Universidade de Lisboa
Faculdade de Farmácia

Variações Epigenéticas Transgeracionais

Marta Sofia Almeida Favita

Monografia orientada pelo Professor Doutor Carolino José Nunes Monteiro, Professor Associado com Agregação.

Mestrado Integrado em Ciências Farmacêuticas

2021

Universidade de Lisboa
Faculdade de Farmácia

Variações Epigenéticas Transgeracionais

Marta Sofia Almeida Favita

Trabalho Final de Mestrado Integrado em Ciências Farmacêuticas apresentado à Universidade de Lisboa através da Faculdade de Farmácia

Monografia orientada pelo Professor Doutor Carolino José Nunes Monteiro, Professor Associado com Agregação.

2021

Resumo

Existe evidência que as experiências vividas e a ambiência podem alterar os perfis psicológicos e comportamentais, de modo a que os seres vivos se adaptem às novas ambiências. Um ponto importante que tem sido discutido é se estas alterações podem ser transmitidas ao longo de várias gerações, através da transmissão epigenética. A epigenética é o conjunto de modificações químicas no DNA, que não alteram a sua sequência, mas codificam informação que, por sua vez, influencia a expressão génica. Nova evidência tem desvendado os mecanismos responsáveis pelas marcas epigenéticas, tais como a metilação do DNA, as modificações das histonas e os RNA não codificantes. Outra questão pertinente que tem sido levantada é como conseguem estas marcas do DNA escapar à reprogramação epigenética que ocorre no feto e nas células germinativas primordiais. A reprogramação epigenética é responsável por eliminar as modificações epigenéticas nos mesmos, permitindo o desenvolvimento de células totipotentes e pluripotentes. Vários estudos têm identificado falhas neste processo que permitem a passagem da informação para a gerações futuras. Todos estes mecanismos mencionados anteriormente podem alterar a regulação génica devido à plasticidade do DNA e dar origem a respostas adaptativas e não-adaptativas que, por sua vez, irão ser transmitidas às próximas gerações devido à acomodação genética.

O stress e as experiências traumáticas são responsáveis por modificações epigenéticas no DNA, que podem aumentar a suscetibilidade para a síndroma do stress pós-traumático (PTSD) e outras alterações no organismo. Nesta monografia são analisados vários estudos sobre PTSD, que mostram a transmissão de alterações fisiológicas ou comportamentais, tais como o aumento de suscetibilidade para a PTSD, durante várias gerações. Também é aqui mencionado o potencial efeito das toxinas ambienciais, tais como o álcool, o tabaco e a morfina, na transmissão de marcas epigenéticas. Por fim, este trabalho refere o conceito de *environmental enrichment*, que consiste na estimulação do cérebro através do contexto social positivo e da atividade física. Este *environmental enrichment* pode estimular marcas epigenéticas positivas e, deste modo, reverter as modificações feitas pelo stress traumático, surgindo assim, como uma possibilidade de tratamento para os indivíduos afetados.

Palavras-chave: Epigenética; mecanismos epigenéticos; metilação do DNA; transmissão epigenética transgeracional; PTSD

Abstract

It is known that experiences and the environment can change the physiological and behaviour traits, in order for living beings to adapt to new environments. The central point of discussion is whether these modifications can be passed across several generations, through epigenetic transmission. Epigenetics refers to chemical modifications in DNA, which do not alter the genetic code, but carry non-genetic information, which in turn will change the gene regulation. New evidence as uncovered the epigenetic mechanism responsible for marking DNA, such as DNA methylation, histone modifications and non-coding RNA. One other question that has been proposed is how these changes in the DNA sequence can escape the epigenetic reprogramming which occurs in the fetus and in the primordial germ cells. This epigenetic reset is responsible for eliminating all the epigenetic marks in the fetus and in the primordial germ cells, in order to clean the epigenetic landscape and generate both totipotent and pluripotent cells. Several researchers have identified gaps in this process, leading to the transmission of non-genetic information to future generations. Together, these mechanisms can alter the gene regulation due to the DNA plasticity, and lead to adaptive and maladaptive changes in humans, that will be transmitted to next generations thought genetic accommodation.

Stress and traumatic experiences are accountable for epigenetic modifications, that can lead to a higher susceptibility to PTSD and other changes in the organism. In this monography reviews several studies about PTSD, which enhanced the transmission of behaviours and physiological changes, such as an increased susceptibility to PTSD, across generations. The potential effects of environmental toxins, such as alcohol, tobacco, and morphine, in epigenetic inheritance are also discussed here. Lastly, this work presents the concept of environmental enrichment, which consists on the stimulation of the brain, through social surrounding, and physical activity. Environmental enrichment can trigger ‘positive epigenetic marks’ and revert the modifications made by traumatic stress, thus emerging as a treatment possibility for the affect individuals.

Keywords: Epigenetic; epigenetic mechanism; DNA methylation; transgenerational epigenetic inheritance; PTSD

Agradecimentos

Após esta caminhada de 5 anos na Faculdade de Farmácia, gostaria de agradecer a todas as pessoas que marcaram o meu percurso e o tornaram nos melhores 5 anos da minha vida.

Primeiro, um agradecimento especial à minha família, que desde sempre me apoiou e acreditou em mim. Obrigada por compreenderem todas as vezes que não pude estar presente por ter de conjugar o estágio, a monografia e tudo o resto.

Um grande obrigado aos meus amigos que me acompanharam, em especial à Mariana Afonso, Margarida Bagorro, Margarida Viegas, Mariana Cardoso, Catarina Martins, Luís Silva e às minhas colegas de casa. Obrigada por todas as conversas, gargalhadas e noites incríveis, quer fossem de festas ou de estudo no caleidoscópio. Sem vocês estes 5 anos não teriam sido tão divertidos, nem eu teria sido tão feliz.

Obrigada, também, aos meus amigos de longa data, que apesar dos nossos caminhos se terem separado, continuaram sempre ao meu lado. Obrigada por compreender que nem sempre era possível estar presente, e por me incentivarem quando era necessário.

Ao meu namorado, André Nogueira, um gigante obrigado, por me ter acompanhado neste percurso, por ouvir as minhas queixas em relação ao mundo sem reclamar, pelo apoio constante e pelas palavras de motivação.

Agradeço ainda à Tuna A Feminina por me ter mostrado o melhor da vida académica, por me ter permitido conhecer Portugal, de norte a sul, com uma companhia incrível. Às grandes amizades que a tuna me proporcionou, um grande obrigada por animarem a minha vida, vão sempre ter um lugar muito especial no meu coração.

Por último, mas não menos importante, um obrigado ao Professor Carolino Monteiro por ter aceitado este desafio comigo. Obrigada por todas as palavras motivadoras, pelas várias reuniões e por estar sempre disponível para mim.

A todos, um enorme obrigada.

Abbreviations

5caC	5-carboxymethylcytosine
5fC	5-formylcytosine
5hmC	5-hydroxymethylcytosine
5mC	Cytosine 5-methylation
ACTH	Adrenocorticotropin hormone
Ago	Argonaute
ASD	Acute stress disorder
BER	Base excision repair
Bp	Base pairs
CpG	Cytosine–guanine dinucleotides
CRH	Corticotropin-releasing hormone
DMR	Differentially methylated region
DNA	Deoxyribonucleic acid
DNMT	DNA methyltransferase
EE	Environmental enrichment
F0	Parent
F1	First generation
F2	Second generation
F3	Third generation
GR	Glucocorticoid receptor
HAT	Histone acetyltransferases
HDAC	Histone deacetylase
HFD	High-fat diet
HMT	Histone methyltransferase
IAP	Intracisternal particle A
ICR	Imprinting control region
IGF2	Insulin-like growth factor II
IVF	*In vitro* fertilization

lncRNA	Long non-coding RNA
miRNA	Micro-RNA
MR	Mineralocorticoid receptor
mRNA	Messenger RNA
MS	Mass spectrometry
MSUS	Maternal separation with unpredictable maternal stress
ncRNA	Non-coding RNA
PGC	Primordial germ cell
piRISC	piRNA-induced silencing complex
piRNA	piwi-interacting RNA
PTGS	Post-transcriptional gene regulation
PTM	Post-translational modifications
PTSD	Post-traumatic stress disorder
RISC	RNA-induced silencing complex
RNA	Ribonucleic acid
siRNA	Small-interfering RNA
sncRNA	Short non-coding RNA
snoRNA	Small nucleolar RNA
TDG	Thymine DNA glycosylase
tDR	tRNA-derived small RNA
TET	Ten–eleven translocation
TGS	Transcriptional gene regulation
tRNA	Transfer RNA
UTR	Untranslated region

Contents

List of Figures 11

1. Introduction 12

1.1. Epigenetics 12

1.2. Intergenerational *versus* transgenerational inheritance 13

2. Objectives 16

3. Materials and Methods 17

4. Epigenetic mechanisms 18

4.1. DNA Methylation 20

4.2. Histone modifications 23

4.3. Non-coding RNA 26

5. Epigenetic Reprogramming 31

5.1. Escaping Intergenerational Reprogramming 33

6. Phenotypic plasticity and genetic accommodation 35

7. Stress as environmental stimuli for epigenetic marks 39

8. Sex-specific effects 41

8.1. Gender and sex contributors to sex differences in PTSD 42

9. Post-traumatic stress disorder 44

9.1. Epigenetics marks induced by famine 45

9.2. Epigenetic marks induced by war 46

10. Epigenetic marks induced by toxins 48

11. Environmental enrichment 50

12. Future Perspectives 52

13. Conclusion 54

14. References 55

List of Figures

Figure 1- Principles of intergenerational and transgenerational inheritance in females and males. From Nagy et. al (4)**.** 15
Figure 2 - Epigenetic modifications. From Moore, DS (1)**.** 19
Figure 3 - DNA Methylation and DNMTs. From Skvortsova, et. al (5)**.** 21
Figure 4 - Active VS Passive demethylation. From Skvortsova, et. al (15)**.** 22
Figure 5 - Chromatin Structure. Adapted from Hansen et. al (36). 24
Figure 6 - Nucleosome structure highlighting the globular domain and N-terminal tails. Adapted from Kebede, et. al (35). 25
Figure 7 – RNA-binding domains of PIWI Proteins. From Kim (62)**.** 27
Figure 8 – Molecular mechanisms of the PIWI-piRNA complex. From Kim (62)**.** 27
Figure 9 – Modes of action of ncRNAs in gene regulation. From Panni et al (52)**.** 29
Figure 10 – Epigenetic reprogramming during mammalian development. From Nagy et. al (4). 33
Figure 11- Adaptive versus maladaptive plastic response. From Storz at al (94)**.** 36
Figure 12 - Evolutionary changes in phenotypic plasticity. From Kelly (95). 37
Figure 13 – Consequences of traumatic stress in HPA axis. From Guillén-Burgos (101)..... 40

1. Introduction

1.1. Epigenetics

Sometimes we question ourselves ‘Why do I have brown hair, this height and eye colour?’ Generally, scientists answer these questions referring to our genes, our deoxyribonucleic acid (DNA) sequence (1,2). We now also know that exists one more factor called epigenetics that can influence these characteristics (1,2).

The concept of epigenetic appeared half a century ago, meaning ‘the branch of biology which studies the causal interactions between genes and their products which bring the phenotype into being’ which was first used by Conrad Waddington (1,3).

Waddington realized that all the living organisms start as a zygote, that will be divided into millions of similar cells which have the same genetic sequence, but with different features (1). For instance, the blood cells and the brain cells have the same genes, however these cells play very different roles and have very different looks (1). These mentioned differences are a direct result of a nongenetic factor, that allows the activation and deactivation of different genes, depending on the environment in which they are inserted (1).

After 50 years, the term has suffered modifications and is now applied into a wide range (3,4). Nowadays, the epigenetic concept refers to heritable chemical modifications in DNA that alters the gene expression induced by environmental factors, which did not directly influence the DNA sequence (3). Heritable, in this context, implies mitotic and meiotic stability, meaning the ability to pass and maintain epigenetic marks across cell divisions in mitosis and meiosis (4,5).

This transmission to future generation of epigenetic information may allow for living organisms to transmit adaptive and maladaptive behaviours which depend on the environment features that parents had experienced (6).

Environmental factors such as nurturing behaviour, social stress, fear, nutrition, environmental toxins, among others, play an important key in generating epigenetic information which will shape the phenotype of progeny across many generations (6). It is known and accepted that the environment can change the genotype, creating epigenetic marks, modelling behaviour and physiological alterations (6).

Epigenetic marks include DNA and histone modifications and also comprised non-coding RNAs (ncRNAs), including micro-RNA (miRNA), small interfering RNA (siRNA), piwi-interacting RNA (piRNA), transfer RNA (tRNA) fragments, among others (6).

Epigenetic marks will function as a "coat" in our genes, carrying the memory of the past events that our parents had experienced (7). Our organism keeps this information across many generations to prepare us to survive in the environment of our ancestors (7). This information or 'acquired' behaviour will pass on to the lineage in a more marked way if the parents lived in a more life-threatening environmental situation, for instance, torture, war, or famine (6).

As stated before, many of our behaviours can influence these chemical modifications on our genes. For example, the food that contains methyl groups, especially the food with choline or B-complex vitamins, can change the epigenetic profiles (1). These methyl groups can enhance the methylation of the DNA (1). Other types of diets can modify the histone acetylation, broccoli sprouts or turmeric (a spice similar to ginger) can influence the acetylation of histones in specified cells (1). In addition, exercise influences these epigenetic marks, affecting the DNA methylation of muscles cells (1).

The knowledge of the transmission of epigenetic marks through generations, such as DNA methylation, miRNA and other mentioned was encouraged by research studies, not only in plants but also in longitudinal epidemiological studies made in humans (4). A report carried in *Arabidopsis thaliana*, has showed changes in DNA methylation, an epigenetic mark that can be passed to next generations (4).

Over the years scientists have been discovering that memory and learning, addiction, nurture, aging, among others are linked to the epigenetic processes. Therefore, some foods, exercise and experiences can affect our epigenome, thereby increasing or lowering the risk of certain diseases (1).

1.2. Intergenerational *versus* transgenerational inheritance

After discovering the epigenetic effect on animals, the following question arouse: How does the transmission of epigenetic marks on the germline influences the future progeny and how many generations can be affected by this epigenetic information (8). The terms 'Intergenerational' and 'Transgenerational' are often used when we mention the transmission of epigenetic effects, and it is important to clarify the difference between them (8–10) (Fig 1).

Intergenerational effects take place when a stimulus affects a mother, and directly influences her germ cells or the fetus (including the primordial germ cells (PGCs) of the fetus), transmitting the epigenetic marks to the child and grandchild (9). Otherwise, transgenerational inheritance occurs when the epigenetic information did not result from the direct stimulus on the living being (4).

For example, if a pregnant mother (F0) is subjected to a stress environment, such as famine, the trigger will affect the fetus (F1) and their PGCs (which have the capacity to generate the progeny) (F2), modifying the phenotype of the child (F1) and of the grandchild (F2) (4,5,9,10). This inheritance is called intergenerational, due to a trigger exposure in the utero (4,5,9,10). Furthermore, if the great-grandchildren (F3) of this pregnant mother present the same epigenetic information, we consider that exists transgenerational inheritance (4,5,9,10). In the male gender, a man (F0) who was submitted to the same environment as this pregnant woman, his germline (F1), which would participate in the formation of the offspring, likely would also have been influenced by the stimulus, suffering this intergenerational inheritance (4,5,9,10). In this case, in the second generation, grandchild (F2), we could access the transgenerational effects (4,5,9,10) (Fig.1). Effects with shorter timescale, which result from the direct exposure to the trigger are referred as parental or intergenerational (10). However, many of the mechanisms of transmission are shared between intergenerational and transgenerational inheritance (10) (Fig. 1).

