

## Chromosomal and environmental contributions to sex differences in the vulnerability to neurological and neuropsychiatric disorders: Implications for therapeutic interventions

Patrick N. Pallier<sup>a,\*</sup>, Maria Ferrara<sup>b,c,d</sup>, Francesca Romagnolo<sup>b</sup>, Maria Teresa Ferretti<sup>d</sup>, Hermona Soreq<sup>e</sup>, Andrea Cerase<sup>f,g,h,\*\*</sup>

<sup>a</sup> Blizard Institute, Centre for Neuroscience, Surgery and Trauma, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK

<sup>b</sup> Institute of Psychiatry, Department of Neuroscience and Rehabilitation, University of Ferrara, Ferrara, Italy

<sup>c</sup> Department of Psychiatry, Yale University, School of Medicine, New Haven, CT, United States

<sup>d</sup> Women's Brain Project (WBP), Switzerland

<sup>e</sup> The Edmond and Lily Safra Center of Brain Science, The Hebrew University of Jerusalem, 9190401, Israel

<sup>f</sup> EMBL-Rome, Via Ramarini 32, 00015 Monterotondo, RM, Italy

<sup>g</sup> Blizard Institute, Centre for Genomics and Child Health, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 2AT, UK

<sup>h</sup> Department of Biology, University of Pisa, SS12 Abetone e Brennero 4, 56127 Pisa, Italy

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### ABSTRACT

Neurological and neuropsychiatric disorders affect men and women differently. Multiple sclerosis, Alzheimer's disease, anxiety disorders, depression, meningiomas and late-onset schizophrenia affect women more frequently than men. By contrast, Parkinson's disease, autism spectrum condition, attention-deficit hyperactivity disorder, Tourette's syndrome, amyotrophic lateral sclerosis and early-onset schizophrenia are more prevalent in men. Women have been historically under-recruited or excluded from clinical trials, and most basic research uses male rodent cells or animals as disease models, rarely studying both sexes and factoring sex as a potential source of variation, resulting in a poor understanding of the underlying biological reasons for sex and gender differences in the development of such diseases. Putative pathophysiological contributors include hormones and epigenetic regulators but additional biological and non-biological influences may be at play. We review here the evidence for the underpinning role of the sex chromosome complement, X chromosome inactivation, and environmental and epigenetic regulators in sex differences in the vulnerability to brain disease. We conclude that there is a pressing need for a better understanding of the genetic, epigenetic and environmental mechanisms sustaining sex differences in such diseases, which is critical for developing a precision medicine approach based on sex-tailored prevention and treatment.

**Abbreviations:** AD, Alzheimer's disease; ADHD, Attention deficit hyperactivity disorder; ALS, amyotrophic lateral sclerosis; ASC, Autism spectrum condition; BNST, Bed nucleus of the stria terminalis; CNS, Central nervous system; CRH, Corticotropin (Corticotropin-Releasing Factor); DHA, Docosahexaenoic acid; EAE, Experimental autoimmune encephalomyelitis; FCG, Four core genotypes; GABA, Gamma-aminobutyric acid; HPA, Hypothalamic-pituitary-adrenal axis; MIA, Maternal immune activation; MS, Multiple sclerosis; NP, Neuropsychiatric disorder; PD, Parkinson's disease; PUFA, Polyunsaturated fatty acid; PVN, Paraventricular nucleus of the hypothalamus; SRY/SRY, Sex-determining region Y; TH, Tyrosine hydroxylase; XCI, X chromosome inactivation; Xi, Inactive X chromosome; Xist, X-inactive specific transcript.

\* Corresponding author.

\*\* Corresponding author at: Department of Biology, University of Pisa, SS12 Abetone e Brennero 4, 56127 Pisa, Italy.

E-mail addresses: [p.pallier@qmul.ac.uk](mailto:p.pallier@qmul.ac.uk) (P.N. Pallier), [a.cerase@qmul.ac.uk](mailto:a.cerase@qmul.ac.uk), [andrea.cerase@unipi.it](mailto:andrea.cerase@unipi.it) (A. Cerase).

