

Gender and complex diseases: insights into sex-specific epigenetics

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Summary. Gender is responsible for lifetime differences between males and females, conferring variable severity and susceptibility to complex diseases. The different genetic architecture between men and women can be found in human complex traits in relation to the onset of diseases such as glioma and Alzheimer's disease (AD). However, epigenetic events occurring soon after fertilization and modulated over a lifetime in response to external conditions may contribute as well. Besides an illustration of the epigenetic mechanisms involved in sexual differentiation, an overview of the knowledge concerning sex-specific epigenetics is provided for cardiovascular diseases, neurological disorders and cancer, given their dramatic impact on patients' quality of life. Knowledge of gender-related epigenetics needs more focus and, in this regard, the possibility of applying next generation approaches will provide a comprehensive overview of sex-related differences. The combination of genetic and epigenetic information with molecular and phenotypic data will improve the knowledge of different complex disorders and enable identification of diagnostic, therapeutic, prognostic and predictive biomarkers.

Key words: gender, epigenetics, complex diseases, sexual dimorphism.

Genere e malattie complesse: meccanismi epigenetici sesso-specifici

Riassunto. Il genere contribuisce fortemente alle differenze che intercorrono tra individui di sesso maschile e femminile. Nel campo delle malattie complesse è nota una diversa incidenza legata al rapporto fra i sessi, che ne suggerisce un ruolo importante nel rischio e nella progressione di numerose malattie. Fattori genetici sesso-specifici sono coinvolti nell'insorgenza e sviluppo di patologie come il morbo di Alzheimer (AD) e il glioma. Inoltre, è ormai noto che i meccanismi epigenetici sesso-specifici possono influenzare in maniera rilevante diverse malattie multifattoriali. Il ruolo dell'epigenetica nel differenziamento sessuale, ad esempio, è descritto nello sviluppo di malattie cardiovascolari, nel cancro e in alcune patologie neurodegenerative. A tal proposito, è necessario avere una maggior conoscenza sui meccanismi epigenetici che influenzano queste patologie e ne evidenziano una differenza nell'incidenza fra uomini e donne. La possibilità di applicare protocolli di nuova generazione e combinare successivamente le informazioni ottenute con dati molecolari e fenotipici condurrà verso l'identificazione di nuovi meccanismi coinvolti nell'eziopatogenesi delle diverse malattie, nonché

all'identificazione di nuovi bersagli terapeutici e di nuovi biomarcatori diagnostici, prognostici e predittivi.

Parole chiave: genere, epigenetica, malattie complesse, dimorfismo sessuale.

Introduction

To date, several studies have highlighted some differences between males and females concerning brain activity (in terms of processing, chemistry, structure and activity), severity and susceptibility to different diseases. In this regard, cardiovascular diseases (CVDs), cancer, neurodegenerative and ocular diseases have shown a sex-specific incidence. Concerning neurodegenerative disorders, multiple sclerosis (MS) and Alzheimer's disease (AD) have a higher incidence in females^{1,2}. On the other hand, males have been found to be at higher risk of Parkinson's disease (PD)³. Similarly, several cancer types, including melanoma, colon cancer, squamous cell carcinoma, hematological malignancies (especially lymphomas), exhibit a higher incidence and a more severe progression in males. Concerning brain-related cancers, the incidence of glioma is 50% higher in male than female individuals^{4,5}. Among ocular diseases, a higher risk of age-related macular degeneration (AMD) has been found in women with respect to men in a study performed on the Italian population⁶. Gender-related effects may arise from specific genetic interactions as well as from the contribution of sex-related epigenetic events, which occur early after fertilization and can also be influenced by environmental conditions. Epigenetic modifications indicate those changes occurring at chromatin level, without altering the DNA sequence⁷. In particular, transcription can be regulated by covalent DNA modifications, including the methylation of the promoter regions of genes and histone modifications, both of which can affect chromatin conformation; microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) exerting a post-transcriptional regulation on the expression of target-genes. lncRNAs can also bind miRNAs and take part in protein-protein interactions⁸. Given the strong impact of epigenetics on physiological and biological processes, this review is di-

rected at providing interesting insights into gender-specific (epi)genetic mechanisms that may affect many complex traits, while pointing out the present limitations and the future perspectives of research into gender epigenetics.

