Ital J Gender-Specific Med 2018; 4(1): 3-12

Epigenetics and gender-specific medicine

Lucia Migliore, Vanessa Nicolì

Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Pisa, Italy. *Received 1 May 2018; accepted 21 May 2018*

Summary. Males and females show large differences in their susceptibility to many complex diseases, including autoimmune diseases, some forms of cancer and neurological diseases. Autoimmune diseases are characterized, for example, by a greater female component, which for some diseases, such as systemic lupus erythematosus, even reach 80%.
It is believed that in many cases the susceptibility to these diseases can arise early during the life, in agreement with the DOHaD (Developmental Origins of Health and Disease) theory.
The two well known cellular processes involving epigenetic mechanisms dealing with gender differences and arising very early during the embryo development, are genomic imprinting and X-chromosome inactivation in females.
Genomic imprinting affects eutherian mammals and consists in conferring a subclass of homologous *loci* (imprinted genes, such as *IGF2*), a specific monoallelic expression with respect to the parent from which they are inherited. The process is therefore essential for the correct development of the fetus and, in humans, the loss of imprinting of the differentially methylated regions is the cause of diseases such as the syndromes of Silver-Russell, Angelman and Prader-Willi.
The brain has emerged as a main target of genomic imprinting, generating great interest on how this epigenetic regulation provides stable transcriptional control of neural development and behavior. Many of neurodevelopmental disorders may originate from defective signaling during fetal or perinatal brain development, which may affect males and females differently.
The random inactivation of one of the two X-chromosomes in female mammalian cells during early embryogenesis represents a well known epigenetic mechanism of gene regulation. An explanation for the female predominance in autoimmune diseases has been proposed by means of an enhanced skewed X-chromosome inactivation.
The inactivation of one X chromosome in mammalian females is often incomplete and over 15% of X-linked genes escape the inactivation and are expressed from both the active and inactive X chromosomes. Genome-wide analysis is revealing roles for escape genes in an increasing number of diseases. Also disturbances in the normal inactivation pattern of miRNAs on the X chromosome, by means of silencing escape or inactivation skewing, could affect miRNAs-driven gene regulation and result in gender-specific responses.
It is likely that the expression of specific genes related to complex diseases can be modified by various mechanisms influenced by gender and also by environmental factors, likely acting in critical windows, during maternal pregnancy and interfering with fetal programming.

Key words: epigenetics, sex ratio deviation, genome imprinting, skewed X chromosome inactivation.

Epigenetica e medicina genere-specifica

Riassunto. Molte patologie complesse tra cui malattie autoimmuni, malattie cardiovascolari, malattie del neurosviluppo e alcune forme di cancro, presentano una deviazione del rapporto tra i sessi; le malattie autoimmuni sono caratterizzate, ad esempio, da una maggior prevalenza femminile, che per alcune malattie, come il lupus eritematoso sistemico, arriva anche all'80%. Si ritiene che in molti casi la suscettibilità a queste malattie possa insorgere precocemente nel corso della vita, in accordo con la teoria della DOHaD, secondo cui la suscettibilità negli adulti a diverse malattie complesse verrebbe determinata precocemente durante l'embriogenesi in conseguenza dei diversi stimoli ambientali a cui è sottoposto il feto.
I due più noti meccanismi epigenetici coinvolti nella determinazione delle differenze di genere sono l'imprinting genomico e l'inattivazione di uno dei due cromosomi X nelle femmine di mammifero. L'imprinting genomico interessa i mammiferi euteri e consiste nel conferire ad una sottoclasse di *loci* omologhi (geni imprinted, come *IGF2*), un'espressione monoallelica specifica rispetto al genitore da cui sono ereditati. Il processo risulta dunque fondamentale per il corretto sviluppo del feto e, nell'uomo, la perdita di imprinting delle regioni differenzialmente metilate è causa di patologie come le sindromi di Silver-Russell, Angelman e Prader-Willi.
Il cervello sta emergendo come uno dei più importanti bersagli dell'imprinting genomico. Molti disturbi del neurosviluppo potrebbero originarsi da alterazioni dell'imprinting durante lo sviluppo del cervello fetale o perinatale, con diversa influenza su maschi e femmine.
L'inattivazione casuale di uno dei due cromosomi X nelle cellule delle femmine durante l'embriogenesi precoce rappresenta un ben noto meccanismo epigenetico di regolazione genica. Una spiegazione per la prevalenza del genere femminile nelle malattie autoimmuni vede coinvolta un'inattivazione preferenziale di uno dei due cromosomi X. L'inattivazione di uno dei due cromosomi è però spesso incompleta e oltre il 15% dei geni X-linked sfugge all'inattivazione. Analisi a livello genomico stanno rivelando, per i geni

che sfuggono all'inattivazione, ruoli importanti in un numero crescente di patologie. Sul cromosoma X sono presenti anche molti miRNA e alterazioni del normale pattern di attivazione di questi miRNA, o perché sfuggono al silenziamento o perché coinvolti in inattivazione preferenziale dell'X, potrebbero influenzare la regolazione genica mediata dai miRNA e determinare risposte genere-specifiche.
È probabile che l'espressione di geni specifici legati a malattie complesse possa essere modificata da vari meccanismi influenzati dal genere e anche da fattori ambientali, probabilmente agendo in finestre temporali critiche, nel corso della gravidanza materna, con conseguente alterazione della programmazione fetale.

Parole chiave: epigenetica, deviazione del rapporto tra i sessi, imprinting genomico, inattivazione preferenziale del cromosoma X.

Introduction

Differences in the sex ratio occur in most complex diseases, including autoimmune diseases, cardiovascular diseases, neurodevelopmental and neurological disorders, cancer. It is believed that in many cases the susceptibility to these diseases can arise early during the life, in agreement with the DOHaD (Developmental Origins of Health and Disease) theory[1]. This theory postulates that early-life environmental exposures, including maternal diet, can alter disease risk across the life course into adulthood. Epigenetic modifications are the key effectors because they can be maintained throughout cell division. Epigenetic mechanisms, such as DNA methylation, histon tail modifications and the non-coding RNAs interventions, finely regulate gene expression levels without inducing DNA sequence changes, and play a fundamental role in embryonic development, in the differentiation and maintenance of cell identity, as well as in many other physiological processes.