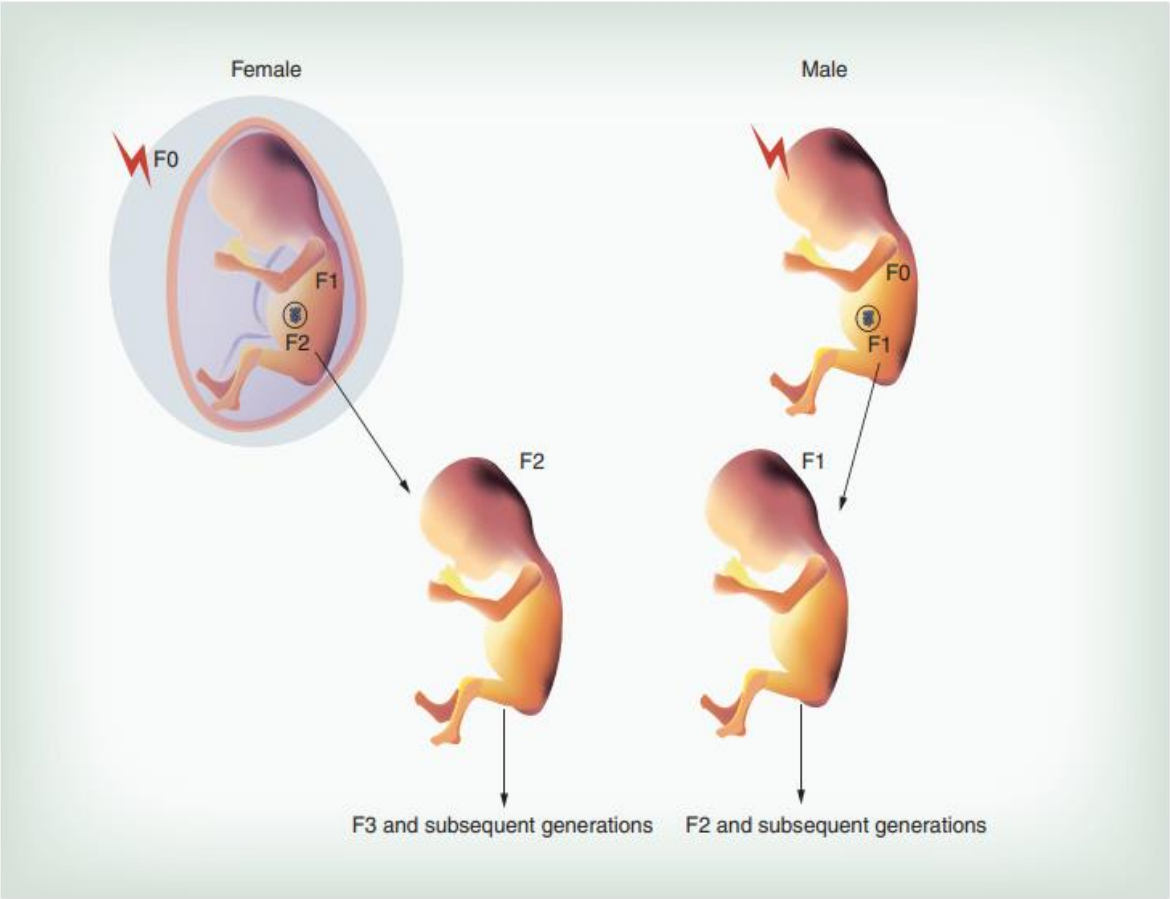

Figure 1- Principles of intergenerational and transgenerational inheritance in females and males. From Nagy et. al (4)**.**

On the right side of the picture, is represented a pregnant female (F0) who suffered a stimulus, that will affect the developing fetus (F1) and his germline (F2) (4,9). On the other side, is a male who is affected by a trigger (F0), influencing his germline (F1) (4,9). This image shows the significance of studying the F2 generation in males and the F3 generation in females to avoid intergenerational epigenetics (4,9). Only by studying these generations, we can access the real transgenerational effects (4,9).

2. Objectives

The main objective of this monography is to understand how behaviour exposure marks can be presented into offspring for several generations. In addition, this work intends to evince that traumatic stress can be inherited by germline transmission through gametes, even if the individual has not experienced a traumatic experience.

The highlights of this work are to understand:

- How can DNA store the traumatic stress memories;
- How memories can interact with DNA and regulate gene expression and other functions;
- Which is the relationship between epigenetic marks, and how can they interact with each other;
- How can these memories be able to be transmitted across generations, without previous stimuli;
- In what extent can these memories influence the life and well-being of the subsequent generations;
- How environmental toxins are also responsible for modifying our epigenome;
- How can we reverse this modifications on DNA and histones in order to discover new treatment strategies.

3. Materials and Methods

The main source of information in this research was PubMed, Google Schoolar and ScienceDirect. The papers referenced in this monography were from scientific journals related to genetic, epigenetic, psychology, neuroscience, among others.

The research was predominantly done in English. Some examples of keywords used in this search were: transgenerational epigenetic inheritance, PTSD, DNA methylation, histone modifications, epigenetic reset, non-coding RNA, DNA plasticity, genetic accommodation, traumatic stress, famine and smoke transgenerational epigenetic. The selected papers were mainly published between 2015 and 2021, however some articles were less recent in order to make historical context.

4. Epigenetic mechanisms

Epigenetic marks contain information over the DNA sequence (in Greek *epi* means above, over), which controls the profiles of gene expression during embryological development and are responsible for establishing cell type-specific patterns (11). These marks are dynamically influenced by different environmental stimuli, such as social environment, nutrition, climate change and seasonal periods, among others (12). These marks on DNA can be dynamic or remain relatively stable and can be transmitted to next generations, through the germline (for example, imprinting genes, X inactivation, etc)(12–14). Epigenetic modifications are considered dynamic when a behaviour or a heritable alteration in gene expression is induced by a previous trigger (14). DNA sequence must have plasticity to control higher-order physiological responses and consequently, behaviour (14).

The epigenetically-regulated cell-type-specific responses enable the locomotor, neuroendocrine and metabolic circuits (12). This is due to the gene expression modification, that ultimately results in behaviour outcomes (12). We distinguish the epigenetic mechanisms according to how they can influence the gene expression (12). These can be: (Fig. 2)

1. Chromatin compaction states, thereby affecting the accessibility to the DNA;
2. The gene expression, through chemical marks (the DNA methylation is the best studied phenomena);
3. Post-transcriptional/pre-translational interference via nc-RNAs.

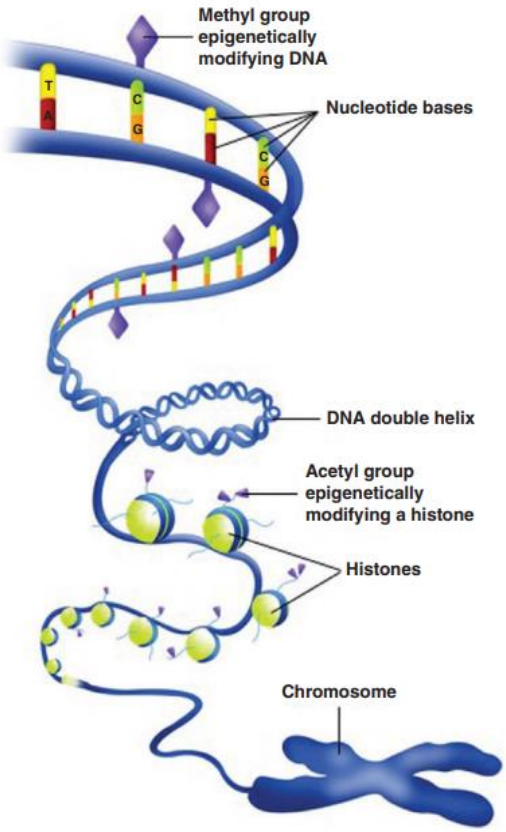

Figure 2 - Epigenetic modifications. From Moore, DS (1)**.**

Illustration of a DNA pulled from a chromosome, showing the double helix curled around the histones, nucleotide bases and the epigenetic modifications that affect the histones and the DNA.

4.1. DNA Methylation

DNA methylation is one of the most abundant and well-studied epigenetic mechanisms, which is fundamental to cellular biology and in normal cell development (6,15). Modifications in the DNA methylation landscape can influence the transcriptome and contribute to the deregulation of cellular pathways (15). These epigenetic modifications remain stable across cell divisions, leading to transgenerational effects and influences in cell memory (12,16).

There is widespread attention to DNA methylation because it is a mechanism involved in gene expression, retroelement silencing, centromere stability and chromosome segregation in mitosis, X-chromosome inactivation and monoallelic silencing of imprinted genes (15,17–19).

In eukaryotes, the most abundant mark of DNA methylation is the methylation of the fifth carbon of cytosines (cytosine 5-methylation), which is controlled by methyltransferases (DNMTs) and ten–eleven translocation (TET) enzymes (13,15,17,20–23). DNA methylation plays an important role during embryogenesis and influences physiological pathways during the lifetime (12).

In mammals, there are three enzymes responsible for DNA methylation, DNMTs which are divided in DNMT3A, DNMT3B and DNMT1 (6,12,22–24). DNMTs catalyse the transfer of a methyl group from S-adenosyl-l-methionine (SAM) to the C5 position of the cytosine base (20,22).

DNMT1 is a methyltransferase with a higher affinity for hemi-methylated cytosine–guanine dinucleotides (CpGs), which catalyse the DNA methylation during cell division, maintaining the DNA methylation landscape from the paternal to the daughter strand (6,12,22,25). DNMT3A and DNMT3B are responsible for catalysing the *de novo* methylation during development, and has higher affinity for CpGs (6,12,22,26). (Figure 3)

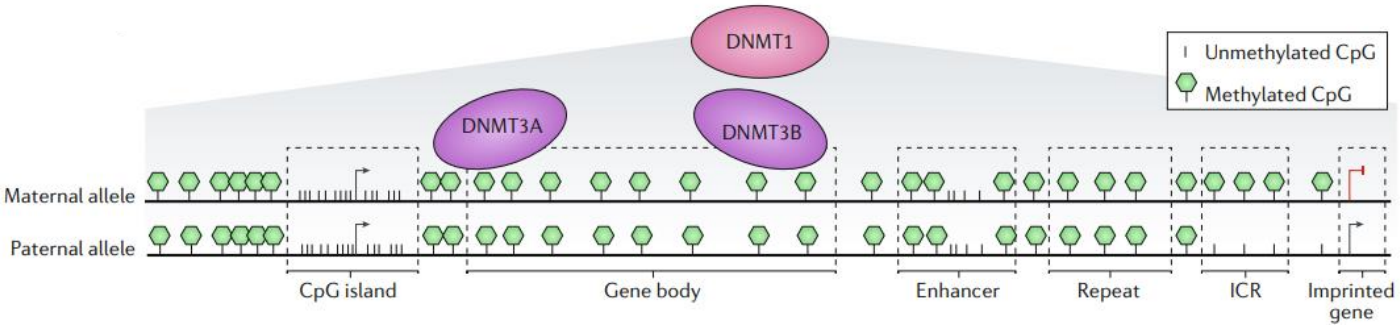

Figure 3 - DNA Methylation and DNMTs. From Skvortsova, et. al (5)**.**

In vertebrates**,** DNA methylation takes place, preferentially, at CpG dinucleotides and the reaction is catalysed by DNMTs (6,20,22,24). DNMT1 is responsible for keeping the DNA landscape, while DNMT3A and DNMT3B is involved in *de novo* methylation (5,6,22). Most of CpG sites in vertebrates are methylated, despite certain CpG-rich regions, such as, CpGs islands, being poorly methylated (5). Active enhancers are generally unmethylated. (5,27) Imprinted genes, which are expressed and regulated in a parent-of-origin-specific manner, present allele-specific methylation at imprinting control regions (5,28).

During early embryogenesis, *de novo* methylation is greatly active, which increases the susceptibility during this period to environmental stimuli. (12) Due to the dynamic character of DNA methylation, it exists both passive and active demethylation (12,29). Active demethylation consists in removing or modifying the methyl groups from 5mC, with TET enzymes (6,29). Whereas passive DNA demethylation refers to loss of 5mC during the dilution of DNA upon the rounds of cell division due to the lack of functional DNA methylation maintenance enzymes (12,29). (Fig. 4)

The methylation mark 5mC is catalysed by DNMT proteins and can be oxidised by TET enzymes to 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC) and 5-carboxymethylcytosine (5caC) (15,29). Thyamine DNA glycosylase (TDG) can excise 5caC and repair with a non-methylated cytosine through base excision repair (BER) (15). DNA demethylation pathway through BER is called active demethylation (15,29). Conversely, passive demethylation occurs when, the DNMT1 enzyme during replication is compromised, resulting in the gradual loss of 5hmC and 5aC across cell divisions (15,29) (Fig.4).

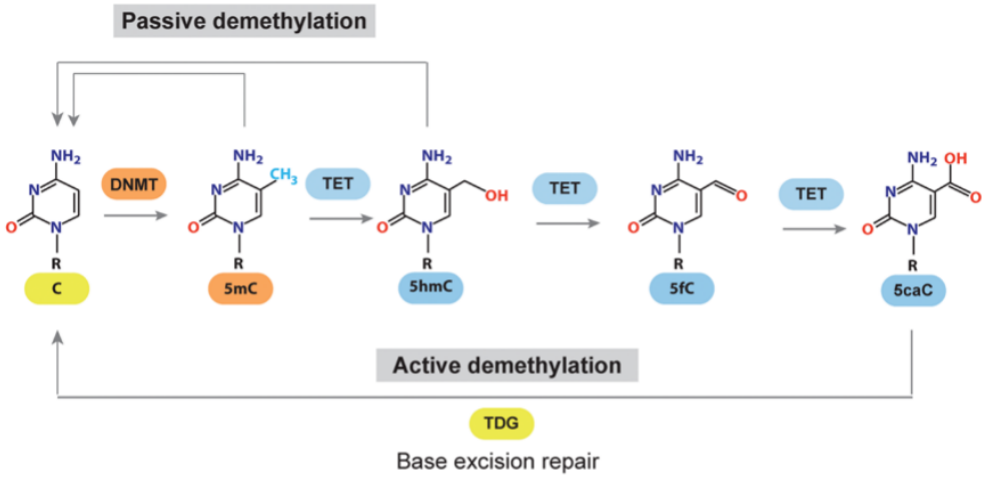

Figure 4 - Active *versus* Passive demethylation. From Skvortsova, et. al (15)**.**

As it has already been mentioned, DNA methylation occurs mainly on the sequence 50-C-phosphate-G-30, which means that a cytosine and guanine are separated by only one phosphate group (6,20). The majority of CpGs are closer to one another in specific *loci*, such as regulatory regions, for example in promoters, exons and in a reduced extent of introns (12). These regions are called CpGs islands, which have 500-1000 base pairs (bp) in length and in contrast to the rest of the genome are generally unmethylated in normal somatic cells (15). DNA of CpGs islands exists in permissive chromatin state and present, as well, combinations of post-translational histone modifications and a different nucleosome organization (15,30). In mammals, the majority of promoters are found in CpGs islands (approximately 70%) and most of the promoters of housekeeping genes are located in these regions (12,15). Therefore DNA methylation in these regions, with gene promoters, is one of the most important epigenetic markers (12).

DNA methylation effects depend on the genome region where the methylation happens (12). The methylation mark 5mC, in promoters, usually results in reduced gene expression (12,15,20,29). This decrease in gene expression is mediated by repressive methyl-binding proteins and/or by access restriction of transcription machinery to the DNA (12). Furthermore, DNA methylation can interfere in gene expression by promoting the formation of heterochromatin (12).

DNA methylation of gene bodies can occur in exons and introns (12,20). Methylation of gene bodies are usually extent, alternatively to CpGs promoters and shores, and seems to be associated with active gene expression (15,20). A study shows that exons are more methylated than introns, suggesting that DNA methylation could affect the alternative splicing (20,31) This leads to result differences in the methylation in promoters and gene bodies. (20,31). However, DNA methylation at promoters is the most studied marker of gene expression regulation (12).