Gender-specific genetic association in human complex diseases

Gender determination can be strongly affected by genetic variability, leading to peculiar allelic architectures observed among men and women which can be responsible for specific sex-by-gene (GxS) interactions within the human population⁹. These kinds of interactions have been investigated in order to better understand how they impact on complex traits in health and disease conditions. In particular, a large-scale study on a British population analyzed the genetic heterogeneity among sexes considering 19 complex traits. In particular, sex-specific genotypes have been found to contribute with a moderate-effect size to the phenotype of a proportion of traits, including blood pressure, height, waist and hip circumference. Concerning the potential role of sex-specific interactions in the pathogenesis and severity of diseases, genome wide association studies (GWAS) provided intriguing information in this regard. In particular, polymorphisms located in *SERPINB1* (6p25.2, *Serpin peptidase inhibitor, clade B, member 1* which regulates neutrophil cell death expressed in microglia¹⁰), *GMNC* (3q28, *Geminin Coiled-Coil Domain-Containing Protein*, which has been associated with intracranial volume¹¹), and *APOE* (19q13.32, *Apolipoprotein E*, which is one of the most relevant susceptibility factor for AD¹²), have been associated with amyloidosis and increased total levels of Tau in the cerebrospinal fluid of women¹³. Ostrom et al. (2018) found different genetic variants associated with glioma: for instance, the rs11979158 polymorphism located within *EGFR* (7p11.2, *Epidermal Growth Factor Receptor* and known to be implicated in several cancer types) was strongly associated with the susceptibility to glioma in males⁵. Moreover, a stronger association was found between female sex and rs55705857 (8q24.21), which is the main genetic susceptibility factor for glioma^{5,14}. Interestingly, polymorphisms of the *EGFR* gene (such as rs2227983) have been associated with a better overall survival rate in females with metastatic colon cancer, while a worse prognosis was observed in males carrying the same genetic variants¹⁵. Gender-specific genetic factors therefore suggest the potential existence of molecular pathways that may differentially trigger the onset of complex diseases among sexes. Nevertheless, the differential susceptibility and progression of disease depending on the gender can be explained not only by genetic factors, but also when considering the role of epigenetic modifications in males and females.

Epigenetics and sexual differentiation

Epigenetic mechanisms play a crucial role in sexual differentiation, especially to ensure the permanent inactivation of one copy of the X-chromosome in women and compensate gene copy differences in male cells^{16,17}. The inactivation of the X-chromosome is controlled by a regulatory locus known as X-inactivation centre (Xic). This region encodes the lncRNA XIST and other regulators of the X-inactivation process. XIST is essential to allow the “coating” of the X-chromosome to be inactivated and leads to chromatin modifications and spatial rearrangement of the chromosome which is permanently silenced in the end. Moreover, this mechanism is regulated by other factors, including: the repressor transcript TSIX, which is the XIST antisense RNA and counteracts XIST activity; the E3 ubiquitin protein ligase *RNF12* (Xq13.2, *Ring Finger Protein 12*), which is an activator of the X-inactivation process together with different lncRNAs (such as JPX and FTX) that work as positive regulators of XIST¹⁸. On the other hand, on the Y chromosome, the *SRY* (Yp11.2, *Sex-determining Region Y*) activation is required for the development of testes and the consequent release of testosterone under the control of DNA demethylation or histone modifications¹⁹. Moreover, genes encoding epigenetic modifiers, such as lncRNAs, miRNAs and histone demethylases, are located within the sex chromosomes and may modulate the differential expression of genes on autosomes in a sex-dependent way. In particular, X-linked miRNAs can participate in the sex-specific regulation of the immune response, by targeting immune-related genes^{17,20,21}. Genomic imprinting leads to the silencing of either paternal or maternal alleles through DNA methylation, thereby modulating the differential expression among males and females. It is widely known how sex differences depend on the hormonal milieu, especially the steroid hormone-dependent mechanisms. The signaling hormonal pathways include epigenetic events that contribute in modulating the subsequent cellular responses. As a matter of fact, the signal transduction of steroids, namely progesterone, estrogens and glucocorticoids, requires intracellular receptors that act as ligand-dependent transcription factors. Following the binding of the hormone, steroid receptors translocate to the nucleus where they directly recognize steroid responsive elements on the DNA sequence and recruit histone modifiers that contribute in modulating the expression of genes targeted by the sex hormone. One of the most important co-activators of this mechanism is *NCOA1* (2p23.3, *Nuclear receptor co-activator 1*), which acts as a histone acetyltransferase and modulates gene expression by enhancing chromatin relaxation²². Moreover, the activity of sex hormones also involves other epigenetic levels (such as DNA methylation and chromatin organization)⁴, highlighting thereby