The two well known cellular processes involving epigenetic mechanisms dealing with gender differences and arising very early during the embryo development, are genomic imprinting and X-chromosome inactivation in females.

Genomic imprinting

Genomic imprinting is an epigenetic regulatory mechanism that results in the monoallelic expression in a parent-of-origin-dependent fashion of a subset of genes, located in specific regions, called differentially methylated regions (DMRs) (Figure 1). These sites of differential methylation between the maternal and paternal alleles are protected from the wave of global demethylation that occurs immediately after fertilization, providing a parent-of-origin-specific epigenetic signature. The imprinted expression of those *loci* has an essential role in normal growth and development in placental mammals.

In humans, loss-of-imprinting of specific DMRs results in a number of diseases often associated with fetal growth and neurological behaviour such as Angelman, Prader-Willi, Beckwith-Wiedemann and Silver-Russell syndromes.

It is becoming increasingly clear that imprinted gene function has a wider role in maternal physiology during

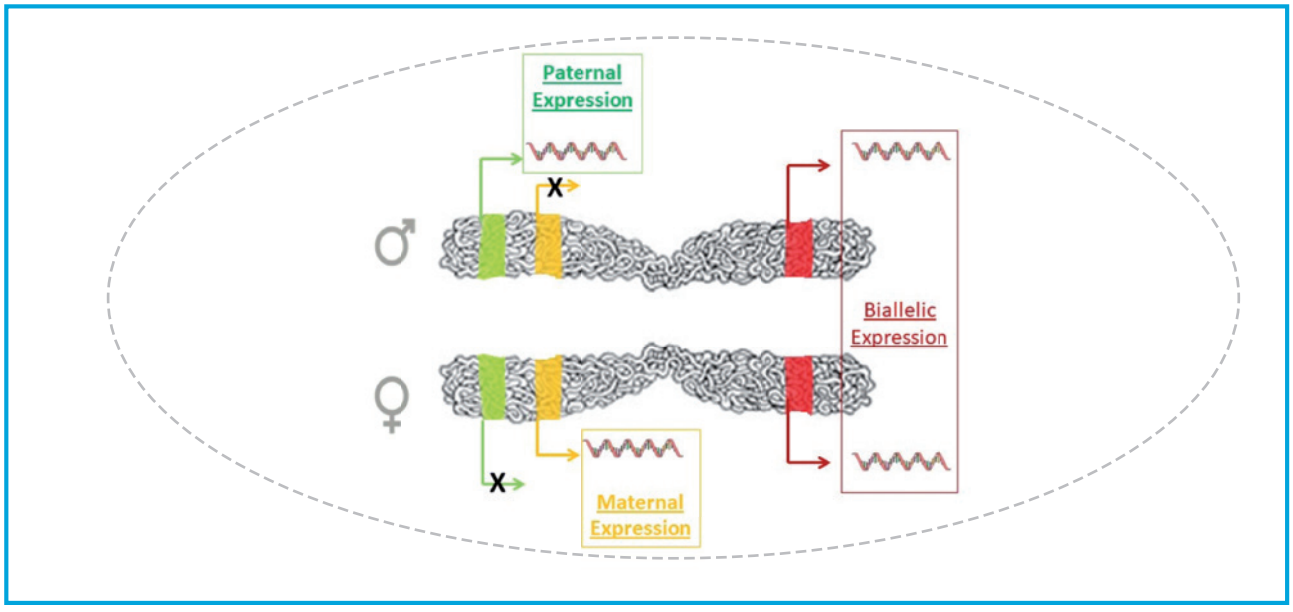

Figure 1. Genomic imprinting consists of the differential expression of alleles paternally or maternally inherited.

