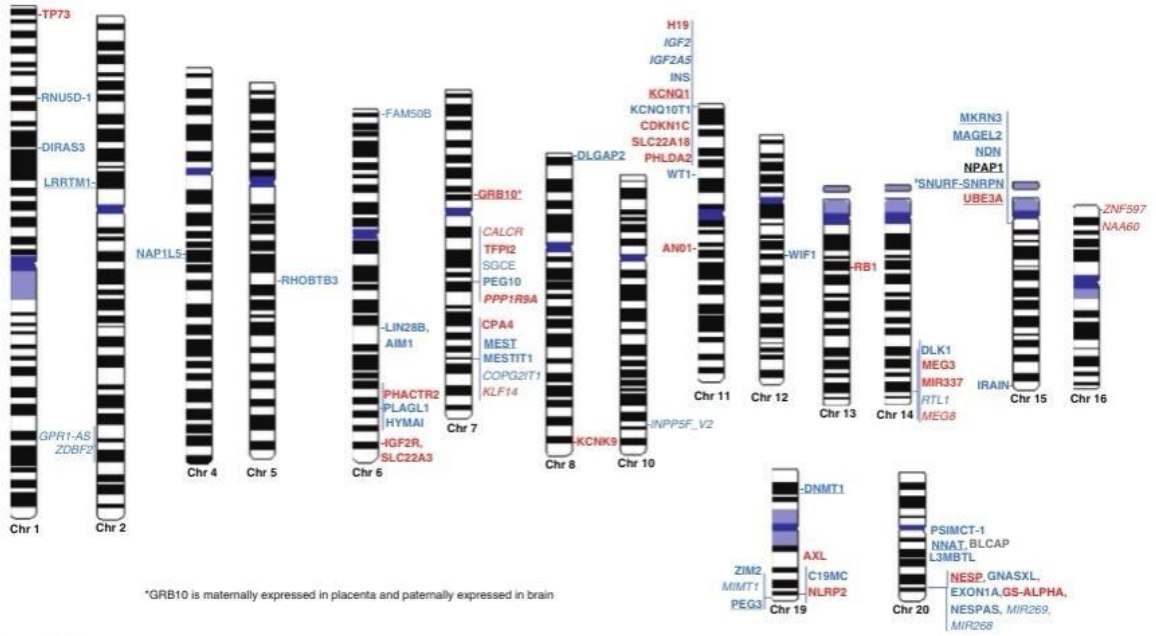


Figure 8.3 **Ideograms of human imprinted genes.** Ideograms were generated using http://www.dna-rainbow.org/ideograms/. An ideogram of each human chromosome known to have an imprinted gene based on the imprinted gene catalogue (http://igc.otago.ac.nz) and GeneImprint portal (http://www.geneimprint.com) is shown. Imprinted genes are listed on each ideogram if they were designated as imprinted in both of the aforementioned human imprinted gene catalogs. *Blue* genes are paternally expressed, red genes are maternally expressed, *black* genes have unknown parent-of-origin expression, *gray* genes have parental expression that is isoform dependent. *Bold* genes are implicated in growth, *underlined* genes play roles in neurodevelopment. Genes in *italic* have no reported function in growth or neurodevelopment.

In this figure

Blue genes: paternal express, only the copy that present at the paternal chromosome will be expressed while
the other copy that will be present at the maternal chromosome doesn't express.

Red genes: maternal express, only the copy that present at the maternal chromosome will be expressed while the other copy that will be present at the paternal chromosome doesn’t get expressed.

UBE3A gene is a maternal expressed, only the copy that was inherited from the mother is expressed while the other copy that was inherited from the mother doesn’t get expressed (imprinted) , it has an imprint other genes work in the opposite side.
In Default conditions, both alleles are expressed, for example the genes of hemoglobin, both alleles of genes are expressed.

The Majority of genes show biallelic expression.

Let's say you have two copies of chromosome 1 derived only from father what is the reflection?
Nothing

But if there are 2 copies of chromosome 15 derived from one parent it gives us prader-Willi syndrome or Angelman, this is because chromosome 15 has imprinted genes so sensitive to Development, so when the two copies of chromosome 15 comes From one paternal cause problem in the health and they will create disease.

so it depends (UBE) what chromosomes have (UBE), some chromosomes that have (UBE) will not be translated into phenotype, but if others it will translated depending on the genes that are present, so chromosome (6, 7, 11, 14, 15), UBD of one of these chromosomes lead to disease.

Sometimes without UPD, problems in imprinting process for certain genes leads to problems.