the fundamental role of epigenetics in the physiological sex-differentiation mechanisms.

Sex-specific epigenetics in cardiovascular diseases (CVDs)

CVDs represent one of the leading causes of death²³ and differences between men and women are well-established. Men develop CVDs at a younger age than women, although women often develop CVD in concomitance with comorbidities and manifest a more severe coronary artery disease¹⁷. Gender-specific epigenetic modifications may contribute in conferring a differential susceptibility to the onset of disease. On this subject, differential methylation of genes associated with CVD and involved in aging, lipid metabolism and blood lipid levels has been reported. For instance, the methylation of promoters of *CETP* (16q13, *Cholesteryl Ester Transfer Protein*), *LPL* (8p21.3, *Lipoprotein Lipase*), *PLTP* (20q13.12, *Phospholipid Transfer Protein*) was found to be higher in females^{24,25}. Conversely, *ABCG1* (21q22.3, *ATP-Binding Cassette, Subfamily G, Member 1*) displayed higher methylation in men. The search for differences in DNA methylation provided interesting data concerning aging and methylation levels in human tissues and fluids. During aging, males showed a higher degree of DNA methylation changes in blood, brain and saliva²⁶. Concerning CVDs, various methylation differences have been found: in *MMP2* (16q12.2, *Matrix Metalloproteinase 2*), a higher hypomethylation of the promoter has been reported for males suffering from ischemic stroke²⁷. Conversely, DNA methylation of *INS* (11p15.5, *Insulin*) and *GNASAS* (20q13.32, *Gnas Complex Locus, Antisense Transcript 1*) genes, which are both expressed in prenatal life, has been found to be higher in the leukocytes of females affected by myocardial infarction. Intriguingly, this association suggests that sex-specific susceptibility may be established in the early stages of life²⁸. Similarly, for coronary artery disease, methylation of *PLA2G7* (6p12.3, *Phospholipase A2, Group VII*) was found to be differently expressed in females²⁹ whereas *PTX3* (3q25.32, *Pentraxin 3*) methylation was associated with a higher neutrophil to lymphocyte ratio in men, which may be an inflammatory hallmark of CVDs^{30,31}. Interestingly, *F2RL3* (19p13.11, *Coagulation Factor II Receptor-Like 3*) hypomethylation correlates to CVDs-related mortality, with a stronger association for males³². Despite the data concerning the alteration of DNA methylation of gene promoters, very little is known about the role of other epigenetic events in CVDs¹⁷. Nevertheless, epigenetic modifications may provide interesting insights concerning the influence of sex on CVDs and may be utilized as biomarkers to predict the susceptibility, severity and, eventually, the prognosis of these disorders^{33,34}.