The methylation patterns of cells will depend on its stress response, as different genes are activated or repressed (20). For example, a study has shown that after the postnatal period, 131 genes lost, and 127 genes gained DNA methylation of their transcriptional region on the nuclei of mouse cardiomyocytes (32).

DNA methylation is an important mechanism in the study of the transgenerational epigenetic inheritance because of the semi-conservative method of DNA replication. DNA methylation pattern of newly CpG sequences is maintained through copy of the paternal strand methylated CpG, duplicating the epigenetic mark (2,5,6).

4.2. Histone modifications

Histones are a specialized class of proteins that are known to carry nongenetic information across generations (2). These proteins are important to the maintenance of the chromatin structure and play an important role in the dynamic and long term regulation of genes (20,33).

In eukaryotic cells, nuclear genetic material is organised in the form of chromatin, which is a nuclearprotein complex whose main function is to pack the DNA (34,35). Chromatin is a dynamic structure that opens, closes, wraps, unwraps, shrinks and stretches depending on the cell cycle phase and the epigenetic marks (35,36). The chromatin structure will influence gene regulation, expression, and other biological functions (12,13,35). Chromatin consists of 147 bp of DNA sequence tangled around a globular protein octamer forming a nucleosome core particle, the fundamental repeating unit of chromatin (2,35–38). Nucleosome core particles are composed of two copies of the four core histones (H2A, H2B, H3 and H4) (34–37,39). Core histones are composed of a structured domain that binds DNA and an N-terminal tail that is projected into solution (35,40). Nucleosome arrays are connected by linker DNA with the help of H1 histone that bounds to the outside of nucleosome core particles, forming a full chromosome and high-order chromatin structures (34,36,37,41). Nucleosomes are separated by

200 or 40 bp and tend to form a characteristic ‘beads on a string’ structure with coating DNA (37). (Fig. 5)

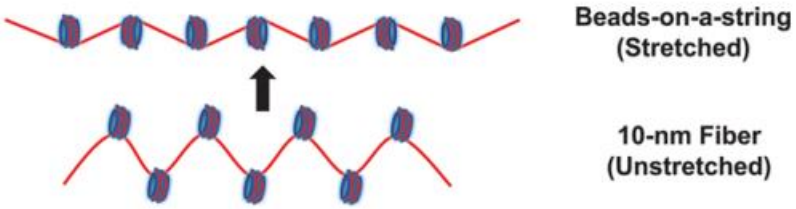

Figure 5 - Chromatin Structure. Adapted from Hansen et. al (35).

Chromatin is formed by an array of nucleosome core particles (represented by blue circles) connected with linker DNA (represented by the red line) with the help of linker histones Depending on environmental conditions the 10-nm fibre can stretch and take a conformation beads-on-a-string or can be free in solution and take an extended zigzag conformation (35).

The chromatin structure opens (euchromatin) and closes (heterochromatin), allowing the expression or silencing of certain genes, respectively, depending on the biological circumstances of the cell (12,13,34,36). Post-translational modifications (PTMs) of histones play a key role in regulating this complex structure and ultimately gene regulation (36,42). Due to this role in gene regulation, PTMs, can be related to pathogenic process as carcinogenesis and physiological process such as ageing (13,34,36).

More than eight types of histones PTMs have been discovered trough different approaches, such as mass spectrometry (MS), antibody-based detection techniques, and metabolic-labelling studies (20,36,42).

A histone PTMs consists in a chemical change made to the amino acid, that and can be both nonenzymatic or enzymatic (36,42).

These PTMs locations influence the structure fluctuation on the nucleosome (43). These chemical alterations can happen in the histones N-terminal tails regions and in the globular domain of histones (34,43). So far the best-studied modifications are the modifications that take place at the N-terminal tails (34,37). These N-terminal tails are important in, stimulating chromatin globule formation and interactions established between nucleosomes (35,37).

Histones globular domain can also suffer PTMs, within globular domain, the lateral surface (for example near the dyad region or the DNA entry/exit) shows a large number of

modifications sites (34,37). The lateral surface, which is the outer region of the histone octamer, comprises the region beneath the DNA, therefore changing the nucleosome dynamic and structure and modulate process such as transcription (34,37) (Fig. 6).

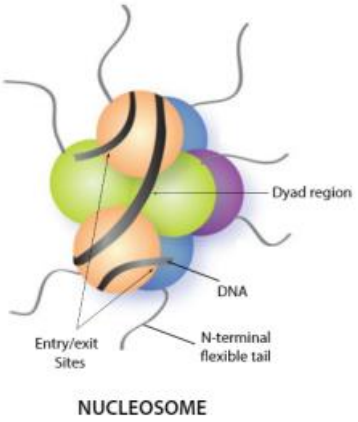

Figure 6 - Nucleosome structure highlighting the globular domain and N-terminal tails. Adapted from Kebede, et. al (34).

Histone modifications comprise acetylation, phosphorylation, methylation, SUMOylation, ADP ribosylation, ubiquitination, among others (2,6,12,34–37,43).

Acetylation is the best studied PTM that functions *in vivo* and influences the chromatin structure, regulating chromatin accessibility. Overall, high levels of histones acetylation are directly linked to euchromatin (12,35,43).

Methylation consists in an addition of a methyl group mainly onto a lysine or arginine amino acid, but the methylation of other residues can also occur in mammals (44–46). Effects on gene activity of histones methylation depend on the specific residue which is modified, the degree and pattern of methylation and the genomic context where methylation occurs (45).

Ubiquitination consists in an isopeptide bond between a terminal glycine of ubiquitin and the ε-amino group of a lysine residue of a protein (47,48). Histone SUMOylation is a reversible PTM, which results from covalent attachment of the small ubiquitin-like modifier (SUMO) protein to proteins, altering their functional properties, especially modifying their binding to other proteins (49,50).

The majority of sperm histones are removed and replaced by highly basic proteins, called protamines, during spermatogenesis. Despite this information, histone modifications are

an important mark in transgenerational epigenetic inheritance, due to several potential mechanism which allows histone modifications to be maintained across generations (2,8). Histones can be replicated by a semiconservative method, the nucleosome can be removed at the replication fork and immediately reapplied to alternating daughter strands, histones could be added to specific newly synthesized DNA from a pool of pre-modified histones, these are some examples of the potential mechanism involved in inheritance of histone modifications (2). Although the specific inheritance mechanism is still very elusive, there is already some studies proving the correlation between histone modifications and transgenerational epigenetic inheritance (2).

4.3. Non-coding RNA

Over the past decade, various studies have reported the importance of ncRNA regulating gene expression, and next-generation sequencing technologies together with bioinformatic analyses have shown the transcription of an unexpected variety of RNA molecules (51). 60% of the transcriptional production of the human genome correspond to ncRNA, which plays a role in regulating gene expression (52), genome stability (53), development (54), differentiation (55) and defence against foreign genetic elements (2,56,57).

NcRNAs are untranslated RNA molecules that regulate gene expression through multiples pathways (58). For example, ncRNAs can interact with chromatin and cause gene silencing due to direct interaction with epigenetic factors (58). Thereby the cellular epigenetic landscape is influenced by ncRNAs (59).

This diverse group of ncRNA includes two subgroups based on their length: long non-coding RNAs (lncRNA) which have more than 200 nucleotides and short non-coding RNAs (sncRNA), that contain less than 200 nucleotides (2,12,59,60). SncRNA can also be divided into various classes: micro RNAs (miRNA), small-interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), transfer RNAs (tRNAs), tRNA-derived small RNAs (tDRs) and small nucleolar RNAs (snoRNAs) (2).

PiRNAs are short non-coding regulatory RNAs and are overall, the most diverse class of regulatory RNAs (61). These ncRNAs form a complex with PIWI protein, often called piRNA-induced silencing complex (piRISC), and are usually present in both vertebrates and invertebrates (61). They are different from miRNA: in their length, piRNAs have 24 to 32 nucleotides of length; they lack sequence conservation and have independence from DICER

biogenesis (61,62). PiRNAs are derived from the RNA transcripts of transposons, protein-coding genes and specific intergenic *loci* (61). PIWI proteins are a part of the same clade as the Argonaute (Ago) family, which are mostly expressed in the germline (61,62). These PIWI proteins have two RNA-binding domains which are the N-terminal PAZ and C-terminal Piwi domain, this last one has endonuclease activity that will allow the cleaving of RNA (Fig. 7) (61).

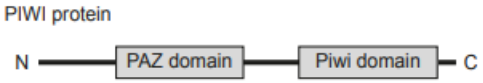

Figure 7 – RNA-binding domains of PIWI Proteins. From Kim (61)**.**

The specificity of PIWI-piRNA is provided by the piRNA sequence that allows the recognising of RNA targets through base-pairing complementarity, while the PIWI protein functions as the effector (61,63–65). The PIWI-piRNA complex can silence its targets at two levels: transcriptional gene regulation (TGS) or post-transcriptional gene regulation (PTGS) (61,62). Gene silencing at the transcriptional level is achieved due to the recruitment of repressive chromatin modifications to genomic target *loci* and *de novo* DNA methylation (61). At the post-transcriptional level, gene silencing is mediated by the cleavage of targeted mRNA transcripts by PIWI's endonuclease activity (61). (Fig. 8)

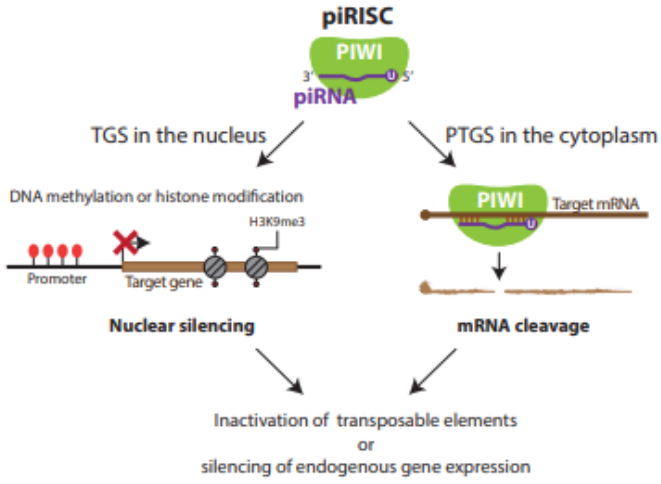

Figure 8 – Molecular mechanisms of the PIWI-piRNA complex. From Kim (61)**.**

miRNAs are small and single-stranded molecules of about 22 nucleotides bases, which are encoded by exonic regions or intronic regions of coding or non-coding transcripts (51,57,58).

In mammals, miRNA is loaded onto a member of the Ago protein family, forming a big ribonucleoprotein effector complex, named RNA-induced silencing complex (RISC) (51,57,58). The binding of miRNA to Ago protein helps to stable the strand (57).

miRNA can regulate the expression of the mRNA at post-transcriptional levels, frequently by binding to a small complementary sequence usually located in the 3′ untranslated region (UTR) of the mRNA (51). If there is a complete homology of the miRNA sequence and the target mRNA, this can be cleavaged by an Ago protein to degrade the target mRNA (57). In the case of an incomplete homology, the RISC complex can induce translational repression (57).

PTGS and mRNA elimination allow miRNAs to control the epigenome due to the downregulation of key epigenetic modifiers and to change the chromatin landscape (58,66). Examples of miRNA-interacting epigenetic factors include HDACs, HMTs and DNMTs (58,66). In addition to this, miRNAs themselves can also be influenced by epigenetic modifications. For example, CpG islands that are present at gene promoters, are also found in approximately half of all miRNA genes, which can suffer aberrant DNA methylation and deregulated expression (58,66).

MiRNAs interact with the other families of regulatory RNAs, the lncRNAs (51). Such as mRNA, lncRNAs are usually spliced, polyadenylated and relatively stable (60). In contrast to miRNAs, lncRNAs they are very different in size and have only a few structural and mechanism features in common (57). Most of these lncRNAs are transcribed by RNA polymerase II from intergenic regions, exonic or distal protein-coding regions, and a broad fraction are generated by divergent transcription upstream and antisense of mRNA promoters (51,57,60). Based on their function mechanism they can be divided into three types: competitor, recruiter or miRNA precursor (57,67).

As mentioned before, lncRNAs are a wide class, which includes many molecules with different functions (51). The action modes of lncRNAs depend on their subcellular localization: nuclear lncRNAs are principally involved in the epigenetic gene regulation or the maintenance of the nuclear architecture, on the other hand, cytoplasmic lncRNAs are mainly involved in post-transcriptional gene regulation (57). Interestingly, lncRNA can also regulate gene

expression by acting as miRNA sponges, which means that lncRNA may sequester miRNAs and influence their ability to modulate post-transcriptional silencing (51,57). (Fig.9) LncRNAs have physiologically important roles such as X-chromosome inactivation, imprinting and the general remodelling of the chromatin landscape (58,67).

Recently, it was discovered another interesting class of lncRNA, the circRNAs, which can influence gene regulation (51,57). CircRNAs are single-stranded RNA molecules, in which the 3′ and 5′ ends are covalently linked originating circular molecules, that avoid exonucleolytic degradation by RNase R (51,57). These ncRNAs can mediated gene regulation by various pathways such as sponging miRNAs or interact with proteins (57). (Fig. 9)

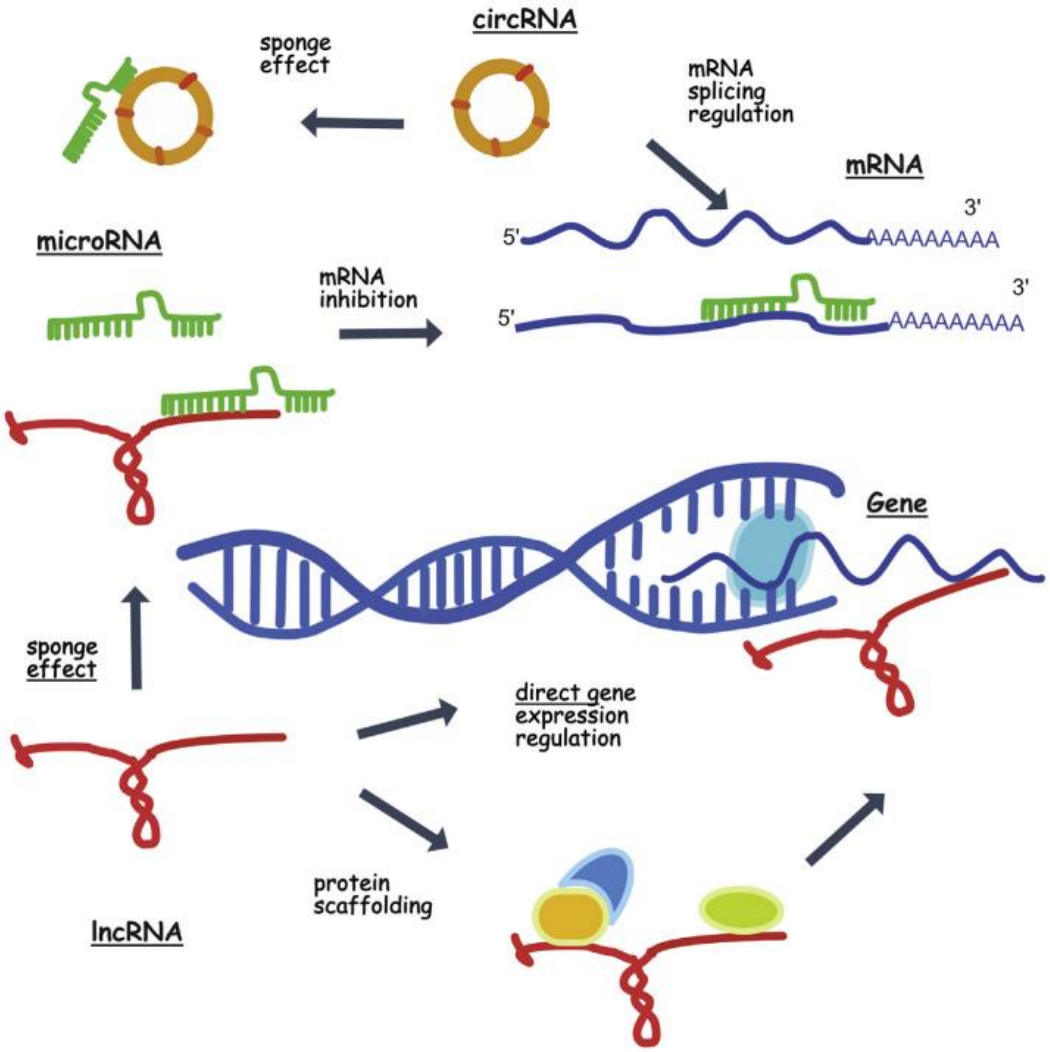

Figure 9 – Modes of action of ncRNAs in gene regulation. From Panni et al (51)**.**

MiRNAs (green) and lncRNAs (red) can directly influence gene expression by interacting with mRNA (miRNAs), to the gene/nascent transcript (lncRNAs) or histone modifiers (lncRNAs).