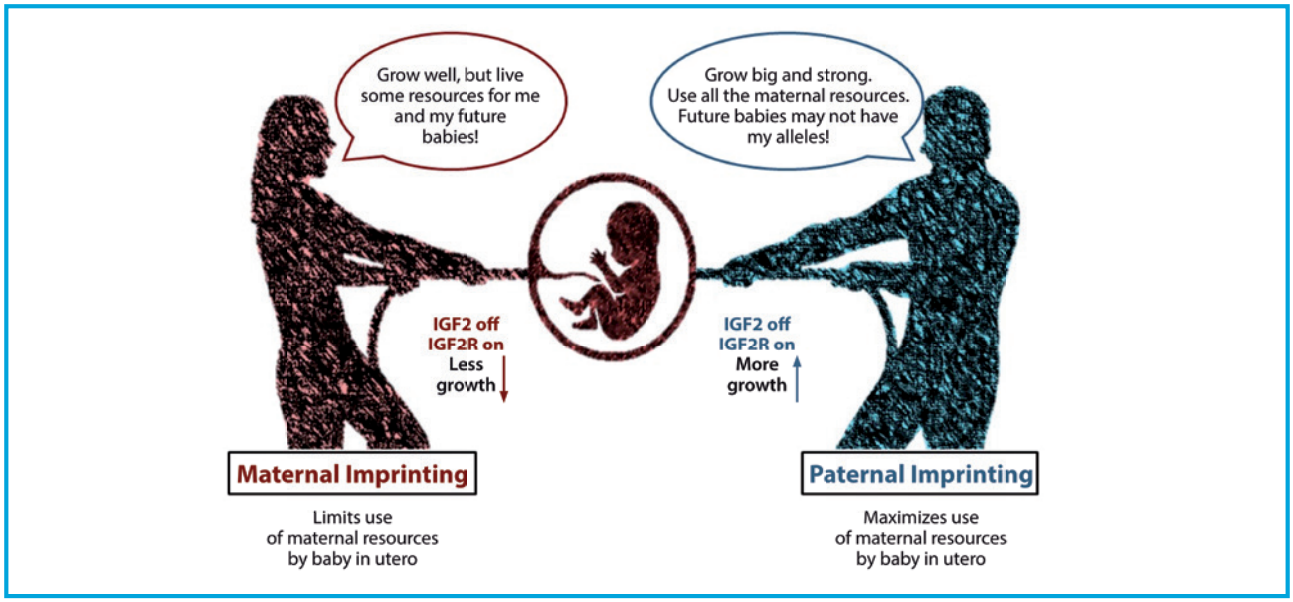

Figure 2. The conflict hypothesis predicts that imprinting has evolved in mammals because of the conflicting interests of maternal and paternal genes in relation to the transfer of nutrients from the mother to her offspring. Modified from Jirtle and Wiedman, 2007.

reproduction. Imprinted genes are required for the development of a functional placenta, the organ that mediates the exchange of nutrients between mother and fetus. Given that abnormal birthweight correlates with adverse adult metabolic health, including obesity and cardiovascular disease, it is likely that the modulation of this dosage-sensitive, epigenetically regulated class of genes can contribute to fetal and postnatal growth, with implications for lifelong health and disease[2].

Therefore it seems that aberrant imprinting could also contribute to a variety of complex diseases including cancer, widening the functional role of imprinted genes in humans[3].

The phenomenon of genomic imprinting is observed predominantly in eutherian mammals (mammals with long-lived placenta, which give birth to live young), but not in prototherians (egg-laying mammals, such as platypus), birds or reptiles[4].

Imprinted genes thus appeared on the evolutionary stage with the advent of live birth, perhaps because of inherent conflicts in the reproductive strategies of mothers and fathers[5]. The close association between the acquisition of imprinting and placenta during the course of evolution has led to several hypotheses to explain the reason for the emergence of genomic imprinting. The parental conflict theory[6] is considered the most widely accepted theory. It suggests that imprinting arose because of a genomic tug-of-war between mothers and fathers over the use of maternal resources by the fetus. In mammals that bear live offspring, the male evolutionary fitness is maximized if his offspring monopolizes the female energy reserves during gestation. The female best strategy requires that she does not invest all of her resources in a single offspring, but ensures her survival and the equal allocation of nutrients among her offspring. Both to the common aim of producing the maximum number of viable offspring carrying their genes[5] (Figure 2).

Consistently, imprinting is observed to occur predominantly in genes influencing fetal growth, particularly through placental growth, suckling and nutrient metabolism[7].

The conflict theory is supported by the example of the prototypical mouse imprinted gene *Igf2* and its receptor *Igf2r*. *Igf2* is a paternally expressed potent growth enhancer, whereas maternally expressed *Igf2r* products suppress growth by mediating the degradation of IGF-II proteins. Mouse knockout of these genes exhibite opposite growth phenotypes; *Igf2-null* mice are growth deficient whilst *Igf2r-null* mice show overgrowth phenotypes. It is now argued that paternally expressed genes tend to promote fetal growth whereas maternally expressed genes restrict fetal growth[3].

The imprintome

The concept of the imprintome represents "the environmentally labile cis-acting imprint regulatory elements in the human genome"[8]. Imprintome creation involves both DNA methylation and histone modifications, with methylation being more studied for a variety of technical reasons. It was assumed that there will be 100-200 genes that are subject to imprinted expression in mammals, with many being tissue specific[9]. About 100 im-

printed genes have now been discovered and the functions of many of these genes have been assessed in murine models[2]. A high-resolution mapping of human imprinted methylation has been obtained by Court and collegues[10]. They could define methylation profiles at known imprinted domains at base-pair resolution and catalogued regions of parentally inherited methylation associated with imprinted regions. They have extensively characterized imprinted methylation in a substantial range of normal human tissues and observed that the extent of imprinted DMRs is extremely similar between tissues, however with differences between somatic and placental tissues. The placenta often presents a different methylation profile compared to somatic tissues[11]. On the other hand, as we discussed above, imprinting has been proposed to be a mechanism that regulates parental resource allocation and ultimately can influence fetal growth, with the placenta being the key in this process[11].

It is becoming increasingly clear that imprinted gene function has a wider role in maternal physiology during reproduction, both by modulating fetal and placental endocrine products that signal to alter maternal energy homeostasis, and by altering maternal energetic set points, thus producing downstream actions on nutrient provisioning.

Recently Cassidy and Charalambous proposed a central role of potential mediator for leptin given the fact that imprinted gene products act at multiple levels in the adipose-hypothalamic axis to modulate set points of energy homeostasis. The dosage of imprinted genes in developing and mature adipose tissue would act in modulating leptin secretion and an impaired imprinted gene dosage in females may influence their resource allocation as mothers[2].

Placental-specific imprinting

Court and coworkers[10] observed that the extent of imprinted differentially methylated regions is extremely similar between tissues, with the exception of the placenta. They found that, in contrast to ubiquitous imprints, the majority of placenta-specific imprinted DMRs are unmethylated in sperm and all human embryonic stem cells. Therefore, placental-specific imprinting provides evidence for the hypothesis that a novel imprinting mechanism occurs in the placenta, which is one of the first examples of methylation-independent epigenetic inheritance in mammals[10].