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## 1. Introduction

Men and women differ in their vulnerability to neurological and neuropsychiatric disorders (NPDs). Women are more affected by Alzheimer's disease (AD), multiple sclerosis (MS), anxiety disorders, depression, meningioma and late-onset schizophrenia. Men are more likely to suffer from Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS), autism spectrum condition (ASC), attention deficit hyperactivity disorder (ADHD), Tourette's syndrome, and early-onset schizophrenia compared to women (Pinares-Garcia et al., 2018; Du et al., 2014) (Fig. 1A). In patients with schizophrenia, symptoms differ depending on sex, with female patients exhibiting predominantly affective symptoms and more likely to have a better overall functioning by the time they receive a diagnosis, compared to males, who show mostly negative symptoms at all times and are more likely to live alone and be out of school or unemployed (Ochoa et al., 2012; Thorup et al., 2014). Prevalence based on sex might also vary with age. For instance, ASC has a male bias from childhood onwards and the onset of schizophrenia is seen early in life in men (Ochoa et al., 2012) but, in women, schizophrenia is more frequent when they are in their 50's (Jongsma et al., 2018; Ferrara and Srihari, 2020; Folsom et al., 2006), whereas the prevalence of anxiety and depression disorder doubles in girls during adolescence (Rutter et al., 2003). Early in life, throughout adolescence, and after the age of 30, stroke is most prevalent in men (Wilson, 2013; Szychala et al., 2017); young adult women are at risk for stroke when using oral contraception but they are protected by estrogens past the age of 30; after menopause, by contrast, the incidence not only increases in women but its outcome worsen compared to age-matched males (Qi et al., 2021).

Despite the extensive efforts that have been aimed at identifying the underlying causes of neurological and NPDs, and the successful strategies implemented to foster the inclusion of sex and gender in health and biomedical research, the contribution of health-relevant sex differences to the etiology of these conditions is still largely unknown and remains an important knowledge gap (Johnson et al., 2014). Because of a multifactorial origin, clear gene-disease causative associations are missing in most cases. Altered epigenetic profiling has been associated with several mental health conditions (such as depression, bipolar disorder, and schizophrenia) (Akbarian and Nestler, 2013; Chase et al., 2015; Houtepen et al., 2016; Sun et al., 2013), but the influence of the sex chromosome complement and external factors (environmental, social, and cultural) is poorly understood (Alarcon, 2009; Agid et al., 2000). A comprehensive review on genetic and environmental contributions to sex differences in the vulnerability to neurological and NPDs was thus necessary to foster new research and alert on this important topic. We have focused on the most recent developments in the field, from the role of sex chromosome complement, the interplay of sex hormones and X chromosome inactivation, to epigenetics and the influence of modifiable components of modern lifestyles, such as psychological stress, exposure to stress/infectious disease during gestation, and poor dietary habits (Fig. 1A). We must note that the sex terminology that is used in this review refers to the "biological sex", that is, the dichotomy between the biological male and female categories, a classification that is assigned at birth and classically based on reproductive organs and gametes (Evan and Garofalo, 2020). This instrumental definition does not negate the existence of biological (chromosomal, genetic, and hormonal) sex variants, but it allows us to differentiate gender as a non biologically determined identity associated with social roles and constructs that does not necessarily match the social expectations commonly associated with the assigned biological sex (Evan and Garofalo, 2020).