CircRNAs (orange) influence gene regulation expression through mRNA splicing. Furthermore miRNAs, lncRNAs and circRNAs can also regulate each other due to the sponge effect.

A high number of ncRNAs from different classes have been identified in growing oocytes (6). Despite spermatozoa having a condensed nucleus with little cytoplasm, a considerable portion of ncRNA was found in sperm (6,68,69). Thereby a substantial portion of ncRNAs both maternally and paternally is transmitted, indicating that it could be involved in the transmission of acquired phenotypes (2,6). However, how these ncRNAs could act in the embryo to regulate early development is still unclear (5). Different environmental stimuli, such as nutrition, physiological stress, can influence the quantity and composition of ncRNAs in the germline, therefore disturbing embryogenesis and leading to phenotypic changes (5).

Examples of intergenerational transmission of small RNAs in mammals with clear implications to offspring phenotypes involve miRNAs and tRFs (2,5,6). The inheritance of these small RNAs is restricted to 3-5 generations (2). A molecular characterization of how this transgenerational clock works is necessary for a better understanding of how non-genetic information is transmitted through small RNAs (2).

An example of intergenerational transmission of metabolic phenotypes mediated by ncRNAs is the tRNA methyltransferase, DNMT2. The tRNA methyltransferase, DNMT2, might have a major role in the transmission of paternally acquired metabolic disorders in mice, partly through its enzymatic activity (70). The elimination of mouse DNMT2, inhibits the elevation of RNA modifications (m5C) in small RNA fractions of sperm, which are induced by a high-fat diet (HFD). Offspring generated from oocytes injected with RNA sperm from fathers submitted to HFD with the deletion of DNMT2 resulting in reduced phenotypes associated with HFD-induced metabolic disorders (70). It is still unknown if the loss of tRNA methylation can have broad, indirect effects on another RNA species present in the sperm (70).

5. Epigenetic Reprogramming

Generally, the epigenetic marks are maintained across generations in somatic tissues, being a crucial component of the cellular memory mechanism (71). Nonetheless, both the chromatin structure and DNA methylation display dynamic changes during development (4,71). In order to achieve the totipotent and pluripotent state, creating an appropriate environment for the development of next generations is necessary remove the environmental marks, diminishing the possibility of memory inheritance (1,4,6,71,72). However, it is now known that, although the erasure of epigenetic marks is extensive, it is not complete (1,6). Thereby allowing the intergenerational and transgenerational inheritance of environmental modifications (1,6).

Since 1987, Monk and colleagues discovered the existence of two waves of DNA demethylation and remethylation, which affects the entire genome, during the embryo development (73,74). (Fig.10)

The first wave of reprogramming takes place during developing germline, specifically on the primordial germ cells (PGCs) residing on the genital ridges, which undergo a genome wide demethylation, and subsequent establishment of germ cell-specific epigenomes (5,71,72). The PGCs DNA demethylation occurs in two different moments (71,75). In the first phase, results in genome wide demethylation, at approximately all genome sequences (71). Passive demethylation is the primary responsible for this process, owing to a deficiency in major components of the methylation machinery (71). The second phase is responsible for demethylation at specific *loci*, such as CpG islands of the inactive X chromosome in females, imprinted genes, and germline-specific and meiosis-specific genes (71,72,76,77). Active demethylation is the process that occurs at this phase, due to the protection of passive demethylation at this *loci* (5,71). At this point, the epigenome contains the lowest amount of epigenetic information (71,72).

After this demethylation, male and female germ cells suffer remethylation of the genome at different times during gametogenesis, including acquisition of new epigenetic information and gender-specific re-establishment of imprinting in gonads (5,71,72,74). The male germline suffers remethetylaion during mitotic expansion in the developmental gonad, and the methylation patterns are established by the time of the birth (71,78). On the other hand, methylation levels of primary oocytes before birth persist low, and the remethylation occurs after birth in the oocyte growing phase (71,78). In addition, to this asymmetry of remethylation

time, the extent and distribution of DNA methylation is also very different between the genders (71). Oocyte genomes have lower methylation levels (~40%CpGs), with these methylation marks being restricted to intragenic regions of active genes, although sperm genomes are broadly methylated (~90% of CpGs) except CpG islands (71,72,79).

The second wave occurs during early embryogenesis, which includes the elimination of most DNA methylation inherited from the gametes in PGCs during preimplantation embryos and establishment of new methylation patterns upon implantation (71).

In the zygote, the maternal and parental genomes are spatially separated, with maternal genes having reduced methylation levels than the paternal genome (71). Few hours after fertilization the paternal pronuclei suffers a genome-wide loss of 5mC affecting single copy genes and also repetitive elements. This process occurs later in maternal genome (4,72). Notwithstanding, not all the genomic *loci* are reprogrammed with same efficiency, some retrotransposons and differentially methylated regions (DMRs) of imprinting control regions (ICRs) escape these wave of demethylation, thereby allowing the transmission of epigenetic memories through generations (4,5,72). Some studies suggest that parental genome reprogramming is mediated by both, active and passive demethylation (71,80–82). In contrast, the maternal genome is protected from TET-mediated 5mC oxidation, therefore the loss of methylation patterns mainly results from DNA replication-dependent dilution during preimplantation development (71).

After implantation, a wave of *de novo* methylation takes place in the epiblast, in order to settle a new methylation landscape and start a new life cycle (71). *De novo* methylation patterns are catalysed by DNMT3A and DNMT3B (71). A study shows that a DNMT3A KO mice can survive until birth, but become sick and die within four weeks after the term, inactivation of DNMT3B culminates in embryonic lethality, and embryos lacking for both DNMT3A and DNMT3B exhibit a more severe developmental phenotype and die earlier than DNMT3B KO embryos (83).

Parental genomes, not only, undergo a DNA demethylation, they also experience active chromatin remodelling and erasure of histone modifications (4,8). This is particularly clear in the male germline (4,8). In human spermatogenesis, 85 or 86% of histones are replaced by protamines culminating in a tenfold compaction (4,84). Most of histone modifications are lost during this process, whereas the histones that are not replaced by protamines are believed to codify genes expressed in early development (4). This extreme dynamics of chromatin and

histone modification, makes them less probable to operate as a mechanism of transgenerational inheritance (4).

Lastly, it exist an intriguing asymmetry between the parental genomes, which remains ununderstood (4,71,72). As mentioned before, the timing of *de novo* methylation in PGCs and demethylation after fertilization are different between the genders (4,71,72). In male germline, histones are replaced by protamines, while female genome maintains histones methylation (4,8,72). This asymmetry can explain why some features are only transmitted thought one parental line and the sex-differences effects (4).

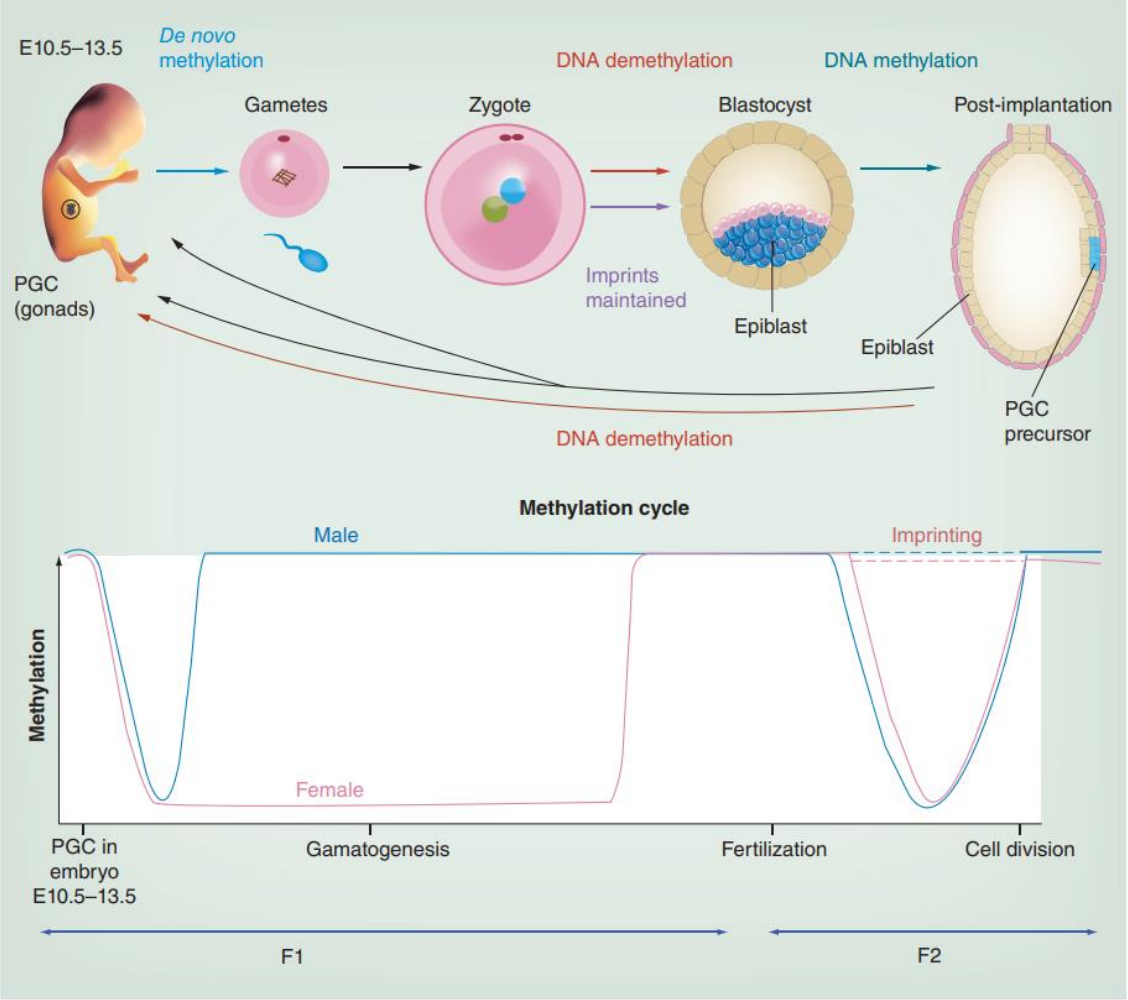

Figure 10 – Epigenetic reprogramming during mammalian development. From Nagy et. al (4).

5.1. Escaping Intergenerational Reprogramming

In mammals, the marks that escape the two waves of epigenetic reprogramming during development represent, a plausible carrier for inheritance of transgenerational information (6).

The inheritance of epigenetic marks and epigenetic reprogramming represent two sides of the same coin (6). In light of epigenetic reprogramming the central question is how, certain epigenetic marks (in the form of histone modifications and DNA methylation), can escape this prototypical intergenerational reprogramming (4).

Imprinting genes are a classical example of genome sequences that can escape the wave of demethylation that occurs in embryos, thereby transmitting epigenetic information to the progeny. Dr. Barlow was one of the researchers that discovered imprinting genes, which are expressed and regulated in a parent-of-origin-specific manner (28). Imprinting genes show monoallelic expression, due to epigenetic silencing of the inactive allele involving DNA methylation (5,85). Most of the imprinting genes are located in clusters, which are regulated by ICRs (28). Deletion of these regions or perturbating DNA methylation of these ICRs can lead to loss of imprinting and were shown to account for various diseases, such as Prader-Willi and Angelman syndrome (28).

The first study to document intergenerational inheritance was the classic experiment of agouti yellow mouse (Avy) (4,86). If a repeated element named an intracisternal particle A (IAP) inserts itself upstream of the agouti *locus*, can account for obesity and altered coat colour (86). Like imprinted genes, IAPs resist the wave of demethylation in developmental embryos and this DNA methylation is inversely related to IAPs transcriptional activity (2). This epiallele seems to be passed along the maternal germline but not the paternal (86). Although the insertion of the IAP it is not random, it does not result from the effects of environmental factors (87). Nonetheless, after the insertion of the IAP, environmental factors can influence the methylation of IAP (86). This argument has been demonstrated by changing the food source of pregnant dams (86). Diets rich in methyl groups, can swich off the Avy *locus*, through the methylation of the promotor region of IAP (88). Feeding the mother with a diet-contain supplemental methyl groups increases the hypothesis of born pups developing burnish coats and healthy constitutions, due to the silencing of the IAP (86). It is an example of a direct environmental exposure on the germline, and thus the result of parental influences, more exactly named intergenerational inheritance (4).

6. Phenotypic plasticity and genetic accommodation

The relation between genotype (genes that are inherited) and phenotype (the target of natural selection) is influenced by environmental inputs on gene expression, phenotypic integration, and trait development (89).

Phenotypic plasticity is what enables a single genotype to originate different phenotypes, depending on the environmental stimuli, during individual development (94,95). Plasticity allows an organism to adapt to distinct environmental conditions, and it is very important for species survival, for example in climate alterations and in colonizing new environments (93,94). In the animal kingdom, phenotypic plasticity has been demonstrated in distinct contexts, such as food availability, social interactions, predator avoidance and seasonal changes (94). Plasticity can thus generate a broad pool of phenotypic diversity, even inside the same population (94). This environmental-induced flexibility can happen in any trait such as morphology, life history, and behaviour (94).

Phenotypic plasticity can be depicted through the term of a norm of reaction, where the level or value of a phenotype generated by a particular genotype can be represented as it changes across environments, such that, the slope of the norm of the reaction, represents the degree of plasticity, for instance, a steeper slope means greater plasticity (90,91)

Not all phenotypic adaptations can lead to a phenotype with increased fitness and with more chances to survive. Therefore, it is important to distinguish the plastic responses between adaptive and maladaptive plasticity (94,97). Adaptive plasticity is when a phenotypic response to a certain environment, leads to increased fitness and moves the phenotype closer to the environment-specific optimum, favoured by selection (94,97). This adaptive plasticity can lead to two different scenarios (97). For a specific feature, if plasticity alone is sufficient to reach the mean phenotype to the optimum of a newly colonized environment, then this variation induced by the environment, prevents an opportunity for selection, to produce genetically-based adaptation (97) (Fig. 11.B). On the other hand, the plastic response can shift the mean phenotype closer to the new optimum, then selection on genetically-based trait changes reaches at this new value of optimum (97). Otherwise, plasticity maladaptive happens when a phenotype derived from the environment stimuli, confers no increased fitness and moves the mean phenotype further from the optimum, which leads to the selection pressure to counterattack the environmentally induced effect (94,97) (Fig. 11.D).

The process by which the environment-induced plastic responses are selected, results in inheritable variation, influencing the allele frequency and expression of these phenotypes, is named genetic accommodation (98). Genetic accommodation can lead to reduced plasticity, resulting in genetic assimilation or increased plasticity of the trait (including the formation of polyphenisms) (98). Genetic assimilation consists in reduced or complete loss of the plasticity of the trait, either from selection against developmental machinery underlying plasticity or because of relaxed selection when alternative environments are not frequently encountered (95) (Fig. 12.B).