Likely the placental-specific imprinting, but also the influence of fetal sex, can have a differential impact in modifying the course and complications related to pregnancy and may also have an impact on maternal health and well-being both during and after pregnancy. Al-Qaraghouli and Fang[12] summarize findings on the effects of male sex on the course of pregnancy and delivery: higher incidence of preterm labor, failure of progression in labor, true umbilical cord knots, cord prolapse, nuchal cord, higher cesarean section rate, higher heart rate variability with increased frequency, and duration of decelerations without acidemia and increased risk of gestational diabetes mellitus through the poor beta cells function. On the other hand female fetal sex has been found to modify pregnancy and delivery outcomes including altered fetal cardiac hemodynamics, increased hypertensive diseases of pregnancy, higher vulnerability of developing type II diabetes after pregnancy possibly because of influences on increased maternal insulin resistance[12]. Therefore placental sex (XX vs XY) seems a major determinant in the magnitude and functional responses of the placenta to perturbations during pregnancy, where the male, but not female, placentas seem consistently so responsive to changes in the maternal environment (e.g. in response to maternal stress, infection, and diet[13]).

Very few studies investigated the relationship between fetal sex and the susceptibility to diseases. For instance women who were pregnant with females were 2.55 at higher risk for placental malaria infection[14].

Placental PGC-1a/TFAM/mitochondrial biogenesis pathway is affected by maternal diabetes and offspring sex. Decreased PGC-1a in response to maternal diabetes plausibly contributes to impaired mitochondrial biogenesis in placenta of male offspring, which may affect long-term health and explain some of enhanced risk of future metabolic diseases in males[15].

We can conclude that probably there is a gender-specific maternal-placental-fetal interaction, including sex specific transplacental signals to the developing brain, with potential significant biological implications, even at longer times, certainly with genetic and epigenetic mechanisms involved.

Imprinting dysregulation, brain disorders and gender differences

The development of new mapping approaches applied to the growing abundance of genomic data has demonstrated that imprinted genes can be important contributors to complex trait variation[16].

The fraction of known and predicted imprinted genes involved in growth and development results in many of these genes also being involved in mental and physical developmental disorders.

The development of the human brain requires a very fine-tuned orchestration of diverse spatial and temporal cues modulating a regulatory interconnected network. Even a slight interference with a proper regulation may have neurodevelopmental consequences resulting in different outcomes, such as varying degrees of cognitive or

psychiatric disorders[17]. Moreover, one implication of the conflict hypothesis is that different brain regions are maternally or paternally influenced in development[18].

This can have important implications for different susceptibility, gender-specific, in developing neurodevelopmental or behavioural disorders.

The importance of imprinted genes in brain function is evidenced by the devastating neurological and behavioral conditions resulting from mutations in these *loci* in humans and mice. Although early studies of genomic imprinting highlighted its roles during embryonic and placental growth, its pleiotropic influences on brain development and function have emerged more recently[19].

Consistent with the conflict hypothesis of genomic imprinting, paternally expressed genes promote suckling and growth in newborns, and to a lesser extent, maternally expressed genes reduce growth and increase metabolic rate in both newborns and adults. These results are also consistent with the roles of imprinted genes in embryonic development[19]. Paternally expressed genes also appear to contribute to the regulation of puberty as well as to reproductive and maternal behaviors. Interestingly, maternally expressed genes appear to be the main contributors of positive and negative regulation of emotional and cognitive behaviors.

In humans, dysregulation of imprinted gene expression resulting from null mutations, deletions, duplications, uniparental disomies, or alterations in epigenetic marks often affects brain function and behavior.

Moreover, the majority of the Mendelian diseases of the epigenetic machinery (carrying mutations in one gene of those that code for enzymes that *write*, *read*, *erase* or *remodel* epigenetic marks) are characterized by brain disorders (from sligth intellectual disability to dementia)[20].

Males are more likely to develop autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), schizophrenia, and dyslexia, whereas females are more likely to be diagnosed with depression and anxiety[21]. Also neurodegenerative diseases such as Parkinson's disease and Alzheimer's disease affect males and females differently, with differences in susceptibility, progression, and severity[22,23] (Table 1).

Sex-specific events, including altered sex-hormone signaling, during early brain development are considered as key processes that can influence the susceptibility for these disorders. The time window of sex hormone actions is vital in view of the long-term effects on neuronal development.

Moreover, sex-specific differences in DNA methylation of CpG islands may be an important epigenetic change able to differentially affect the developmental process of diseases or traits.

Interestingly within-pair profiles of DNA methylation between monozygotic twins are different between male and female pairs which show discordance for disorders and traits. These differences may be associated with different imprinting, influencing susceptibility to some diseases[24].

Table 1. Sex ratio between females and males in some neurodevelopmental and behavioural disorders.

Disease	Sex ratio [F:M]
Autism Spectrum Disorders (ASD)	1:3
Attention Deficit Hyperactivity Disorder (ADHD)	1:3
Schizophrenia	4:1
Dyslexia	1:1.6
Depression	1.7:1
Anxiety	1.7:1
Parkinson's disease (PD)	1:2
Alzheimer's disease (AD)	1.7:1

Saha et al, 2005[49]; Miller et al, 2003[50]; Albert 2015[51]; McLean et al, 2011[52]; Loomes et al, 2017[53]; Schmidt et al, 2008[54].

Additionally in the first reported epigenome-wide analysis by sex at birth, methylation profiles of DMRs were found sex-specific even in autosomal genes[25].

The central role of genomic imprinting in brain function has been supported further by the extensive neural and behavioral phenotypes of mutants of imprinted genes and by the widespread expression of imprinted genes throughout the brain[26,27]. Thus, the brain has emerged as a main target of genomic imprinting, generating great interest on how this epigenetic regulation provides stable transcriptional control of neural development and behavior. Many of neurodevelopmental disorders may originate from defective signaling during fetal or perinatal brain development, which may affect males and females differently.

X-chromosome inactivation in females

In female mammalian cells, one of the two X-chromosomes is randomly inactivated during early embryogenesis in all cells in order to match the X chromosome number of male cells; this represents a well known epigenetic mechanism of gene regulation. Thus, females are mosaics for two cell populations: cells with either the paternal or the maternal X in the active form. Since X-chromosome choice is assumed to be random, the result is generally 50% of cells expressing the paternal and the remaining 50% expressing the maternal genes (Figure 3).