The first human disorders recognized to result from genomic imprinting:

1- Prader-Willi syndrome: absence of paternally Expressed genes

2- Angelman syndrome: absence of maternally expressed genes

Mechanisms include:

1- Chromosome deletions (in short arm chromosome 15)

If the deletion is in the paternal chromosome it will give us Prader willi syndrome, if the deletion is in the maternal chromosome it will give us Angelman syndrome.

2- Uniparental Disomy (chromosome 15)

UPD of maternal chromosome 15 gives us prader willi syndrome, while UPD of paternal chromosome 15 gives us Angelman syndrome

3- Imprinting defects (epimutation)

4- Pathogenic sequence variants

Also, there are two diseases associated with imprinting:

1- Beckwith-Wiedmann syndrome overgrowth :
Means an increase in growth

means an increase in growth

2- Russell-Silver syndrome undergrowth

means a decrease in growth

Overgrowth and undergrowth work the opposite way.

They are genes

IC1 IGF2 & H19, there is imprinting center mutation

IC2 CDKN1C, KCNQ1, KCNQ10T1

IC1 is methylated on the paternal chromosome: IGF2 expression (promotes cell growth and proliferation), H19 silencing

IC2 is methylated on maternal chromosome: KCNQ1 and CDKN1C expression (a negative regulator of cell proliferation), KCNQ10T1 silencing

IC1 is methylated on the paternal chromosomes and gives IGF2 expression, this gene promotes cell growth and proliferation, H19 do silencing

IC2 is methylated on the maternal chromosome, KCNQ1

These conditions are mirror images of each other both clinically and molecularly.

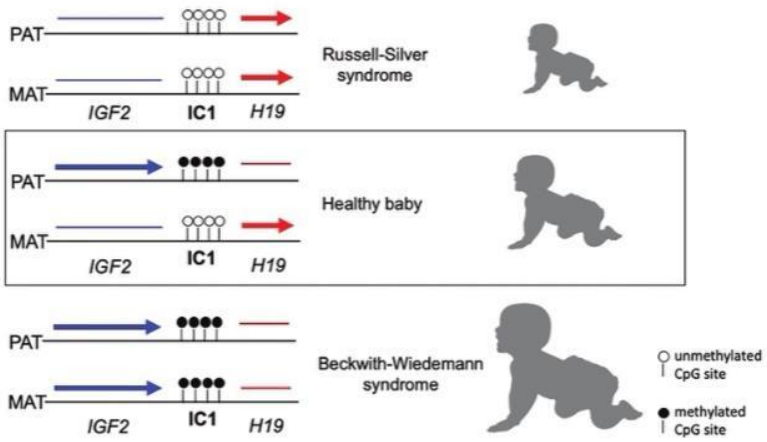


Figure 8.4 **Opposite imprinting alterations on 11p15 can cause opposite phenotypes.** Schematic representation of imprinting regulation at imprinting center 1 (IC1) in the chromosome 11p15 region. The highlighted box *(middle)* represents normal expression in which IC1 is methylated on the paternally derived chromosome and unmethylated on the maternally derived chromosome, resulting in expression of insulin-like growth factor 2 (IGF2) only from the paternal allele. *(Top)* Loss of methylation at IC1 on the paternal allele results in silencing of IGF2; suppression of IGF2 results in reduced growth and causes Russell-Silver syndrome (RSS). *(Bottom)* Gain of methylation at IC1 on the maternal allele results in activation of IGF2, which promotes growth and causes Beckwith-Wiedemann syndrome (BWS). Loss and gain of methylation at IC2 (not shown here) can also lead to BWS and RSS.

The middle is normal, in this situation, there is a methyl group on the paternal chromosome, so we have methylation, this methylation leads to the chromosome that comes from the father doesn't do an expression to (H19) gene otherwise the (IGF2)do an expression, this is the normal, this paternal chromosome, methylation occurs on the IC1 reflection the expression of this gene only the father chromosome.

The second is a hypo methylation means that no methylation occurs, that hypo methylation leads to stop expression on the IGF2 otherwise and the expression on the H19 and gives the normal situation.

At the top, in the Russell-Silver syndrome, there is hypomethylation, no methylation occurs in both chromosome, and it will give the same results as the second chromosome in a normal situation, only the H19 work, and the IGF2 not working in the maternal chromosome, so that gives the Russell-Silver syndrom.