Genetic compensation is a process whereby selection on genetically-based trait variation tries to compensate for a maladaptive plastic response (97). This process can involve canalization of the ancestral phenotype (the slope of the reaction norm changes, but not the Y-interception)(97) (Fig. 12.C). The phenotype will confer a highest fitness and in a long term, results in loss of plasticity (97). In contrast, genetic compensation can happen without canalization, where it exists an adaptive shift in the reaction norm without loss of plasticity (the Y-intercept of reaction norms modify, but not the slope) (97) (Fig. 12.C).

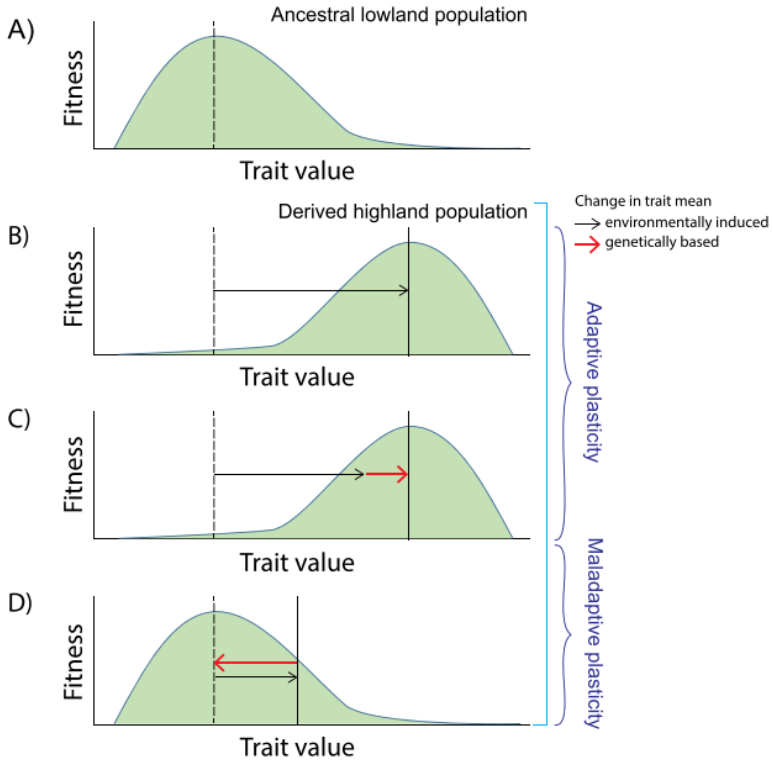

Figure 11- Adaptive *versus* maladaptive plastic response. From Storz at al (92)**.**

The green curve indicates the relationship between trait values and fitness for that population (93)

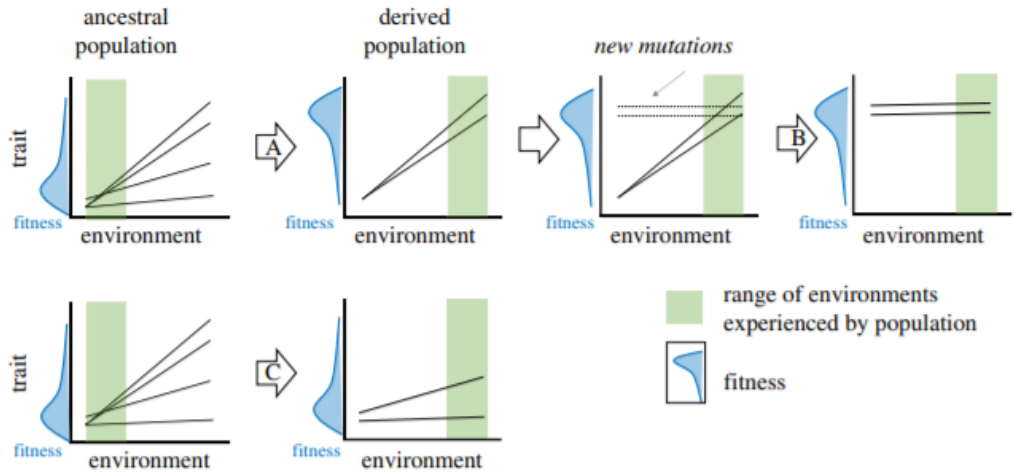

Figure 12 - Evolutionary changes in phenotypic plasticity. From Kelly (93).

This graph represents the different processes involved in evolution, which are genetic accommodation (A), genetic assimilation (B), and genetic compensation, with and without canalization (C). The plots display the hypothetical changes in reaction norms before and after a shift in an environment, succeeding natural selection (93). Green boxes show the spectrum of the environment experienced by a population, and the blue curve indicates the relationship between trait values and fitness for that population (93).

A classic example of phenotypic plasticity and genetic accommodation is the hypoxia adaption of high-altitude vertebrates (89,92). Lowland humans show a plastic response after being exposed to a highland environment, changing respiratory (for example, hyperventilation), cardiovascular (for instance, increased heart rate) and metabolic traits (for instance, suppression of digestion)(89,92). Sometimes maladaptive responses may occur in the ancestors who never experienced this environment before (92). In this case, the sensing of reduced cellular O_2 can be misread as an issue related to pulmonary O_2 exchange or convective O_2 transport (92). These maladaptive responses can lead to hypoxic pulmonary hypertension, which in turn can impair O_2 uptake and in more extreme cases, give rise to life-threatening pulmonary edema, right ventricle hypertrophy and heart failure (92,94). This disadvantage is mitigated by genetic compensation (92).

The epigenetic modifications and plasticity are intimately related since both of them can change gene expression without modifying the genetic sequence (89). Drawing a line between these two terms is impossible: a great number of the epigenetic marks are environmentally-induced, plasticity itself can be mediated by epigenetic mechanisms, and transgenerational

(maternal) effects may be due to maternal plasticity (for instance, provisioning) or to epigenetic inheritance (89).

Many scientists defend that phenotypic plasticity, provide another mechanism of adaptation in addition, to mutations (95). In contrast, some scientist argue that plasticity shields the genotype from the direct effects of natural selection (96). Nonetheless, the role of the phenotypic plasticity in shaping evolution is a topic of longstanding interest and with increase relevance (92,95).

7. Stress as environmental stimuli for epigenetic marks

Epigenetic modifications enable organisms to adapt to extreme environmental conditions without mutating the genome (2). These epigenetic marks will passe on to the next generation allowing their progeny to survive in the same conditions as their parents (2).

Epigenetic marks are dependent on lifestyle and environmental factors, resulting in adaptive or maladaptive responses (97). Behaviour, nutrition, exposure to toxins and pollutants are some of the environmental stimuli that are associated with epigenetic modifications (97). Traumatic stress is an important environmental stimulus that can impact cognition, behaviour and physiological functions such as metabolism, across several generations (98).

In biological or medical background, stress is defined as a mental, physical, or emotional factor that can be acute or chronic, giving rise to body and/or mental tension (98). Stress can be an organism's response to an internal (physical illness or injury) or to an external (environmental or psychological) trigger (98). Acute stress is a robust physiological condition due to an exposure to a novel and unpredictable threatening event (98). While chronic stress results from the repetitive and robust enough stimuli, inducing a persistent response, usually more than a week in mice and more than a month in humans (98). Traumatic stress is a particular type of stress, which can be acute or chronic, due to shocking and emotionally overwhelming situations, commonly associated with a threat to one's physical or personal integrity (98). In humans, traumatic stress can be divided into two groups, acute stress disorder (ASD) or post-traumatic stress disorder (PTSD) if the symptoms persist over a month (98). Childhood trauma, such as physical or sexual abuse, deprived parental care, natural disasters, or forced displacement, are important triggers of traumatic stress (98).

Traumatic stress affects the integrity of the hypothalamic–pituitary–adrenal (HPA) axis (98). The HPA axis is responsible for releasing the corticotropin-releasing hormone (CRH), which in turn stimulates the release of adrenocorticotropin hormone (ACTH) from the pituitary (98,99). ACTH will stimulate the release of cortisol by the adrenal gland and other factors, such as angiotensin II, various cytokines and inflammatory mediators (98,99). Individuals with PTSD have gross changes in the HPA axis, including a constant release of CRH, blunted negative feedback of ACTH on CRH release, and hypercortisolism (98). The amygdala and hippocampus will also stimulate and provide critical inputs to the HPA axis (98,99).

It is important to note that the effects of traumatic stress on the brain are pleiotropic and are moderated by complex signalling pathways besides the HPA axis (98). Traumatic stress can

also affect the function and expression of mineralocorticoids, which influences the regulation of basal HPA axis activity, stress resilience, and traumatic memories (98). Oxytocin and arginine vasopressin are also involved in traumatic stress since these stimuli dysregulate the balance between these hormones, which have an important role in regulating social and emotional responses (98). Interestingly, the serotonergic pathway is involved in the susceptibility to long-term effects of traumatic stress (98). Besides, catecholamines and the noradrenergic system are believed as well, to underlie the development of PTSD after trauma exposure (98).

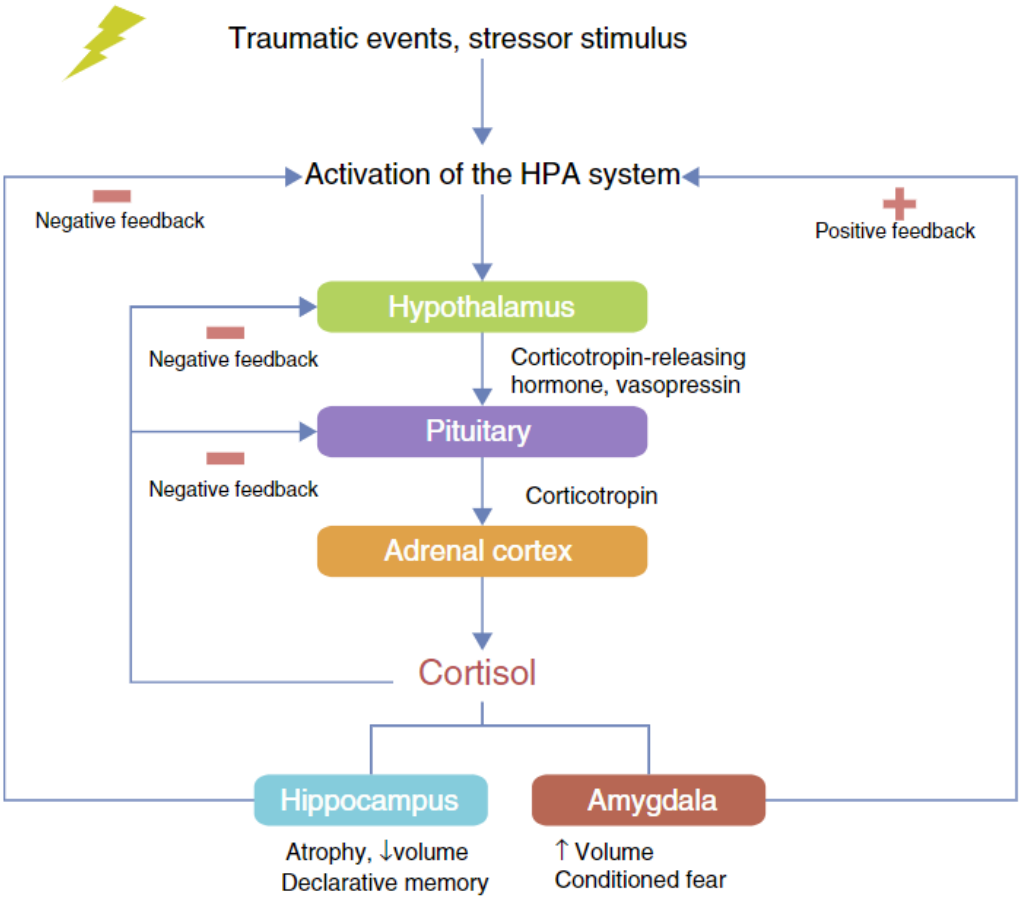

Figure 13 – Consequences of traumatic stress in HPA axis. From Guillén-Burgos (99).

8. Sex-specific effects

New meta-analyses of phenotypic data performed in mice shows that most of the mammalian features are influenced by sex (100,101). These sex-specific differences can be affected by parental stress and may vary depending on stress type as well, as on the behavioural phenotype present on the offspring (100,102). Furthermore, the results of studies are difficult to interpret, since many of these phenotypes have only been examined in the male offspring, probably to avoid the influence of the female oestrous cycle on the variability of the results (100,102). Thereby, it is vital to examine the two sexes in transgenerational studies, since in some cases the parental epigenetic effects on the offspring may be sex-specific (100).

A study carried out in mice about maternal separation with unpredictable maternal stress (MSUS) exhibits an increase of affective behaviours and a reduction of social memory in female offspring, while male offspring results in reduced sociability (100,103–105). These discoveries are in accordance with the model of maternal separation for early life stress, which has been demonstrated to affect males and females in different ways (100). Two studies performed in humans show that mothers with diabetes, obesity and/or overweight during pregnancy have more possibility that their offspring will bear neurodevelopmental disorders, including autism, schizophrenia, and attention-deficit/hyperactivity disorders (106,107). Nonetheless, it is intriguing that male offspring are more susceptible to developing these neurodevelopmental disorders (103). A study done in humans has shown that paternal depression is correlated with increased depression of adolescent males in comparison to girls, while maternal depression affects equal sexes (108). Together, these studies, performed both in mice and humans, demonstrated that different types of stress can lead to distinct sex-dependent responses of the progeny (100).

Several explanations have been suggested to clarify this sex-specific effect. Chromosomal differences between the sexes can affect the levels of gonadal hormones which, in turn, influence many stress-related mechanisms, including the HPA axis, as well differences in epigenetic plasticity during gamete maturation may contribute to these sex differences (6,100). Further, the X chromosome has essentially miRNA genes while the Y chromosome has none, these can affect sexual dimorphism since miRNAs genes are involved in epigenetic transmission and males receive only one X chromosome from their mothers (in contrast to females, who receive two X chromosome, but one of them is inactivated to obviate gene dosage effects) (100). The potential mode of transmission can too affect this dimorphism. Maternal

transmission relies on the complex maternal-fetal/neonatal interaction, in which parturition, lactation, and early maternal care may impact stress sensitivity in future generations (109). While the paternal transmission depends on the epigenetic marks carried by the sperm (109). In addition, a few sex-specific epigenetic-inheritance effects that have been described in mice, are incompatible with XY transmission (6).

Although this sex-specific effects demonstrated in some rodents, present an intriguing possibility that parental experience influence sex differences in stress responsivity, in humans, disease risk and the lack of this effect in other models contradict this hypothesis (109). It will be necessary that further studies of behavioural and physiological phenotypes, both in male and in female progeny, to clarify potential sex-specific vulnerabilities and truly understand the mechanism that are underlying these sex differences (109).

8.1. Gender and sex contributors to sex differences in PTSD

A general study performed in both sexes in the human population shows that the heritability of PTSD is 46%, though in the all-female studies the heritability was 71% (110,111). The sex differences in PTSD are well-established (112). Besides women's being more predisposed to PTSD, also tends to have more severe, chronic and higher comorbidity rates, albeit women tend to respond better to the treatment than men (112,113). Although relatively little research was done to understand how sex differences in PTSD emerge, it is believed that many factors are responsible, such as sociocultural gender and biological sex (112). Considering the potential impact of sex and gender, before, during, and after the traumatic event, it will be necessary a greater understanding of how much sex and gender differences appear, which, in turn, could improve the quality of life of men and women with PTSD and reduce the increased personal, social, and societal costs of this disorder (112).