A skewed inactivation of the X Chromosome (XCI) is a deviation from the 50:50 ratio of X inactivation in

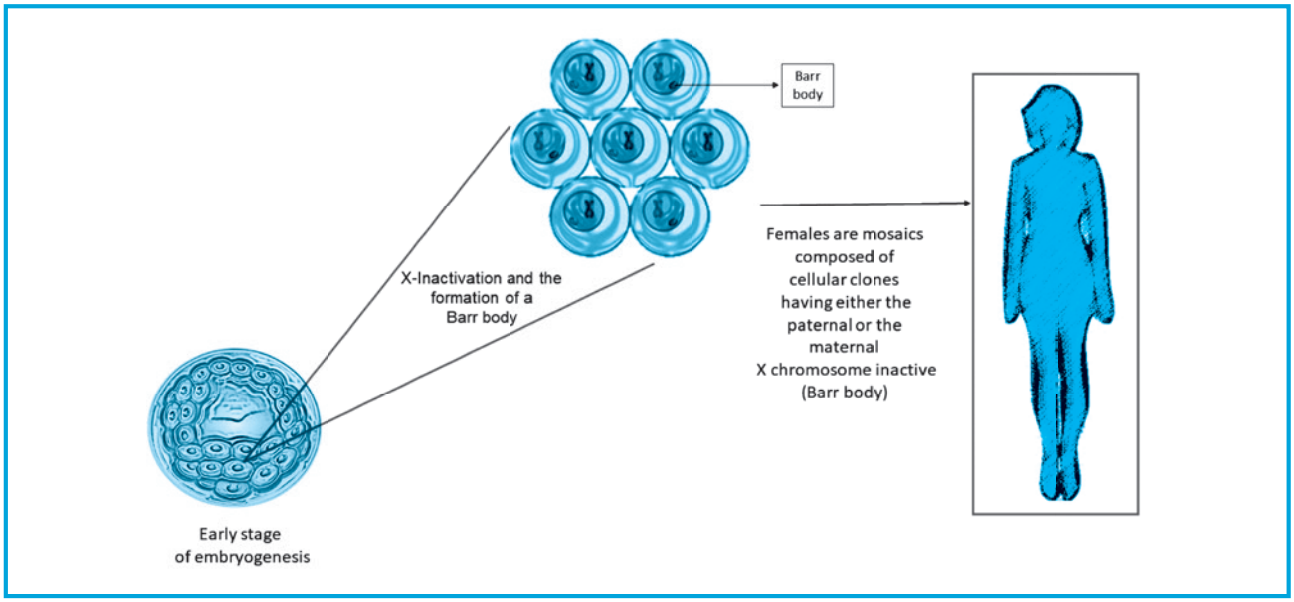

Figure 3. Due to dosage compensation, in females one X chromosome is inactivated in every cell. The inactivation occurs early in development when the embryo consists of only a few cells. The inactivation is random. The inactivated X chromosome forms the Barr body still in all descendent cells. With respect to their X chromosomes, females are chimeras: some areas of their bodies have the paternal X chromosome active and other areas have the maternal X chromosome active.

female cells and is arbitrarily defined, for example, as a pattern where 80% or more of the cells inactivate the same X-chromosome. This deviation may be the result of chance or genetic factors involved in the XCI or a selection process[28].

Males and females show strong differences in their susceptibility to many diseases, such as infectious diseases, autoimmune diseases and some forms of cancer. Generally, females show increased susceptibility to autoimmune disease development and males show increased susceptibility to non reproductive malignant cancers[29].

The most striking sex differences in autoimmune diseases are observed in Sjogren's syndrome, systemic lupus erythematosus, autoimmune thyroid diseases, and scleroderma, with about 80% of patients being females. Also for rheumatoid arthritis, multiple sclerosis and myasthenia gravis there is a lower female prevalence but still 60-75% of the patients are women (Table 2).

The sexually dimorphic prevalence of autoimmune diseases has been suggested to be due to sex hormone influences because the X-chromosome contains a considerable number of sex and immune-related genes such as *AR*, *IL2* receptor gamma chain, *CD40 ligand* and *FOXP3*[28]. These genes are essential in determining sex hormone levels and, more importantly, immune tolerance. An alternative explanation for the female predominance has been proposed by means of an enhanced skewed X-chromosome inactivation found in peripheral blood cells of female patients with autoimmune diseases[28].

Table 2. Sex ratio between females and males in some autoimmune disorders.

Autoimmune disease	Sex ratio [F:M]
Systemic lupus erythematosus	9:1
Hashimoto's thyroiditis	3-5:1
Graves' disease	7:1
Sjögren syndrome	9:1
Rheumatoid arthritis	7-8:1
Myasthenia gravis	3:1

Fairweather et al, 2008[55]; Cooper et al, 2003[56].

Skewed XCI and diseases

A role for skewed XCI has been found in female subjects with autoimmune tyroid diseases (Graves' disease and Hashimoto's thyroiditis)[30], scleroderma[31], rheumatoid arthritis and systemic sclerosis[32] which could, in part, explain the strong female preponderance observed in these diseases. Interestingly, besides the presence of numerous X-located genes having a direct or indirect role in immunity, some of them are responsible for X-linked primary immunodeficiencies[32-34].

Additionally, skewed X chromosome inactivation has been implicated in both the expression and the

suppression of X-linked disease phenotypes and has been reported to occur more frequently in breast and ovarian cancer patients, including *BRCA1* or *BRCA2* mutation carriers, than in control subjects; moreover it is associated with a statistically significant increase in age at diagnosis of breast and ovarian cancer[35]. Extremely skewed X-chromosome inactivation is increased in pre-eclampsia[36].

Erroneous epigenetic modifications due to environmental perturbations such as manipulation and culture of embryos during in vitro fertilization (IVF) are linked to various short- or long-term consequences. Among these, the sex ratio is skewed in animal embryos and even in human IVF newborns. This was found to be a result of female-biased peri-implantation developmental defects that were originated from impaired imprinted X chromosome inactivation (iXCI). Thus impaired XCI represents one of the major epigenetic barriers for the developmental competence of female embryos during preimplantation stage[37].