At the bottom unlike the first two situations, the methylation occurs for two chromosomes, like the duplication events for the paternal chromosome from the normal situation, its effect is increasing the expression for IGF2 also this gene is important for the promotion of cell growth and decreasing the expression of H19, so that gives the Beckwith-Wiedemann syndrome.
The Two diseases with contrasting phenotypes, one disease that has overgrowth in it and its Beckwith-Wiedemann syndrome and other diseases have undergrowth and
its Russlle-Silver syndrome

These conditions can be seen in the same family when the underlying etiology is chromosome duplication/deletion that is transmitted through a male versus a female due to parent-of-origin specific imprinting

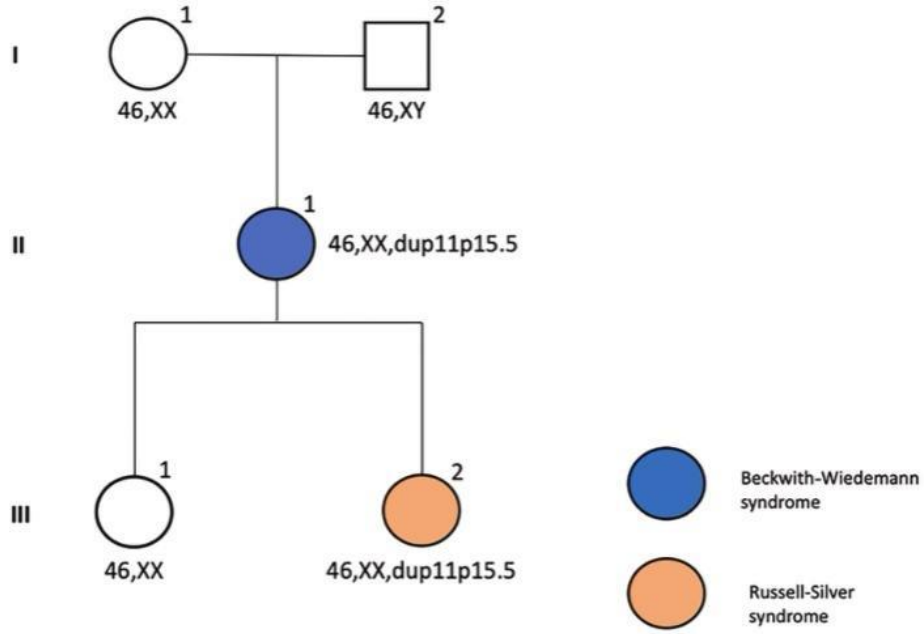


Figure 8.5 **Pedigree of a family in which a chromosome 11p15 duplication is segregating; different phenotypes determined by parent-of-origin–specific imprinting.** Individual II-1 has a diagnosis of Beckwith-Wiedemann syndrome, which is determined to be due to a *de novo* chromosome duplication of chromosome 11p15 encompassing imprinting center 1 (IC1) on her paternally derived chromosome 11. She therefore has two copies of paternally imprinted genes in this region and one copy of maternally imprinted genes, which leads to relative hypermethylation of IC1. When she passes this chromosome duplication on to her children, the parental imprints will be erased and replaced with maternal imprints. Therefore her daughter (III-2) who inherits the chromosome 11p15 duplication will have two copies of maternally imprinted genes in this region and one copy of paternally imprinted genes, which leads to relative hypomethylation of IC1. This is associated with Russell-Silver syndrome.

Here are de novo Duplications of 11p15.5 which contain imprinting genes, II female had back Wiedemann syndrome because it inherited the mutation from his father (paternally mutation), and if you look to III female had a Rusell-silver syndrome because she received the mutation from her mother,

you can ask if she received a mutation from her mother why does she have a different disease?

Because she received the mutation from his mother (maternally mutation)as what we were said previously

Generally For diseases related to imprinting for the same mutation, if you received the mutation from the father, you will express phenotype different from if you received the same mutation from your mother.

Disorders involving unstable repeat expansions

Huntington's disease, fragile X syndrome, and myotonic dystrophy, result from the expansion of repeats and could be present in coding sequence or promoter sequence

Or in 5' UTR or 3' UTR

Fragile X is the most common disease that causes Intellectual disability (Higher Frequency)

In Fragile X syndrome, expansion of FMR1 CGG repeat to full mutation triggers epigenetic events including methylation of FMR1 promotor leading to reduced or absent production of FMRP protein.
Normally the number of repeats is less than 55,
In Fragile X the number of repeats becomes more than 200 which leads to methylation of the FMR1 promoter and no production of FMR1

Epigenetic factors are involved in anticipation;
In diseases that are caused by repeats, we have 3 cases
1- Normal
2- Pre mutation
3- Affected

Generally, as the number of repeats increases the age of onset decreases and the severity of the disease increases.