The gender-related factor that has been more studied is masculinity in male combat-veterans (112). Masculinity may increase the risk of PTSD through distinct ways, refusing to accept psychological trauma and by inhibiting cognitive-emotional processing of the trauma, which prevents man from seeking help (112,113). In addition, affective suppression can cause underreporting of symptoms; toughness, and action-oriented behaviours are likely to increase confrontation with trauma-related triggers and consequently extinction of fear responses (112). One study examining masculine ideals shows that men believing they must possess self-reliance, toughness, restrictive emotionality, avoidance of femininity, dominance, and contribute to sex, was linked with higher PTSD levels in male veterans (114). The only study

that evaluates femininity concludes to be unassociated with PTSD in Latina women in the United States of America (115).

It has long been believed that gonadal hormones contribute to these differences in PTSD outcomes since those gonadal hormones are influenced by stress hormones, dysregulating fear circuitry, giving rise to PTSD (112). Testosterone has been linked to anxiolytic effects, probably due to reducing HPA axis reactivity to stress and trauma (112,116). Increased levels of testosterone are present in competitive situations, such as judo competitions and professional basketball games, probably initiating approach motivation and reducing fear (116).

A variation on oestradiol and progesterone levels influenced by the menstrual cycle has shown to affect many neurotransmitter systems and the HPA axis response to stress (112,117). One study evaluated the variations of psychological symptoms in women with and without PSTD, during the menstrual cycle (117). Results have shown that exists a variation in PTSD symptoms throughout the menstrual cycle, particularly depression and phobic anxiety during the early follicular phase (117).

Lastly, gonadal hormones can also interact with behaviour through epigenetic modifications, leading to sexual dimorphism (112). Sexual differences interesting to stress response and PTSD development have been identified at a molecular level (112). One of the epigenetic marks that is linked to PTSD is DNA methylation, that usually occurs near the binding sites of the oestrogen receptor (112). A study has demonstrated that oestradiol interacts with ADCYAP1R1 leading to activation of this gene, which is believed to be important to healthy stress response and has been linked to PTSD (118). The ADCYAP1R1 codifies for PAC1, which works together with PACAP to activate cortisol production, and therefore regulate the response of the HPA axis to stress and trauma (118). This study concludes that high levels of oestradiol were linked with the intensification expression of ADCYP1R1, which leads to the augmentation of PTSD symptoms (118).

These findings show that epigenetic marks are not only responsible for the heritability of PTSD, but also for the sex differences in PTSD outcomes (112).

In summary, gender and sex can influence PTSD symptoms through different pathways, such as hormonal influences, genetic predisposition and individual gender roles (112). The first step towards specialized treatments is to recognize these variables for PTSD symptoms, which in turn will lead to more effective treatments (112).

9. Post-traumatic stress disorder

PTSD is an anxiety disorder unleashed by an exposure to a traumatic event, which will be developed in a minority of individuals who experience this type of stress (109). Diagnostic and Statistical Manual (DSM-5) define a traumatic event as an exposure to actual or threatened death, serious injury, or sexual violence that is experienced directly, witnessed, experienced vicariously through family or close friends, or experienced repeatedly or with extreme exposure to aversive details of the traumatic event (110). DSM-5 classify PTSD as a "trauma and stressor-related disorder", and is characterized by four symptoms: avoidance of stimuli associated with the trauma; negative cognitions and affects associated with the trauma; re-experiencing (for instance flashbacks, nightmares) hyperarousal symptoms and signs (119–121).

Exposure to a traumatic event is common in the general population, approximately 50 or 60% will experience at least one traumatic stressor, but only 10% of individuals are at a considerable risk of developing PTSD (111,119,122). The question that arises from this data is: why will some individuals develop PTSD after a traumatic stressor while others are resilient? (121). Numerous factors can influence the risk and the phenotype of PTSD, such as type and frequency, intensity, and recurrence of trauma; the age of the individual at the time of the trauma; differences in stress sensitivity and comorbid psychiatric (109,119). There has also been suggested that susceptibility can be inherited, due to epigenetic factors, which can increase or diminish the PTSD risk (119). A study estimated that the heritability of PTSD ranges between 30% and 70% in twin studies (121). The role of the environment experienced by the ancestor has been progressively emphasized, demonstrating that prior exposure to a traumatic stressor is one of the main contributors to later stress sensitivity and PTSD susceptibility (109). These increases in PTSD risk, as well as the behavioural and neuroendocrine consequences of stress exposure, have been reported not only in the individuals who experienced this stressor but also in their progeny (109).

Epidemiological studies performed in the human population suggest that PTSD is associated with depression and anxiety and is linked with a greater prevalence and incidence of dementia (98). This effect may be due to a decrease in hippocampal volume observed in individuals with PTSD (98). Apart from the harmful effects on the brain, traumatic stress during prolonged periods also affect other body functions (98). PTSD is a risk factor for cardiovascular and cerebrovascular diseases, gastrointestinal dysfunctions, rheumatoid arthritis, and cancer (98).

The first study to report transgenerational effects of parental lifetime stress in an animal model, was performed approximately half a century ago. The female rats were exposed to a stressor before mating resulting in altered offspring behavioural stress responses, increasing exploratory behaviour in a novel environment across two successive generations (123). However, these transgenerational effects are not confined to the perinatal period, parental exposure to a traumatic event during adolescence and adulthood can influence offspring stress-related behaviour and physiology (109). For instance, an experience performed in male mice subjected to chronic variable stress either over the pubertal period or only in adulthood results in blunted HPA stress axis response in male and female offspring, a stress phenotype that matches with that is observed in PTSD (124).

Other studies have modelled PTSD through fear conditioning in mice, which consists in associating an aversive stimulus (the trauma) with a neutral context or stimulus (6). An experiment using fear conditioning, conditioned F0 male mice to fear acetophenone by combining this odour with mild foot shocks before mating (125). The unexposed F1 and F2 generations, sired by conditioned F0 mice, showed an elevated fear learning of this odour (125). In addition, these alterations in behavioural sensitivity and neuroanatomics persisted after *in vitro* fertilization (IVF), cross-fostering, and through two generations, demonstrating that these behaviour and structural changes were epigenetically transmitted and were not socially inherited from the F0 mice (125). Sperm DNA of F0 and F1 were analysed and revealed the presence of CpG hypomethylation in the Olfr151 gene, which codifies a known odorant receptor (125), leading to a greater expression of this receptor (125).

Although there are various studies performed in animal models, the literature in humans is sparse (121). Epidemiological studies in humans can provide valuable insight in this area (121).

In conclusion, understanding the epigenetic and genetic bases underlying the vulnerability and resilience to a stressor it will help in the creation of preventive strategies in subsequent generations and new specialized therapeutic interventions for PTSD (111).

9.1. Epigenetics marks induced by famine

Several studies about famine show that experienced starvation results in development of increased risk of type II diabetes mellitus, cardiovascular disease, metabolic disorders, and decreased cognitive function in later life (97,126).

An epidemiological study about the Dutch Famine of 1944-1945 analysed the effects of starvation during periconceptional and late gestation exposure and subsequent health and developmental outcomes (127). The study has revealed that periconceptional exposure to the famine results in hypomethylation of the insulin-like growth factor II (IGF2) DMR, a gene related to insulin metabolism, six decades later, increasing the risk of type 2 diabetes (2,97,127). This result may be due to a deficiency in methyl donors, such as the amino acid methionine (127). It is important to note that other stimuli such as cold and emotional stress cannot be ruled out (127). In contrast, late gestational exposure was not associated with lower methylation levels of IGF2 DMR (127). The children conceived during the Dutch Famine also tended to have lower birth weights (127). The study shows that the periconceptional period and the first months of pregnancy have a considerable effect on disease risk (97).

Several epidemiological studies were carried in three Overkalix cohorts born in 1890, 1905, and 1920 in northern Sweden, exhibiting a link between the nutrition experience in parental obesity and cardiovascular disease (128,129). Interestingly, these studies show a sex-specific transmission, that only the paternal grandfather´s food supply was associated with the mortality risk of grandsons, whereas the maternal grandmother´s food supply was associated only with the mortality risk of granddaughter´s (129). These studies have also demonstrated that there exists an exposure-sensitive window during mid-childhood (the 'slow growth' period during the few years leading up to the prepubertal growth spurt) but not in late childhood (128).

9.2. Epigenetic marks induced by war

From the military to the population living in the conflict zones, individuals who experience war, are constantly exposed to traumatic events. Therefore, these individuals are more likely to develop epigenetic modifications related to stress, making them more susceptible to PTSD and other diseases.

Several studies have been done on Holocaust survivors to understand what the susceptibility of their offspring to PTSD is, and how this susceptibility can be inherited. A study has shown that parental PTSD has an important role in the development of PTSD in adult offspring of survivors with PTSD (130). This study has identified neuroendocrine alterations in the offspring of parents with PTSD (130). It has been demonstrated that progeny of the holocaust survivors with PTSD have significantly lower urinary cortisol excretion and salivary cortisol levels, as well as enhanced plasma cortisol suppression compared with the offspring of survivors without PTSD (130). Nonetheless, these neuro-endocrine alterations were negatively

correlated with the severity of parental PTSD symptoms (130). Another study performed in holocaust survivors, examined transgenerational methylation alterations of FKBP5, which can decrease glucocorticoid levels binding to the glucocorticoid receptor (GR) (131). Analysis of blood samples were done to obtain the quantification of FKBP5 methylation and cortisol levels (131). The analysis found significantly higher methylation of FKBP5 intron 7 in Holocaust survivors (131). In contrast, their offspring were found to have significantly lower FKBP5 intron 7 methylation levels (131). This difference in the methylation levels between the F0 mother and F1 offspring could be a biological accommodation (131). The higher methylation of the gene FKBP5 results in decreased FKBP5 expression and increased sensitivity of GR in the F0 mothers, which in turn leads to lower circulating glucocorticoid levels during pregnancy (131). This effect could increase demethylation in the fetus to optimize or increase glucocorticoid levels (131). If the stimuli does not occur during pregnancy and occurs in the preconception or postnatal period, low cortisol levels may further regulate FKBP5 methylation levels in offspring (131). However, this study cannot examine if the glucocorticoid programming in offspring is due to an intergenerational consequence of parental exposure or offspring recalibration of glucocorticoid regulation (131).

One study has analysed the clinical outcomes of the Tutsi genocide, which happened in Rwanda between April and June 1994 (132). This study shows that offspring of traumatized mothers are at a higher risk of developing PTSD and have a more severe form of PTSD (132). In addition, transmission of PTSD to the offspring was linked with transmission of biological changes of the HPA axis, specifically the blunted plasma cortisol levels (132). Methylation levels of the GR gene NR3C1 and the mineralocorticoid receptor (MR) gene NR3C2 were measured (132). Analysis shows that PTSD in F0 mothers results in hypermethylation of NR3C1 exon 1_F promoter regions of traumatized mothers and their children compared to unexposed mothers (132). Methylation of NR3C2 was not statistically significantly different (132). MR is a receptor with a higher affinity to cortisol, which allows the organism to maintain low basal corticosterone levels during circadian (132). In contrast, GR has less affinity to cortisol, which increases the levels of glucocorticoids during acute stress (132). Via these GR, glucocorticoids suppresses the neuroendocrine stress response through GR in the pituitary, hypothalamus and hippocampus (132). This study emphasises the methylation changes in individuals exposed to a traumatic event and the inheritance of these changes to subsequent progeny (121).

10. Epigenetic marks induced by toxins

Although exposure to alcohol, smoking and opioids, is not a stress caused by a traumatic event, the cellular stress that these toxins generate has been linked with epigenetic modifications in germ cells (109). Therefore, suggesting that molecular signatures in germ cells, besides parental behaviour and experiences, can transmit this information about the parental environment to their progeny (109).

Pre-conception paternal smoking has been linked with greatest prevalence of many disorders and numerous types of cancer and birth defects in offspring (133). Although cigarette smoke contains several mutagenic agents, it can also influence DNA methylation patterns at sperm cells, peripheral blood, buccal cells and lung tissue (133). One study has analysed the sperm of the individuals who smoke and compared them to non-smokers (133). Global methylation levels were not different between the two groups (133). However, the study has identified 141 differentially methylated CpGs in sperm from smokers compared with non-smokers (133). Other studies that have examined the methylation levels in peripheral blood, buccal cells and lung tissue discovered many more differentially methylated *loci* (133–135). Thereby, suggesting, that sperm may be less susceptible to these methylation changes compared with other tissues (133). Nonetheless, the marks that affect germ cells can be subsequently transmitted to their progeny (133).

One study about the effects of smoking in the progeny has shown an increased risk for asthma if the fathers smoked before conception compared to fathers that never smoked in their lifetime (136). This risk was greater especially when fathers smoked during puberty, emphasising a critical and vulnerable period of the sperm's life (136). Maternal smoking during pregnancy and birth was linked to increased early-onset asthma in sons, most pronounced for the non-allergic phenotype (136).

Several studies of opioid exposure have demonstrated the transgenerational effects, even in absence of continuous use (137). Adolescence maternal morphine-exposure results in behaviour modifications, altered response of the dopamine agonists and upregulated the dopamine- and opioid-related gene affecting the first (F1) and second (F2) generation (138). Another study examined the effects of maternal morphine-exposure, followed by a subsequent drug-free period of some weeks (139). Although this drug-free period, morphine exposure still results in induced anxiety and morphine sensitization in the offspring (139).

Alcohol consumption in parents is known to change offspring phenotype, leading to epigenetic modifications, in part by shifts in the reproductive function upstream of the germline development and maintenance (140). A study done in rats examined the DNA fragmentation in sperm-producing altered learning and anxiety-like behaviours in offspring – offspring phenotypes that were also affected in paternal alcohol multi-transgenerational models (140).

11. Environmental enrichment

Until now, it has been evidencing the negative influences of environmental stress in individuals. However, positive environmental influences can also induce epigenetic modifications, which, in turn, can be transmitted to subsequent progeny. A positive environment can include increased physical activity, environmental enrichment (EE), or other forms of enhanced motor, sensory and cognitive stimulation (100,141).

EE refers to the stimulation of the brain, through the social surrounding, and physical activity, which promotes brain plasticity (102). Several studies have demonstrated that EE can rescue the transgenerational influence of traumatic events (98,100).

A study performed in mice, examined the transgenerational effects of maternal separation with unpredictable maternal stress (MSUS), discovered the anxiety-like behaviour in offspring of male mice, who had suffered early life stress through maternal separation, was prevented by exposing the fathers to EE (142). The reverse of these effects of traumatic stress may involve a mismatch between favourable EE conditions and adverse conditions in early life (142). This reversal can also be linked with epigenetic alterations in the GR gene in the hippocampus, consistent with the hypothesis that EE and chronic stress recruit similar pathways within the hippocampus (142). Additional molecular pathways could also be responsible for these effects (142).

Three months of physical exercise in humans has shown to influence the DNA methylation profile in sperm, especially in genes that are responsible for some brain disorders, such as autism, schizophrenia, and Parkinson's disease, indicating that the physical activity can be genetically transmitted to offspring (143).

Early life EE has been shown to protect or remediate the effects of morphine-exposure in rats (144). A study showed that EE during adolescence has prevented the anxiety-like behaviours and dendritic retraction in the adult offspring of the rats exposed to morphine (144). These results suggest that early life EE interacts with brain development and influences individual differences in anxiety and depression (144). Nevertheless, the mechanism responsible for the reversion of anxiety and dendritic plasticity remains unknown (144).

The mechanisms responsible for environmentally mediated effects are complex, and some remain unknown (102). Together, these findings highlight the beneficial properties of EE in reverting the effects of adverse events and preventing them from being transmitted to

offspring (100). This suggests that education given to individuals and their social surroundings can ameliorate the effects of traumatic events (100).

This information can be used to develop new treatment strategies in populations with PTSD and other disorders, and also prevent consequences on subsequent progeny (142).

12. Future Perspectives

An increasing body of evidence has emerged showing that epigenetic modifications can occur and are most likely to be inherited. The mechanism of epigenetic modifications has been identified however, it still exists several questions and challenges to be conquered.