"Escape genes"

Inactivation of one X chromosome in mammalian females achieves dosage compensation between XX females and XY males; however, is often incomplete and over 15% of human X-linked genes are expressed from both the active and inactive X chromosomes (Xa and Xi, respectively). New methods for genomic analysis have improved our identification and characterization of these "escape genes", revealing the importance of DNA sequence, chromatin structure, and chromosome ultrastructure in regulating expression from an otherwise inactive chromosome. Current estimates suggest that 12-20% of human and 3-7% of mouse X-linked genes are exceptions-escape genes that are expressed from both the Xa and the Xi, with the degree of 'escape' from inactivation varying between genes. Genome-wide mutation identification is revealing roles for escape genes in cancer and heritable disease, while mouse models reveal the importance of the Xi, particularly in the brain and metabolism[38].

Recent analyses show that incomplete XCI is mostly shared between individuals and tissues, and extend previous surveys by pinpointing several examples of variability in the degree of XCI escape between cells, chromosomes, and tissues. In addition escape from XCI results in sex-biased expression of at least 60 genes, potentially contributing to sex-specific differences in health and disease[39]. Interestingly epigenetic marks differ between genes subject to and escaping from XCI. Subject genes have many chromatin marks typical of inactive heterochromatin, such as H3K9me3, and are also depleted for active marks such as H3K4me3. By contrast, escape genes retain active histone marks such as H3K4me2 and are depleted for the repressive mark H3K27me3[38].

The biological implications of this phenomenon remain to be fully explored, however likely can explain the between-female and male-female diversity and perhaps different disease susceptibility.

MiRNA

It has been also hypothesized that X chromosome-associated mechanisms, which affect X-linked genes and are behind the immunological advantage of females, may also affect X-linked microRNAs. These small non-coding RNAs regulate gene expression by translational repression and/or messenger RNA degradation. There is a higher concentration of miRNAs on the X chromosome when compared to autosomes. Likely, the human X chromosome contains 10% of all microRNAs detected so far in the human genome. Actually, miRbase[40] reports 4 miRNA on Y chromosome and 118 on X chromosome. Although the role of most of them has not yet been described, several X chromosome-located microRNAs have important functions in immunity and cancer[34]. According to Pinheiro and coworkers[34], disturbances in the normal inactivation pattern of miRNAs on the X chromosome, by means of silencing escape or inactivation skewing, could affect miRNAs-driven gene regulation and result in gender-specific responses. MiRNAs can be located in the intergenic genome (i.e. between genes), or in intronic regions within genes. Moreover, some of them are located within genes that have been shown to escape XCI, such as *DMD*, *CHM*, *ATP11C*, or *IRAK*[34-41]. Therefore female mosaicism, silencing escape or skewed patterns of inactivation of X-linked miRNAs involved in immunity could lead to unbalanced miRNA expression between sexes, and to sex-specific immune responses. Furthermore we must keep in mind that many miRNAs have multiple targets in the genome, this can result in cascade-effect and lead to greater differences between genders in terms of regulation, compared to what was previously thought[34]. Although the role of most X-linked miRNAs is not known, several of them participate in cancer onset and progression, and regulate the immune system at different levels. Amongst the X-linked miRNAs involved in immune regulation, for instance mir-18b has been found to have a role in multiple sclerosis and *ERa* gene is its target[42].

Role for escape genes in male-female differences

The escape genes are most likely to underlie the phenotype found in the X chromosome aneuploidies, but are also being revealed to have a more widespread impact.

Intellectual disability, which affects 1-3% of the human population, is characterized by a considerable gender bias; it is believed to reflect the prevalence of X-linked mutations in about one hundred genes. X-linked mutations causing female intellectual disability are considerably rarer, but have been identified in two genes (*DDX3X* and *USP9X*) known to escape from XCI[38,43,44].

It is likely that dysregulation of these and other miRNAs expression on the X chromosome, by means of irregularities in the process of DNA methylation such as may occur during silencing escape, may be partly responsible for differences for instance in immune responses between genders. Potentially, mutations arising in these miRNAs or in their regulatory sequences could also contribute to gender differences in immune responses.

Environmental factors and gender-specific response

Even more evidence is accumulating from epidemiological studies that there are sex differences in the response to a variety of neurotoxicants. For instance, clinical studies of the effects of lead (Pb) on the neurodevelopment of children show clear sex-dependent effects of developmental Pb exposure on the brain and behavior. Considering the potential impact that Pb exposure may have on epigenetic responses in the brain and on DNA methylation in particular, a study on rats prenatally exposed to Pb was performed. The highest number of significant differentially methylated regions was found in females exposed to Pb at the lowest exposure level. These data reinforce the significant effect that low level Pb exposure may have on gene-specific DNA methylation patterns in brain and underline that this occurs in a sex-dependent manner[45].

The prenatal period is a critical window in development and the potential relationship between environmental exposure and DNA methylation at birth may provide an opportunity for epidemiological research with implications concerning increased disease risk.

A recent study carried out in human infants revealed decreases in methylation of *LINE-1*, *IGF2*, and *PPARA* regions, with increasing endocrine disrupting chemicals (EDCs) concentrations, evaluated in cord blood. A sex-stratified analysis of EDCs and DNA methylation showed that some relationships were female-specific. These findings add to a body of evidence prompting epigenetically labile regions may be the reading key linking early exposures with disease risk later in life. Moreover suggest that toxicoepigenetic susceptibility may be sex specific[46].

From studies in animal models and human birth cohorts key developmental periods have been identified, of high importace for epigenetic programming and vulnerable to environmental insults. Therefore, epigenetic modifications represent a potential mechanism through which sexually dimorphic effects of early-life exposures display[47].