## 2. The importance of sex chromosomes in health and disease

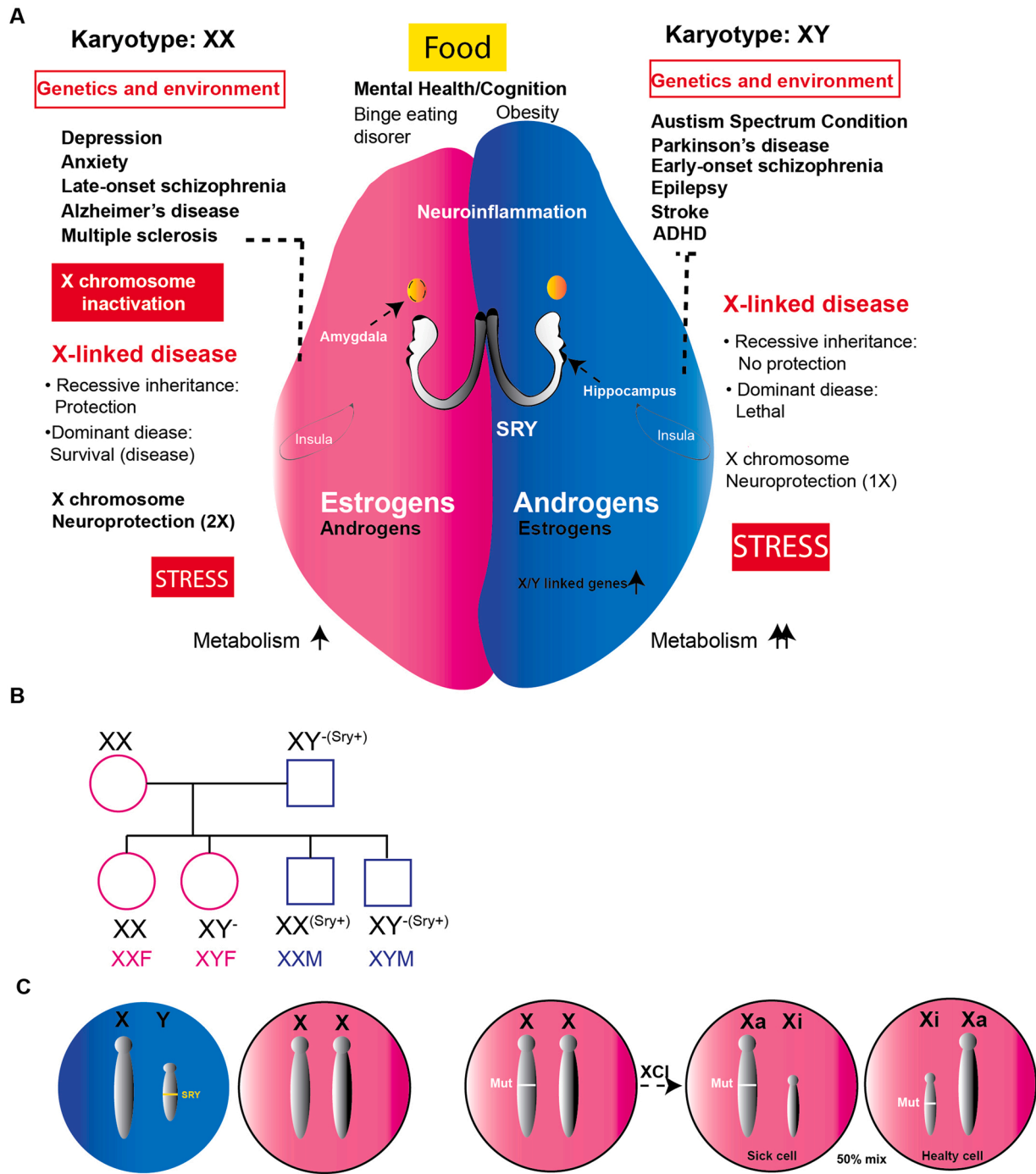
### 2.1. The sex chromosomes

In mammals, biological sex is primarily determined by the sex chromosomes (Graves, 1995). Sex chromosomes are derived from an ancestral pair of autosomal chromosomes called proto-X and proto-Y chromosomes, which diverged during evolutionary time (Gribnau and Grootegoed, 2012). In particular, the rate of Y chromosome contraction and regression accelerated when the Y chromosome gained the *sex-determining region Y (Sry)* gene (whose product is the male sex determinant protein, responsible for the development of the testes –subsequently followed by the production of testosterone) (Rey et al., 2000) and other male-beneficial genes (Gribnau and Grootegoed, 2012). These processes resulted in a Y chromosome carrying about 80–100 genes, while the X chromosome currently sheds about 1000 genes. Only the extremities of the sex chromosomes (called pseudoautosomal regions 1 and 2 or PAR1/2) have been conserved from the original proto-X/proto-Y chromosomes (Gribnau and Grootegoed, 2012). While the Y chromosome acquired male-beneficial genes during evolution, the X chromosomes became enriched in brain-specific genes (Kemkemer et al., 2009) that are generally highly expressed in the brain (Nguyen and Disteche, 2006) (and also in genes related to muscle function, sex, and reproduction (Yang et al., 2006); Gurbich and Bachtrög, 2008). The changes of the proto-X chromosome during evolution became linked to transcriptional upregulation of X-linked genes in females, with the concomitant co-evolution of a process called "X chromosome inactivation" (XCI), a chromosome-wide gene silencing mechanism by which one of the two X chromosomes is randomly silenced in females to achieve a balanced gene expression between males and females (Boeren and Gribnau, 2021; Jachowicz et al., 2022; Markaki et al., 2021). The different karyotypes of the two sexes, the accumulation of brain and muscle function-related genes on the X chromosome, and the random nature of XCI contribute to making the female's biology intrinsically different from that of the male, an essential factor to be considered both in research and in the clinic.

It is now accepted that the male- and female-specific chromosome makeup (the sex chromosome complement) plays a critical role in sex-biased disorders (Goyal et al., 2019). These differences are mediated by both the genetic contribution of the XX vs XY karyotype and hormonal effects (Fig. 1A). As brain development and most bodily functions are strongly influenced by gonadal hormone levels (Fig. 1A), it has been historically difficult to understand the role of sex chromosomes beyond that of hormonal and metabolic effects. Experiments in which these could be independently modulated have been crucial in disentangling the role of hormones from the other genetic contribution of the sex chromosomes (Xu et al., 2002) (see below).