Anticipation: from generation to the next, the number of repeats increase which leads to a decrease in the age of onset (get the disease earlier) and the severity of disease increase.

Disorders of the epigenetic machinery

There are mendelian disorders but they cause a problem in the methylation process so they affect the epigenetic machinery (regulation)

1- Increasing number of mendelian disorders recognized to be caused by sequence variants in genes maintaining normal epigenetic regulation, including writers, erasers, readers, and chromatin remodelers.

2- In contrast to classical imprinting disorders that impact imprinted genes in cis, for this group of disorders the epigenetic dysregulation occurs in trans, impacting multiple genomic-wide targets

Cis means that the gene affects the other genes in the same chromosome, trans means the gene affects other genes in different chromosomes,

so we have a lot of diseases that have mutations in genes that affect the other genes, and the other genes have not any relationship with it, this mendelian disorders affect the way methylation or other epigenetic changes in the DNA or histone.

Figure 8.6 Mendelian disorders of the epigenetic machinery

Over 70 genes with defined epigenetic domains (reader, writer, eraser, remodeler, middle icons) have been linked to Mendelian phenotypes.

The majority of genes cause disease in the heterozygous state (filled circle).

Enzyme domains (writer, eraser, remodeler) are

mutually exclusive in any given factor but many coexist with a reader domain (gray shading). Intellectual disability is seen in the vast majority (blue), as are growth abnormalities (orange). A indicates genes on autosomes; X indicates genes on the X chromosome.

All these genes are different, and related to epigenetics that effect (Eraser, Writer, Reader, Remodeler) that do a mendelian disorders

Disorders of the epigenetic machinery: DNA methylation

1- Methyl-CpG-binding protein 2 (MeCP2) functions as a reader of DNA methylation marks, mutations which causes Rett syndrome

The problem is the baby is born healthy for up to six months to one year, and then the baby starts to get weak, also, you can not recognize less than one year of age after that recognizes intellectual disability and abnormal behavior,

like the hand motion is continuous, and this is the X-linked mean present on the X chromosome and caused the females, possible cause the males but when it causes the males it becomes a very severe phenotype.
Most of the time it affects females, and sometimes males also affected but it is very severe

2- Classic Rett are generally girls heterozygous for loss-of-function Variants
3- Boys with pathogenic MeCP2 mutation exhibit severe infantile encephalopathy with seizures

Disorders of the epigenetic machinery: Histones

Number of genes involved in regulating histone modifications are much larger than for DNA methylation
Disorders often present with overlapping phenotypes, difficult to differentiate clinically
Sotos and Weaver syndromes, overgrowth conditions with overlapping features, caused by NSD1 and EZH2

(epigenetic writers)

Sotos: significant intellectual and behavioral problems

Weaver: mild or no intellectual deficits.

Recurrence risk

Most cases of Sotos have a de novo etiology

Weaver is often familial, with a milder presentation in a

parent

Sotos and Weaver related to histone modifications

Diagnosis of Disease related to epigenetics

By using methylation-sensitive multiplex ligation-

dependent probe amplification

(MS-MLPA) by using enzymes that can recognize

methylated DNA if the methylation is present the enzyme will not cut, if the methylation is absent they

will cut.

Can assess both methylation levels and copy number

variants, distinguish between methylation

abnormalities due to deletion, uniparental disomy, imprinting defect

If a methylation abnormality is detected, additional testing may be required to determine the specific underlying molecular etiology, microarray, or UPD.

If MS-MLPA testing is negative, additional testing should be considered: specifically, sequence analysis of relevant imprinting genes (i.e., UBE3A for Angelman syndrome) and cytogenetic analysis

Identification of etiology is critical to determining recurrence risk
Recurrence risk: if you have a child affected by disease what is the Probability of the second child to is affected by this disease?
Etiology: the causes of disease

If the disease is caused by De novo (during gametogenesis) methylation abnormality, there is

a low probability of the second child being affected by this disease.

If the disease is caused by methylation abnormality due to deletion at the imprinting region could confer a 50% risk of recurrence if inherited, depending on parent of origin.

Current obstacles to fully understanding the role of epigenetic aberrations in disease pathophysiology:

- The Sheer complexity of the epigenome, consisting of many interrelated and context-dependent chemical marks
- A unique epigenome that exists for each cell type and changes across the lifespan, especially during development
- Baseline levels of stochastic (Random) and nonstochastic variation among individuals, similar to that in the human genome

ENCODE project (Encyclopedia of DNA Elements):

explore epigenetic patterns in chromatin genome-wide to better understand the control of gene expression in different tissues or disease states Clinical Epigenetics