Concluding remarks

There is currently an increasing interest in precision medicine, which is "an emerging approach for disease treatment and prevention that takes into account individual variability in genes, environment, and lifestyle for each person"[48].

This approach must necessarily include the genders. For instance drug therapy is not yet optimized for both genders. Although women are now included in clinical trials of drugs, devices and biologics, there remains inadequate analysis of whether outcomes differ between men and women. The different efficacy of drugs in women and men is due to biological differences which may be caused by sex-specific gene expression likely triggered by sex-specific epigenetic modifications. In addition, gender plays a role in drug efficacy as a sociocultural dimension that may lead to differences between women and men.

Likely there are many players for gender differences in human complex diseases. A significant proportion of human imprinted genes are realistically implicated in complex diseases, as well as monoallelic expression of critical regions (due, for instance, to a skewed inactivation of X-linked genes in females or to a X-linked miRNA deregulation) can result in pathological outcomes.

Therefore the expression of specific genes related to complex diseases can be modified by various mechanisms influenced by gender and also by environmental factors, likely acting in critical windows, during maternal pregnancy and interfering with fetal programming.

Key messages

- Males and females show large differences in their susceptibility to many complex diseases, including autoimmune diseases, some forms of cancer and neurological diseases.
- Autoimmune diseases are characterized by a greater female component, which for some diseases, such as systemic lupus erythematosus, even reach 80%.
- The two well known cellular processes involving epigenetic mechanisms dealing with gender differences and arising very early during the embryo development, are genomic imprinting and X-chromosome inactivation in females.
- The brain has emerged as a main target of genomic imprinting, generating great interest on how this epigenetic regulation provides stable transcriptional control of neural development and behavior.
- It is likely that the expression of specific genes related to complex diseases can be modified by various mechanisms influenced by gender and also by environmental factors, likely acting in critical windows, during maternal pregnancy and interfering with fetal programming.

References

1. Gabory A, Roseboom TJ, Moore T, et al. Placental contribution to the origins of sexual dimorphism in health and diseases: sex chromosomes and epigenetics. Biol Sex Differ 2013; 4 (1): 5.
2. Cassidy FC, Charalambous M. Genomic imprinting, growth and maternal-fetal interactions. J Exp Biol 2018; 221: jeb164517.
3. Ishida M, More GR. The role of imprinted genes in humans. Mol Aspects Med 2013; 34 (4): 826-40.
4. Hore TA, Rapkins RW, Graves JA. Construction and evolution of imprinted loci in mammals. Trends Genet 2007; 23 (9): 440-8.
5. Jirtle RL. Interview. Epigenomics, imprinting and disease susceptibility. Pharmacogenomics 2008; 9 (12): 1791-5.
6. Moore T, Haig D. Genomic imprinting in mammalian development: a parental tug-of-war. Trends Genet 1991; 7 (2): 45-9.
7. Frost JM, Moore GE. The importance of imprinting in the human placenta. PLoS Genet 2010; 6 (7): e1001015.
8. Jirtle RL. Epigenome: the program for human health and disease. Epigenomics 2009; 1 (1): 13-6.
9. Morison IM, Reeve AE. A catalogue of imprinted genes and parent-of-origin effects in humans and animals. Hum Mol Genet 1998; 7 (10): 1599-609.
10. Court F, Tayama C, Romanelli V, et al. Genome-wide parent-of-origin DNA methylation analysis reveals the intricacies of human imprinting and suggests a germline methylation-independent mechanism of establishment. Genome Res 2014; 24 (4): 554-69.
11. Monk D. Genomic imprinting in the human placenta. Am J Obstet Gynecol 2015; 213 (4 Suppl): S152-62.
12. Al-Qaraghouli M, Fang YMV. Effect of fetal sex on maternal and obstetric outcomes. Front Pediatr 2017; 5: 144.
13. Bale TL. The placenta and neurodevelopment: sex differences in prenatal vulnerability. Dialogues Clin Neurosci 2016; 18 (4): 459-64.
14. Adam I, Salih MM, Mohmmed AA, et al. Pregnant women carrying female fetuses are at higher risk of placental malaria infection. PLoS One 2017; 12 (7): e0182394.
15. Jiang S, Teague AM, Tryggestad JB, et al. Role of microRNA-130b in placental PGC-1α/TFAM mitochondrial biogenesis pathway. Biochem Biophys Res Commun 2017; 487 (3): 607-12.
16. Lawson HA, Cheverud JM, Wolf JB. Genomic imprinting and parent-of-origin effects on complex traits. Nat Rev Genet 2013; 14 (9): 609-17.
17. Varshney M, Nalvarte I. Genes, gender, environment, and novel functions of estrogen receptor beta in the susceptibility to neurodevelopmental disorders. Brain Sci 2017; 7 (3): pii: E24.
18. Badcock C, Crespi B. Battle of the sexes may set the brain. Nature 2008; 454: 1054-5.
19. Perez JD, Rubinstein ND, Dulac C. New perspectives on genomic imprinting, an essential and multifaceted mode of epigenetic control in the developing and adult brain. Annu Rev Neurosci 2016; 39: 347-84.
20. Bjornsson HT. The Mendelian disorders of the epigenetic machinery. Genome Res 2015; 25 (10): 1473-81.
21. Loke H, Harley V, Lee J. Biological factors underlying sex differences in neurological disorders. Int J Biochem Cell Biol 2015; 65: 139-50.
22. Ruitenberg A, Ott A, van Swieten JC, et al. Incidence of dementia: does gender make a difference? Neurobiol Aging 2001; 22: 575-80.
23. Van Den Eeden SK, Tanner CM, Bernstein AL, et al. Incidence of Parkinson's disease: variation by age, gender, and race/ethnicity. Am J Epidemiol 2003; 157: 1015-22.
24. Watanabe M, Honda C, Osaka, Iwatani Y, et al. Within-pair differences of DNA methylation levels between monozygotic twins are different between male and female pairs. BMC Med Genomics 2016; 9 (1): 55.
25. Yousefi P, Huen K, Davé V, et al. Sex differences in DNA methylation assessed by 450 K BeadChip in newborns. BMC Genomics 2015; 16: 911.
26. Gregg C, Zhang J, Butler JE, et al. Sex-specific parent-of-origin allelic expression in the mouse brain. Science 2010; 329: 682-5.
27. Wilkinson LS, Davies W, Isles AR. Genomic imprinting effects on brain development and function. Nat Rev Neurosci 2007; 8 (11): 832-43.
28. Chabchoub G, Uz E, Maalej A, et al. Analysis of skewed X-chromosome inactivation in females with rheumatoid arthritis and autoimmune thyroid diseases. Arthritis Res Ther 2009; 11 (4): R106.
29. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol 2016; 16 (10): 626-38.
30. Simmonds MJ, Kavvoura FK, Brand OJ, et al. Skewed X chromosome inactivation and female preponderance in autoimmune thyroid disease: an association study and meta-analysis. J Clin Endocrinol Metab 2014; 99 (1): E127-31.
31. Uz E, Loubiere LS, Gadi VK, et al. Skewed X-chromosome inactivation in scleroderma. Clin Rev Allergy Immunol 2008; 34: 352-5.
32. Kanaan SB, Onat OE, et al. Evaluation of X chromosome inactivation with respect to HLA genetic susceptibility. PLoS One 2016; 11 (6): e0158550.
33. Pessach IM, Notarangelo LD. X-linked primary immunodeficiencies as a bridge to better understanding X-chromosome related autoimmunity. J Autoimmun 2009; 33 (1): 17-24.
34. Pinheiro I, Dejager L, Libert C. Bioessays. X-chromosome-located microRNAs in immunity: might they explain male/female differences? The X chromosome-genomic context may affect X-located miRNAs and downstream signaling, thereby contributing to the enhanced immune response of females. Bioessays 2011; 33 (11): 791-802.
35. Lose F, Duffy DL, Kay GF, et al. Skewed X chromosome inactivation and breast and ovarian cancer status: evidence for X-linked modifiers of BRCA1. J Natl Cancer Inst 2008; 100:1519-29.
36. Uz E, Dolen I, Al AR, et al. Extremely skewed X-chromosome inactivation is increased in pre-eclampsia. Hum Genet 2007; 121 (1): 101-5.
37. Tan K, An L, Miao K, et al. Impaired imprinted X chromosome inactivation is responsible for the skewed sex ratio following in vitro fertilization. Proc Natl Acad Sci USA 2016; 113: 3197-202.