Sex-specific epigenetics in brain development and neurodegeneration

Sexual differentiation can impact the structure and function of human brain during human development and lifetime through sex hormones-related processes¹⁹. Moreover, sex hormones can dynamically shape and modulate cognitive function and emotions³⁵, which can also be subjected to environmental stimuli and epigenetic modifications. The role of epigenetics in brain activities and the brain differentiation between the sexes is under investigation in rodent models. In females, the developing brain is supposed to be feminized by default, while it becomes "defeminized" in males by the activation of *SRY* through specific changes in DNA methylation and histone modifications. On this subject, male mice lacking the histone demethylase *Jmjd1*, showed a male-to-female sex reversal process³⁶. Moreover, histone acetylation exerts a function in the testosterone-related masculinization process in two sexually dimorphic brain regions in mice, the bed nucleus of stria terminalis³⁷ and the medial preoptic area³⁸. Concerning the existence of sex-specific environmental effects inducing epigenetic alterations, intriguing evidence has been reported for the bisphenol A (BPA) action on the neurodevelopment. In fact, high maternal BPA levels in utero led to DNA methylation changes of *BDNF* (11p14.1, *Brain-Derived Neurotrophic Factor*), which is involved in synaptic plasticity, in the cord blood of humans and in the hippocampus and blood of mice. In particular, *BDNF* methylation levels were found to be increased in men at birth. Interestingly, *BDNF* expression undergoes alterations in psychiatric disorders related to early-life adversities, such as depression and schizophrenia^{39,40}. In general, environmental factors can impact neuroendocrine and sex-hormones-related pathways, which in turn may exploit epigenetic modifiers to establish sex-specific differences^{19,41,42}. Epigenetic mechanisms underlying sex differences are likely to be involved also in the neuroinflammatory processes whose deregulation leads to neurodegenerative and autoimmune disorders. Sex hormones regulate inflammatory pathways in the brain. In fact, estrogens can downregulate the neuroinflammatory cascade and inhibit the release of pro-inflammatory cytokines, thereby impairing the inflammatory response^{1,43} which is known to be deregulated in autoimmune diseases such as MS. MS is a neurodegenerative and autoimmune disease characterized by sex differences: women show a higher rate of inflammation compared to males, although they also present a slower progression of disease¹. Moreover, a maternal parent-of-origin effect has been found in the inheritance of MS risk⁴⁴. Sex-related differences influence AD and PD incidence and pathogenesis as well. Concerning AD, females experience a faster progression of hippocampal atrophy than males.

In general, males show more aggressive behaviors and comorbidities while females show more affective symptoms and disability but survive longer than males. As previously mentioned, the male sex is regarded as one of the relevant risk factors for PD. Men exhibit more severe motor symptoms than women and a greater improvement of motor function upon levodopa administration was observed in women, suggesting a neuroprotective effect of estrogens^{1,3}. Estrogen treatment has been found to have a protective effect on striatal lesions only in female PD gonadectomized rat models⁴⁵. To date, little is known about the sex-specific epigenetic patterns and epigenetic modifiers involved in these diseases. Nevertheless, the role of sex hormones in neurodevelopment suggests the possible role of sex-specific epigenetic modifications to contribute to or counteract AD, PD or MS etiopathogenesis. The impact of epigenetics in sexual dimorphisms at brain level might therefore shed light on the molecular networks involved in the etiology and treatment of many neurological and psychiatric diseases.

Sex-specific epigenetics in cancer

Gender can have a significant impact on cancer onset and progression, as shown by the higher incidence of different types of cancer recorded among the male population⁴⁶. The main differences between men and women in susceptibility to cancer diseases can be highlighted at the molecular level by the activity of specific sex hormones. Indeed, the signaling hormonal pathways affect cancer susceptibility and severity through multiple mechanisms, including self-renewal mechanisms, tumor microenvironment, immune system and metabolism⁴. However, the contribution of epigenetic events in this regard still needs elucidation. Evidence of sex-specific epigenetic regulation comes from research projects on the role of sex-chromosomes in cancer, especially on the X chromosome. Different epigenetic modifier genes are spread along this chromosome. For instance, *ZMYM3* (Xq13.1, *Zinc Finger, MYM-Type 3*) is a component of histone-deacetylase complexes and is mutated in males affected by medulloblastomas; *KDM5C* (Xp11.22, *Lysine-specific Demethylase 5C*) encodes a histone demethylase and can trigger genomic instability in renal carcinoma cells⁴. Intriguingly, *KDM6A* (Xp11.3, *Lysine-specific Demethylase 6A*) codes for a histone demethylase which evades the X-inactivation process and is expressed by both the chromosomes in females. Male individuals, on the other hand, carry a homologous gene on the Y chromosome, namely *KDM6C* (Yq11.221, *Lysine-specific Demethylase 6C*), which does not produce a completely functional protein⁴⁷. Therefore, loss-of-function mutations within *KDM6A* cannot be compensated in males in contrast to females. These kinds of mutations usu-