One question still remains unanswered, how is complex environmental information assimilated in germ-cells epigenome (100). In addition, how do these epigenetic changes alter the zygote development and specifically the neurodevelopment, leading to influences in offspring behaviour and function cognition (100). It is also crucial to understand how environmental triggers are transduced at cellular and molecular levels, which, in turn, affect the germ-cells epigenome (100). Some studies have proposed, that extracellular vesicles contain small ncRNA which can be responsible for this process (145).

Future research must focus, as well, in understanding how and why some *loci* are resistant to epigenetic reprogramming. Answering to all these questions will help us to better perceive the inheritance mechanism of epigenetic marks and to realize how transgenerational traits are transmitted (100).

Epidemiological studies in humans are relatively few and far between them, due to the longevity of human life. So, another relevant point is understanding how animal studies can be translated to clinical outcomes in the human population (100).

The studies performed in the human population also have some limitations, such as reduced sample sizes, methodological heterogeneity, and other confounding factors (which could have led to epigenetic changes) that were not accounted for by the studies (121). Many of these studies are cross-sectional, whereby causation cannot be inferred from these studies (121,146). All the samples of the studies are from peripheral tissues, mostly blood and saliva, however, we need to analyse the epigenetic marks in brain tissue (119,146). This limitation can be overcome by the creation of human *postmortem* brain collections (146).

Future studies must always analyse the specific time of the trauma, (some of the studies mentioned here have done this) if it was during puberty, adulthood, periconceptional or pregnancy (in pregnancy it may be important to know the trimester) (121). Another factor to take into account is whether the trauma was acute or chronic (121).

Lastly, research needs to be done to investigate the positive outcomes after trauma exposure, such as posttraumatic growth. Understanding these epigenetic changes, which makes

some individuals resilient to PTSD, could lead to the development of new treatment strategies (146). As well, EE needs to be studied, to figure out how this positive epigenetic modification can overcome the environmental trauma.

13. Conclusion

In the past, it was believed that the only contribution of parent's germ cells to their offspring was limited to genes. However, epigenetic studies revealed that environmental factors can also mark the parents' genome, which in turn is transmitted to their progeny.

Here it is analysed how these environmental-triggers modify our genome, through molecular pathways (DNA methylation, histone modifications and ncRNA). It is becoming increasingly clear that these epigenetic changes do not work alone, they are interconnected and form an epigenetic network. It was laid out the epigenetic reprogramming process, identifying the gaps in this epigenome reset, that allows these epigenetic marks to be transmitted across several generations. These transgenerational epigenetic marks are transmitted to offspring in an attempt to prepare the offspring for the challenging environment that the parents have experienced.

In this review our research was focused on the environmental stress, specifically in PTSD, which has showed to cause epigenetic modifications, making the individual subjected to this traumatic event, and subsequent progeny more susceptible to suffer PTSD, and having an increased reactivity of the HPA axis. Several studies were analysed revealing the effects of famine and war on the human population and the consequences to future progeny. It was also studied and tried to understand how traumatic events produce different outcomes between the sexes, making them more or less susceptible to developed PTSD.

Lastly, it was highlighted that, not only negative environmental factors can interact with our genome, but also positive environmental influences can generate adaptive modifications to subsequent generations. Therefore, new therapies involving EE and physical activity can reverse the negative effects of trauma.

It is especially important to rase awareness about this scientific field to the healthcare professional and the community. Monitoring the individuals that are subjected to traumatic stress is crucial to prevent intergenerational inheritance to future generations.

A better understanding of these areas will allow the development of new specialized therapies, targeting a person's necessities, namely environmental enrichment, and strategies to prevent the development of PTSD in subsequent generations.

14. References

1. Moore DS. Behavioral epigenetics. Wiley Interdiscip Rev Syst Biol Med. 2017;9:14–33.

2. Liberman N, Wang SY, Greer EL. Transgenerational Epigenetic Inheritance: From Phenomena to Molecular Mechanisms. Curr Opin Neurobiol. 2019;176:100–106.

3. Deans C, Maggert KA. What do you mean, “Epigenetic”? Genetics. 2015;199:887–96.

4. Nagy C, Turecki G. Transgenerational epigenetic inheritance: An open discussion. Epigenomics. 2015;7:781–90.

5. Skvortsova K, Iovino N, Bogdanović O. Functions and mechanisms of epigenetic inheritance in animals. Nat Rev Mol Cell Biol. 2018;19:774–90.

6. Wang Y, Liu H, Sun Z. Lamarck rises from his grave: parental environment-induced epigenetic inheritance in model organisms and humans. Biol Rev. 2017;92:2084–111.

7. Kellermann NPF. Epigenetic transmission of holocaust trauma: Can nightmares be inherited? Isr J Psychiatry Relat Sci. 2013;50:33–9.

8. Bošković A, Rando OJ. Transgenerational epigenetic inheritance. Annu Rev Genet. 2018;52:21–41.

9. Mørkve Knudsen T, Rezwan FI, Jiang Y, Karmaus W, Svanes C, Holloway JW. Transgenerational and intergenerational epigenetic inheritance in allergic diseases. J Allergy Clin Immunol. 2018;142:765–72.

10. Perez MF, Lehner B. Intergenerational and transgenerational epigenetic inheritance in animals. Nat Cell Biol. 2019;21:143–51.

11. Nemoda Z, Szyf M. Epigenetic Alterations and Prenatal Maternal Depression. Birth Defects Res. 2017;109:888–97.

12. Guerrero TP, Fickel J, Benhaiem S, Weyrich A. Epigenomics and gene regulation in mammalian social systems. Curr Zool. 2020;66:307–19.

13. Zhang W, Qu J, Liu GH, Belmonte JCI. The ageing epigenome and its rejuvenation. Nat Rev Mol Cell Biol. 2020;21:137–50.

14. D’Urso, Agustina, Brickner JH. Mechanisms of Epigenetic Memory. Trends Genet.

2014;30:230–6.

15. Skvortsova K, Stirzaker C, Taberlay P. The DNA methylation landscape in cancer. Essays Biochem. 2019;63:797–811.

16. Tung J, Barreiro LB, Johnson ZP, Hansen KD, Michopoulos V, Toufexis D, et al. Social environment is associated with gene regulatory variation in the rhesus macaque immune system. Proc Natl Acad Sci U S A. 2012;109:6490–5.

17. Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. Nat Rev Mol Cell Biol. 2019;20:590–607.

18. Reik W, Collick A, Norris ML, Barton SC, Surani MA. Genomic imprinting determines methylation of parental alleles in transgenic mice. Nature. 1988;328:248–51.

19. T Mohandas, R S Sparkes LJS. Reactivation of an inactive human X chromosome: evidence for X inactivation by DNA methylation. Science (80-). 1981;1:35–8.

20. Stoll S, Wang C, Qiu H. DNA methylation and histone modification in hypertension. Int J Mol Sci. 2018;19:1–17.

21. Zemach A, McDaniel IE, Silva P, Zilberman D. Genome-wide evolutionary analysis of eukaryotic DNA methylation. Science (80-). 2010;328:916–9.

22. Jurkowska RZ, Jurkowski TP, Jeltsch A. Structure and Function of Mammalian DNA Methyltransferases. Vol. 12, ChemBioChem. 2011. p. 206–22.

23. Li E, Zhang Y. DNA methylation in mammals. Cold Spring Harb Perspect Biol. 2014;6:1–22.

24. Bestor TH. The DNA methyltransferases of mammals. Vol. 9, Human Molecular Genetics. 2000. p. 2395–402.

25. Sharif J, Muto M, Takebayashi SI, Suetake I, Iwamatsu A, Endo TA, et al. The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature. 2007;450:908–12.

26. Smith ZD, Meissner A. DNA methylation: Roles in mammalian development. Nat Rev Genet. 2013;14:204–20.

27. Stadler MB, Murr R, Burger L, Ivanek R, Lienert F, Schöler A, et al. DNA-binding

factors shape the mouse methylome at distal regulatory regions. Nature. 2011;480:490–5.

28. Bartolomei MS, Oakey RJ, Wutz A. Genomic imprinting: An epigenetic regulatory system. PLoS Genet. 2020;16:6–8.

29. Kohli RM, Zhang Y. TET enzymes, TDG and the dynamics of DNA demethylation. Vol. 502, Nature. 2013. p. 472–9.

30. Heintzman ND, Stuart RK, Hon G, Fu Y, Ching CW, Hawkins RD, et al. Distinct and predictive chromatin signatures of transcriptional promoters and enhancers in the human genome. Nat Genet. 2007;39:311–8.

31. Jones PA. Functions of DNA methylation: Islands, start sites, gene bodies and beyond. Vol. 13, Nature Reviews Genetics. 2012. p. 484–92.

32. Gilsbach R, Preissl S, Grüning BA, Schnick T, Burger L, Benes V, et al. Dynamic DNA methylation orchestrates cardiomyocyte development, maturation and disease. Nat Commun. 2014;5:5288.

33. Millis RM. Epigenetics and hypertension. Curr Hypertens Rep. 2011;13:21–8.

34. Kebede AF, Schneider R, Daujat S. Novel types and sites of histone modifications emerge as players in the transcriptional regulation contest. Vol. 282, The FEBS Journal. 2015. p. 1658–74.

35. Tolsma TO, Hansen JC. Post-translational modifications and chromatin dynamics. Essays Biochem. 2019;63:89–96.

36. García-Giménez JL, Romá-Mateo C, Pallardó F V. Oxidative post-translational modifications in histones. BioFactors. 2019;45:641–50.

37. Lawrence M, Daujat S, Schneider R. Lateral Thinking: How Histone Modifications Regulate Gene Expression. Trends Genet. 2016;32:42–56.

38. Dawson MA, Kouzarides T. Cancer epigenetics: From mechanism to therapy. Cell. 2012;150:12–27.

39. Weber CM, Henikoff S. Histone variants: Dynamic punctuation in transcription. Vol. 28, Genes and Development. 2014. p. 672–82.

40. McGinty RK, Tan S. Nucleosome structure and function. Chem Rev. 2015;115:2255–

73.

41. Biterge B, Schneider R. Histone variants: Key players of chromatin. Cell Tissue Res. 2014;356:457–66.

42. Sabari B, Sabari D, Allis CD, Zhao Y. Metabolic regulation of gene expression through histone acylations. Nat Rev Mol Cell Biol. 2016;176:100–106.

43. Bowman GD, Poirier MG. Post-translational modifications of histones that influence nucleosome dynamics. Chem Rev. 2015;115:2274–95.

44. Michalak EM, Burr ML, Bannister AJ, Dawson MA. The roles of DNA, RNA and histone methylation in ageing and cancer. Vol. 20, Nature Reviews Molecular Cell Biology. 2019. p. 573–89.

45. Jambhekar A, Dhall A, Shi Y. Roles and regulation of histone methylation in animal development. Vol. 20, Nature Reviews Molecular Cell Biology. 2019. p. 625–41.

46. Gong F, Miller KM. Histone methylation and the DNA damage response. Mutation Research - Reviews in Mutation Research. 2019. p. 37–47.

47. Aquila L, Atanassov BS. Regulation of Histone Ubiquitination in Response to DNA Double Strand Breaks. Vol. 9, Cells. 2020. p. 1–29.

48. Jeusset L, McManus K. Developing Targeted Therapies That Exploit Aberrant Histone Ubiquitination in Cancer. Cells. 2019;8:165.

49. Ryu HY, Zhao D, Li J, Su D, Hochstrasser M. Histone sumoylation promotes Set3 histone-deacetylase complex-mediated transcriptional regulation. Nucleic Acids Res. 2020;48:12151–68.

50. Niu D, Lin XL, Kong X, Qu GP, Cai B, Lee J, et al. SIZ1-Mediated SUMOylation of TPR1 Suppresses Plant Immunity in Arabidopsis. Mol Plant. 2019;12:215–28.

51. Panni S, Lovering RC, Porras P, Orchard S. Non-coding RNA regulatory networks. Biochim Biophys Acta - Gene Regul Mech. 2020;1863:194417.

52. Molnár A, Schwach F, Studholme DJ, Thuenemann EC, Baulcombe DC. miRNAs control gene expression in the single-cell alga Chlamydomonas reinhardtii. Nature. 2007;447(7148):1126–9.

53. Moazed D. Small RNAs in transcriptional gene silencing and genome defence. Nature.

2009;457:413–20.

54. Giraldez AJ, Cinalli RM, Glasner ME, Enright AJ, Thomson JM, Baskerville S, et al. MicroRNAs regulate brain morphogenesis in zebrafish. Science (80-). 2005;308:833–8.

55. Melton C, Judson RL, Blelloch R. Opposing microRNA families regulate self-renewal in mouse embryonic stem cells. Nature. 2010;463:621–6.

56. Rechavi O, Minevich G, Hobert O. Transgenerational inheritance of an acquired small RNA-based antiviral response in C. elegans. Cell. 2011;147:1248–56.

57. Filardi T, Catanzaro G, Mardente S, Zicari A, Santangelo C, Lenzi A, et al. Non-coding RNA: Role in gestational diabetes pathophysiology and complications. Int J Mol Sci. 2020;21:4020.

58. Kumar S, Gonzalez EA, Rameshwar P, Etchegaray JP. Non-coding RNAs as mediators of epigenetic changes in malignancies. Cancers (Basel). 2020;12:1–32.

59. Dong Y, Xu S, Liu J, Ponnusamy M, Zhao Y, Zhang Y, et al. Non-coding RNA-linked epigenetic regulation in cardiac hypertrophy. Int J Biol Sci. 2018;14:1133–41.

60. Kaikkonen MU, Adelman K. Emerging Roles of Non-Coding RNA Transcription. Trends Biochem Sci. 2018;43:654–67.

61. Kim KW. PIWI Proteins and piRNAs in the Nervous System. Mol Cells. 2019;42:828–35.

62. Luteijn MJ, Ketting RF. PIWI-interacting RNAs: From generation to transgenerational epigenetics. Nat Rev Genet. 2013;14:523–34.

63. Gou LT, Dai P, Yang JH, Xue Y, Hu YP, Zhou Y, et al. Pachytene piRNAs instruct massive mRNA elimination during late spermiogenesis. Cell Res [Internet]. 2014 May 2 [cited 2021 Jun 26];24(6):680–700. Available from: www.nature.com/cr

64. Shen EZ, Chen H, Ozturk AR, Tu S, Shirayama M, Tang W, et al. Identification of piRNA Binding Sites Reveals the Argonaute Regulatory Landscape of the C. elegans Germline. Cell. 2018;172:937-951.e18.

65. Zhang D, Tu S, Stubna M, Wu WS, Huang WC, Weng Z, et al. The piRNA targeting rules and the resistance to piRNA silencing in endogenous genes. Science (80-).

2018;359:587–92.

66. Bianchi M, Renzini A, Adamo S, Moresi V. Coordinated actions of microRNAs with other epigenetic factors regulate skeletal muscle development and adaptation. Vol. 18, International Journal of Molecular Sciences. 2017. p. 1–14.

67. Dhanoa JK, Sethi RS, Verma R, Arora JS, Mukhopadhyay CS. Long non-coding RNA: Its evolutionary relics and biological implications in mammals: A review. J Anim Sci Technol. 2018;60:1–10.

68. Sharma U, Conine CC, Shea JM, Boskovic A, Derr AG, Bing XY, et al. Biogenesis and function of tRNA fragments during sperm maturation and fertilization in mammals. Science (80-). 2016;351:391–6.

69. Chen Q, Yan M, Cao Z, Li X, Zhang Y, Shi J, et al. Sperm tsRNAs contribute to intergenerational inheritance of an acquired metabolic disorder. Science (80-). 2016;351:397–400.

70. Zhang Y, Zhang X, Shi J, Tuorto F, Li X, Liu Y, et al. Dnmt2 mediates intergenerational transmission of paternally acquired metabolic disorders through sperm small non-coding RNAs. Nat Cell Biol. 2018;20:535–40.