38. Balaton BP, Brown CJ. Escape artists of the X chromosome. Trends Genet 2016; 32 (6): 348-59.
39. Tukiainen T, Villani AC, Yen A, et al. Landscape of X chromosome inactivation across human tissues. Nature 2017; 550: 244-8.
40. http://www.mirbase.org/
41. Carrel L, Willard HF. X-inactivation profile reveals extensive variability in X-linked gene expression in females. Nature 2005;434: 400-4.
42. Sanders KA, Benton MC, Lea RA, et al. Next-generation sequencing reveals broad down-regulation of microRNAs in secondary progressive multiple sclerosis CD4+ T cells. Clin Epigenetics 2016; 8 (1): 87.
43. Snijders BL, Madsen E, Juusola J, et al. Mutations in DDX3X are a common cause of unexplained intellectual disability with gender-specific effects on Wnt signaling. Am J Hum Genet 2015; 97 (2): 343-52.
44. Reijnders MR, Zachariadis V, Latour B, et al. De novo loss-of-function mutations in USP9X cause a female-specific recognizable syndrome with developmental delay and congenital malformations. Am J Hum Genet. 2016 Feb 4;98(2):373-81.
45. Singh G, Singh V, Sobolewski M, et al. Sex-dependent effects of developmental lead exposure on the brain. Front Genet 2018; 9: 89.
46. Montrose L, Padmanabhan V, Goodrich JM, et al. Maternal levels of endocrine disrupting chemicals in the first trimester of pregnancy are associated with infant cord blood DNA methylation. Epigenetics 2018; 18: 1-9.
47. McCabe C, Anderson OS, Montrose L, et al. Sexually dimorphic effects of early-life exposures to endocrine disruptors: sex-specific epigenetic reprogramming as a potential mechanism. Curr Environ Health Rep 2017; 4 (4): 426-38.
48. https://ghr.nlm.nih.gov
49. Saha S, David Chant D, Welham J, et al. A systematic review of the prevalence of schizophrenia. PLoS Med 2005; 2 (5): e141.
50. Miller IN, Golomb AC. Gender differences in Parkinson's disease: clinical characteristics and cognition. Mov Disord 2010; 25 (16): 2695-703.
51. Albert PA. Why is depression more prevalent in women? J Psychiatry Neurosci 2015; 40 (4): 219-21.
52. McLean CP, Asnaani A, Litz B, et al. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. J Psychiatr Res 2011; 45 (8): 1027-35.
53. Loomes R, Hull L, Mandy WPL. What is the male-to-female ratio in autism spectrum disorder? A systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2017; 56 (6): 466-74.
54. Schmidt R, Kienbacher E, Benke T, et al. Sex differences in Alzheimer's disease. Neuropsychiatr 2008; 22 (1): 1-15.
55. Fairweather DL, Frisancho-Kiss, Rose N R. Sex differences in autoimmune disease from a pathological perspective. Am J Pathol 2008; 173 (3): 600-9.
56. Cooper GS, Stroehla BC. The epidemiology of autoimmune disease. Autoimmun Rev 2003; 2 (3): 119-25.

Conflict of interest statement: the Authors declare no financial disclosures related to the content of this article.

Correspondence to:
Lucia Migliore
Department of Translational Research and New Technologies in Medicine and Surgery
University of Pisa
Via Roma 55
56126 Pisa
email lucia.migliore@med.unipi.it