ally occur in T-acute lymphocyte leukemia⁴⁸ and renal cell carcinoma⁴⁹, which are two of the most prevalent cancers among males⁴. Moreover, the X-chromosome harbors several miRNAs that have a function in oncogenetic processes. MiR-221 and miR-222 map in the same genomic region of *KDM6A*, being thereby susceptible to evading X-inactivation, may be involved in the onset of several cancers. These miRNAs are able to alter the expression of p27^{Kip1} (*CDKN1B*, 12p13.1, Cyclin-Dependent Kinase Inhibitor 1B), which is a cell cycle inhibitor. Therefore, modulation of miR-221 and miR-222 expression results in an impairment of p27^{Kip1} activity, which may be involved in the development of tumors. However, this data needs further investigation^{4,50}. Other X-linked miRNAs, such as miR-20b, miR-361 and the miR-506 cluster, are involved in pathways known to be deregulated in tumors, including PI3K/AKT. However, any eventual sex-specific action of these miRNAs needs to be investigated⁴. In conclusion, sex hormones and sex chromosomes encode information that can impact on several stages of the cancer process and are likely to involve epigenetic modifications that, in turn, might account for sex-specific differences which cannot be fully explained by environmental or genetic factors.

Conclusion

Sexual dimorphism influences molecular pathways and networks leading to the establishment of lifetime differences among males and females. Sex-specific epigenetic modifications contribute to these differences although the epigenetic landscape can be shaped and changed during lifetime in response to environmental stimuli. Therefore, the expression of specific genes related to complex traits and diseases may be modulated by epigenetic mechanisms under the influence of sex and external conditions. Understanding the role of sex-specific epigenetic markers concerning the susceptibility, onset and progression of multifactorial disease is intriguing but difficult to resolve. Indeed, more comprehensive and higher resolution epigenomic studies should be implemented, in order to analyze sex differences on a large cohort of subjects. Given the difficulties in the realization of epigenome studies on human subjects, the utilization of rodent models remains fundamental, especially for neuroepigenome research. However, the utilization of "in vitro" models of human diseases provided by iPSCs (human Induced Pluripotent Stem Cells)-derived cell types may be helpful. Moreover, the role of the chromatin structure and organization due to epigenetic events and gender should be taken into account, performing ChIP (Chromatin Immunoprecipitation) assay and Chromatin Conformation Capture (3C, 4C and the omic version Hi-C) sequencing assay at genome-

wide level¹⁷. In fact, the spatial organization of genome and chromatin topological interactions may impact the biological mechanisms underlying the pathogenesis of complex diseases and has not been yet dissected in the context of sexual dimorphism. The future of this research may involve an integration of large scale epigenomic data, which combines information on DNA methylation, histone modifications, non-coding RNAs and 3D genome organization, with genomic, transcriptomic, metabolomic and proteomic data in order to achieve a deeper knowledge of the pathogenesis of multifactorial disorders. This data should be further integrated with clinical and phenotypic data to enable a precision medical approach. In addition, this approach should also consider gender as a variable, given the different incidence and severity of complex disorders and cancers between the sexes. Indeed, sex-related epigenetic modifications could possibly shed light on novel pathways involving protein-protein interactions, RNA and DNA-protein interactions that affect pathogenesis and might become potential therapeutic targets. Moreover, the sex-specific epigenetic landscape may influence response to drugs and treatments that are rarely optimized according to gender. In conclusion, gender epigenetics will in future be a promising resource in gaining a better understanding of the pathogenetic mechanisms underlying different complex diseases and could be used for the development of novel therapeutic approaches for diseases, especially in the case of cancer, cardiovascular and neurodegenerative disorders.

Key messages

- Gender accounts for differences between males and females in the susceptibility, the onset and progression of several complex diseases.
- Genetic and epigenetic factors contribute to the establishment of sex-specific differences in disease status between males and females.
- Sex-specific epigenetic events may contribute to cardiovascular diseases, neurological and neurodegenerative disorders and to several cancer types.
- A major knowledge of sex-related epigenetic mechanisms underlying the risk and the pathogenesis of these diseases is needed and can be improved by the application of next-generation analysis platforms.
- The integration of epigenomic data with data from genomics, metabolomics, transcriptomics and proteomics and with phenotypic and clinical data will lead to the identification of diagnostic and prognostic biomarkers as well as novel therapeutic pathways and targets.

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