71. Zeng Y, Chen T. DNA methylation reprogramming during mammalian development. Genes (Basel). 2019;10:1–18.

72. Hajkova P. Epigenetic reprogramming in the germline: Towards the ground state of the epigenome. Philos Trans R Soc B Biol Sci. 2011;366:2266–73.

73. Monk M, Boubelik M, Lehnert S. Temporal and regional changes in DNA methylation in the embryonic, extraembryonic and germ cell lineages during mouse embryo development. Development [Internet]. 1987;371–82. Available from: https://pubmed.ncbi.nlm.nih.gov/3653008/

74. Edwards JR, Yarychkivska O, Boulard M, Bestor TH. DNA methylation and DNA methyltransferases. Epigenetics and Chromatin. 2017;10:1–10.

75. Guibert S, Forné T, Weber M. Global profiling of DNA methylation erasure in mouse primordial germ cells. Genome Res. 2012;22:633–41.

76. Hackett JA, Sengupta R, Zylicz JJ, Murakami K, Lee C, Down TA, et al. Germline

DNA demethylation dynamics and imprint erasure through 5-hydroxymethylcytosine. Science (80-). 2013;339:448–52.

77. Yamaguchi S, Hong K, Liu R, Inoue A, Shen L, Zhang K, et al. Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. Cell Res. 2013;23:329–39.

78. Kota SK, Feil R. Epigenetic Transitions in Germ Cell Development and Meiosis. Dev Cell. 2010;19:675–86.

79. Kobayashi H, Sakurai T, Imai M, Takahashi N, Fukuda A, Yayoi O, et al. Contribution of Intragenic DNA Methylation in Mouse Gametic DNA Methylomes to Establish Oocyte-Specific Heritable Marks. PLOS Genet. 2012;8:e1002440.

80. Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. Nature. 2000;403:501–2.

81. Inoue A, Zhang Y. Replication-dependent loss of 5-hydroxymethylcytosine in mouse preimplantation embryos. Science. 2011;334:194.

82. Inoue A, Shen L, Dai Q, He C, Zhang Y. Generation and replication-dependent dilution of 5fC and 5caC during mouse preimplantation development. Cell Res. 2011;21:1670–6.

83. Okano M, Bell D, Haber D, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. Cell. 1999;99:247–57.

84. Brunner AM, Nanni P, Mansuy IM. Epigenetic marking of sperm by post-translational modification of histones and protamines. Epigenetics Chromatin. 2014;7:2.

85. Thamban T, Agarwaal V, Khosla S. Role of genomic imprinting in mammalian development. J Biosci. 2020;45:1–21.

86. Morgan HD, Sutherland HGE, Martin DIK, Whitelaw E. Epigenetic inheritance at the agouti locus in the mouse. Nat Genet. 1999;23:314–8.

87. Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, Richmond TA, Sapio F De, et al. Somatic retrotransposition alters the genetic landscape of the human brain. Nature. 2011;479:534–7.

88. Wolff GL, Kodell RL, Moore SR, Cooney CA. Maternal epigenetics and methyl

supplements affect agouti gene expression in Avy/a mice. FASEB. 1998;949–57.

89. Schlichting CD, Wund MA. Phenotypic plasticity and epigenetic marking: An assessment of evidence for genetic accommodation. Evolution (N Y). 2014;68(3):656–72.

90. Renn SCP, Schumer ME. Genetic accommodation and behavioural evolution: Insights from genomic studies. Vol. 85, Animal Behaviour. 2013. p. 1012–22.

91. Dingemanse NJ, Kazem AJN, Réale D, Wright J. Behavioural reaction norms: animal personality meets individual plasticity. Trends Ecol Evol. 2010;25:81–9.

92. Storz JF, Scott GR. Phenotypic plasticity, genetic assimilation, and genetic compensation in hypoxia adaptation of high-altitude vertebrates. Comp Biochem Physiol -Part A Mol Integr Physiol [Internet]. 2021;253(December 2020):110865. Available from: https://doi.org/10.1016/j.cbpa.2020.110865

93. Kelly M. Adaptation to climate change through genetic accommodation and assimilation of plastic phenotypes. Philos Trans R Soc B Biol Sci. 2019;374:20180176.

94. Shimoda LA, Laurie SS. Hypoxia: HIF and pulmonary vascular responses to hypoxia. J Appl Physiol [Internet]. 2014 Apr 1 [cited 2021 Jul 26];116(7):867. Available from: /pmc/articles/PMC3972740/

95. Jones BM, Robinson GE. Genetic accommodation and the role of ancestral plasticity in the evolution of insect eusociality. J Exp Biol. 2018;221(23):1–11.

96. Schneider RF, Meyer A. How plasticity, genetic assimilation and cryptic genetic variation may contribute to adaptive radiations. Mol Ecol. 2017;26(1):330–50.

97. Tiffon C. The impact of nutrition and environmental epigenetics on human health and disease. Int J Mol Sci. 2018;19(11).

98. Jawaid A, Roszkowski M, Mansuy IM. Transgenerational Epigenetics of Traumatic Stress. Progress in Molecular Biology and Translational Science. 2018. 273–298 p.

99. Guillén-Burgos HF, Gutiérrez-Ruiz K. Genetic advances in post-traumatic stress disorder. Rev Colomb Psiquiatr (English Ed. 2018;47:108–18.

100. Yeshurun S, Hannan AJ. Transgenerational epigenetic influences of paternal environmental exposures on brain function and predisposition to psychiatric disorders.

Mol Psychiatry. 2019;24:536–48.

101. Karp NA, Mason J, Beaudet AL, Benjamini Y, Bower L, Braun RE, et al. Prevalence of sexual dimorphism in mammalian phenotypic traits. Nat Commun. 2017;8:15475.

102. Dezsi G, Ozturk E, Salzberg MR, Morris M, O'Brien TJ, Jones NC. Environmental enrichment imparts disease-modifying and transgenerational effects on genetically-determined epilepsy and anxiety. Neurobiol Dis. 2016 Sep 1;93:129–36.

103. Piyali M, Subramaniam G. Sex-biased transgenerational effect of maternal stress on neurodevelopment and cognitive functions. J Genet. 2018;97:581–3.

104. Franklin TB, Russig H, Weiss IC, Gräff J, Linder N, Michalon A, et al. Epigenetic Transmission of the Impact of Early Stress Across Generations. Biol Psychiatry. 2010;68:408–15.

105. Franklin TB, Linder N, Russig H, Thöny B, Mansuy IM. Influence of Early Stress on Social Abilities and Serotonergic Functions across Generations in Mice. PLoS One. 2011;6:e21842.

106. Ornoy A, Ratzon N, Greenbaum C, Wolf A, Dulitzky M. School-age children born to diabetic mothers and to mothers with gestational diabetes exhibit a high rate of inattention and fine and gross motor impairment. J Pediatr Endocrinol Metab. 2001;14:681–9.

107. Torres-Espinola FJ, Berglund SK, García-Valdés LM, Segura MT, Jerez A, Campos D, et al. Maternal Obesity, Overweight and Gestational Diabetes Affect the Offspring Neurodevelopment at 6 and 18 Months of Age--A Follow Up from the PREOBE Cohort. PLoS One. 2015;10(7):e0133010.

108. Mikkonen J, Moustgaard H, Remes H, Martikainen P. Intergenerational transmission of depressive symptoms – The role of gender, socioeconomic circumstances, and the accumulation of parental symptoms. J Affect Disord. 2016;204:74–82.

109. Rodgers AB, Bale TL. Germ cell origins of PTSD risk: the transgenerational impact of parental stress experience. Biol Psychiatry. 2015;78:307–14.

110. Howie H, Rijal CM, Ressler KJ. A review of epigenetic contributions to post-traumatic stress disorder. Dialogues Clin Neurosci. 2019;21:417–28.

111. Daskalakis NP, Rijal CM, King C, Huckins LM, Ressler KJ. Recent Genetics and Epigenetics Approaches to PTSD. Curr Psychiatry Rep. 2018;20:1–12.

112. Christiansen DM, Berke ET. Gender- and Sex-Based Contributors to Sex Differences in PTSD. Curr Psychiatry Rep. 2020;22:1–9.

113. Tolin DF, Foa EB. Sex differences in trauma and posttraumatic stress disorder: a quantitative review of 25 years of research. Psychol Bull. 2006;132:959–92.

114. Cox DW, O'Loughlin J. Posttraumatic stress mediates traditional masculinity ideology and romantic relationship satisfaction in veteran men. Psychol Men Masculinity. 2017;18:382–9.

115. Cuevas CA, Sabina C, Picard EH. Posttraumatic Stress among Victimized Latino Women: Evaluating the Role of Cultural Factors. J Trauma Stress. 2015;28:531–8.

116. Reijnen A, Geuze E, Vermetten E. The effect of deployment to a combat zone on testosterone levels and the association with the development of posttraumatic stress symptoms: A longitudinal prospective Dutch military cohort study. Psychoneuroendocrinology. 2015;51:525–33.

117. Nillni YL, Pineles SL, Patton SC, Rouse MH, Sawyer AT, Rasmusson AM. Menstrual cycle effects on psychological symptoms in women with PTSD. J Trauma Stress. 2015;28:1–7.

118. Mercer KB, Dias B, Shafer D, Maddox SA, Mulle JG, Hu P, et al. Functional evaluation of a PTSD-associated genetic variant: estradiol regulation and ADCYAP1R1. Transl Psychiatry 2016 612. 2016;6:e978.

119. Blacker CJ, Frye MA, Morava E, Kozicz T, Veldic M. A review of epigenetics of PTSD in comorbid psychiatric conditions. Genes (Basel). 2019;10(2):1–15.

120. Sheerin CM, Lind MJ, Bountress K, Nugent NR, Amstadter AB. The Genetics and Epigenetics of PTSD: Overview, Recent Advances, and Future Directions. Curr Opin Psychol. 2017;14:5.

121. Youssef NA, Lockwood L, Su S, Hao G, Rutten BPF. The effects of trauma, with or without PTSD, on the transgenerational DNA methylation alterations in human offsprings. Brain Sci. 2018;8(5).

122. Kessler RC, Aguilar-Gaxiola S, Alonso J, Benjet C, Bromet EJ, Cardoso G, et al. Trauma and PTSD in the WHO World Mental Health Surveys. Eur J Psychotraumatol. 2017;8:1353383.

123. WEHMER F, PORTER RH, SCALES B. Pre-mating and Pregnancy Stress in Rats affects Behaviour of Grandpups. Nat 1970 2275258 [Internet]. 1970 [cited 2021 Aug 2];227:622–622. Available from: https://www.nature.com/articles/227622a0

124. Rodgers AB, Morgan CP, Bronson SL, Revello S, Bale TL. Paternal Stress Exposure Alters Sperm MicroRNA Content and Reprograms Offspring HPA Stress Axis Regulation. J Neurosci. 2013;33:9003–12.

125. Dias BG, Ressler KJ. Parental olfactory experience influences behavior and neural structure in subsequent generations. Nat Neurosci 2013 171. 2013;17:89–96.

126. Barker DJP, Clark PM. Fetal undernutrition and disease in later life. Rev Reprod. 1997;2(2):105–12.

127. Heijmans BT, Tobi EW, Stein AD, Putter H, Blauw GJ, Susser ES, et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. Proc Natl Acad Sci U S A. 2008;105:17046.

128. Pembrey M, Saffery R, Bygren LO, Epidemiology N in E. Human transgenerational responses to early-life experience: potential impact on development, health and biomedical research. J Med Genet. 2014;51:563–572.

129. Pembrey ME, Bygren LO, Kaati G, Edvinsson S, Northstone K, Sjöström M, et al. Sex-specific, male-line transgenerational responses in humans. Eur J Hum Genet. 2005;14:159–66.

130. Yehuda R, Bierer LM. Transgenerational transmission of cortisol and PTSD risk. Prog Brain Res. 2007 Jan 1;167:121–35.

131. Yehuda R, Daskalakis NP, Bierer LM, Bader HN, Klengel T, Holsboer F, et al. Holocaust Exposure Induced Intergenerational Effects on FKBP5 Methylation. Biol Psychiatry. 2016;80:372–80.

132. Perroud N, Rutembesa E, Paoloni-Giacobino A, Mutabaruka J, Mutesa L, Stenz L, et al. The Tutsi genocide and transgenerational transmission of maternal stress: epigenetics and biology of the HPA axis. World J Biol Psychiatry. 2014;15:334–45.

133. Jenkins T, James E, Alonso D, Hoidal J, Murphy P, Hotaling J, et al. Cigarette Smoking Significantly Alters Sperm DNA Methylation Patterns. Andrology. 2017;5:1089–1099.

134. Ambatipudi S, Cuenin C, Hernandez-Vargas H, Ghantous A, Calvez-Kelm F Le, Kaaks R, et al. Tobacco smoking-associated genome-wide DNA methylation changes in the EPIC study. Epigenomics. 2016;8:599–618.

135. Teschendorff AE, Yang Z, Wong A, Pipinikas CP, Jiao Y, Jones A, et al. Correlation of Smoking-Associated DNA Methylation Changes in Buccal Cells With DNA Methylation Changes in Epithelial Cancer. JAMA Oncol. 2015;1:476–85.

136. Svanes C, Koplin J, Skulstad SM, Johannessen A, Bertelsen RJ, Benediktsdottir B, et al. Father's environment before conception and asthma risk in his children: A multi-generation analysis of the Respiratory Health In Northern Europe study. Int J Epidemiol. 2017;46:235–45.

137. Vassoler FM, Byrnes EM, Pierce RC. The impact of exposure to addictive drugs on future generations: Physiological and behavioral effects. Neuropharmacology. 2014;76:269–75.

138. Byrnes JJ, Johnson NL, Carini LM, Byrnes EM. Multigenerational effects of adolescent morphine exposure on dopamine D2 receptor function. Psychopharmacol 2013 2272 [Internet]. 2013 Jan 13 [cited 2021 Aug 14];227(2):263–72. Available from: https://link.springer.com/article/10.1007/s00213-012-2960-1

139. Byrnes EM. Transgenerational consequences of adolescent morphine exposure in female rats: effects on anxiety-like behaviors and morphine sensitization in adult offspring. Psychopharmacology (Berl). 2005;182:537–44.

140. Zeid D, Gould TJ. Impact of nicotine, alcohol, and cocaine exposure on germline integrity and epigenome. Neuropharmacology. 2020;173:108127.

141. Ohline SM, Abraham WC. Environmental enrichment effects on synaptic and cellular physiology of hippocampal neurons. Neuropharmacology. 2019;145:3–12.

142. Gapp K, Bohacek J, Grossmann J, Brunner AM, Manuella F, Nanni P, et al. Potential of Environmental Enrichment to Prevent Transgenerational Effects of Paternal Trauma. Neuropsychopharmacology. 2016;41:2749–58.

143. Denham J, O'Brien BJ, Harvey JT, Charchar FJ. Genome-wide sperm DNA methylation changes after 3 months of exercise training in humans. Epigenomics. 2015;7:717–31.

144. Li C-Q, Luo Y-W, Bi F-F, Cui T-T, Song L, Cao W-Y, et al. Development of Anxiety-Like Behavior via Hippocampal IGF-2 Signaling in the Offspring of Parental Morphine Exposure: Effect of Enriched Environment. Neuropsychopharmacology. 2014;39:2777–87.

145. Eaton SA, Jayasooriah N, Buckland ME, Martin DI, Cropley JE, Suter CM. Roll over Weismann: extracellular vesicles in the transgenerational transmission of environmental effects. http://dx.doi.org/102217/epi1558. 2015;7:1165–71.

146. Zannas AS, Provençal N, Binder EB. Epigenetics of Posttraumatic Stress Disorder: Current Evidence, Challenges, and Future Directions. Biol Psychiatry. 2015;78:327–35.