### 2.2. Studying the role of sex chromosomes – The "four core genotypes" mouse model

The four core genotypes (FCG) mouse model is a series of mouse systems where genetic and hormonal sex can be largely separated. This mouse genetic manipulation system has been highly instrumental in separating the role of sex chromosomes from hormonal activities at different developmental stages (Burgoyne and Arnold, 2016) (Fig. 1B). The model involves knocking out the *Sry* gene (XY<sup>-</sup> mice) and moving it onto an autosome, in the same mice, through the insertion of a *Sry* transgene (XY<sup>(Sry)</sup> mice). The four core genotypes, XX (XX gonadal females), XY<sup>-</sup> (XY gonadal females), XX<sup>(Sry)</sup> (XX gonadal males), and XY<sup>(Sry)</sup> (XY gonadal males) are produced by crossing XX gonadal females with XY<sup>(Sry)</sup> gonadal males, and the sex of the resulting mice is



**Fig. 1). Males and females are differently predisposed to neurological and neuropsychiatric phenotypes and show brain sexual dimorphism.** 1A) Examples of sex-biased neurological and NPDs are shown in bold. Representative examples of sexual dimorphic regions are shown on the schematic: hippocampus, in grey; amygdala, in orange (the black circles in females indicate the change in size compared to males over the development); insula, grey line. Gonadal hormones, X-linked predisposition to disease, and X chromosome neuroprotective effects are also shown. Examples of sex-biased eating disorders are given in the Food section (highlighted in yellow), differences in metabolic rate during aging and HPA axis activation in response to stress are shown. The sex divergent effects of these factors are discussed in the text. The size of the text or the number of arrows is linked to the sex-bias evidence discussed in the main text. 1B) Illustration of the crossing used for generating the four core genotypes. Pink circles represent female animals, blue squares represent male animals. XY<sup>(Sry)</sup> represents a male line in which the Sry gene has been moved onto an autosome. XX (XXF), XY (XYF) are female animals; XX<sup>(Sry)</sup> (XXM) and XY<sup>(Sry)</sup> (XYM) are male animals. 1 C) XCI and disease-causing mutations. Illustration of the mechanism that occurs in females for balancing gene expression between the two X chromosomes: one of the X chromosomes is randomly silenced and becomes inactive (Xi), therefore females are a living mosaic of cells expressing either the paternal or the maternal X chromosome. This process generally protects females from adverse X-linked mutations.

defined by the type of gonads present (since once the primitive gonads are differentiated into testes or ovaries, under the influence of the sex chromosomes, the presence of specific testicular hormones drives internal and external genitalia to follow the male pathway, or the female pathway in their absence) (Rey et al., 2000). Using this model, the effect of the sex chromosome complement (sex effect) can be separated from the sex-specific hormonal influences. Studies using the FCG model have been critical in revealing that sex chromosomes themselves play a major role in sex development (Boczkowski, 1981), differences in anatomical features between sexes, and the expression of complex behavioral phenotypes (De Vries et al., 2002; Quinn et al., 2007; Barker et al., 2010). For example, in an MRI study by Corre and colleagues, 62 brain regions were compared across the four core models, 16 regions showed differences linked to the gonadal sex and 11 showed differences linked to the sex chromosome complement (Corre et al., 2016). XX mice demonstrated faster food-reinforced instrumental habit formation than XY mice, regardless of gonadal phenotype (Quinn et al., 2007), while XY mice demonstrated rapid habitual responding in an alcohol reinforcement paradigm compared to XX mice, whose behavior remained goal-directed over the same period of testing (Barker et al., 2010). This study also concluded that different alcoholism-related behaviors were determined independently by either gonadal influences (alcohol drinking) or chromosomal sex (habitual responding to alcohol reinforcement). The FCG model also contributed to proving that XX and XY neurons do not respond in the same way to sex hormones, these responses being independent of gonadal sex but linked to the sex chromosome complement differences (Cisternas et al., 2015) (Fig. 1B). However, a recent paper suggested that XX and XY organoids do not show significant differences in response to sex hormones (Kelava et al., 2022) – more work is needed to understand these discrepancies. Finally, the FCG model has helped to elucidate the role of XCI-escapee genes in the ischemic sexual dimorphism, through differential regulation of inflammation in microglia (Qi et al., 2021) (see also below). For more information on studies that have used this model, the reader is referred to this excellent review (Arnold, 2020).

### 2.3. Influence of sex chromosomes and gonadal hormones on brain anatomy and function

There is a wealth of evidence showing that male and female brains have subtle differences in global and regional anatomy (Fig. 1A) as a result of different exposure to gonadal hormones interacting with different sex chromosome genes (McCarthy and Arnold, 2011; McEwen and Milner, 2017; Liu et al., 2020). In humans, total brain volume is larger in males than in females (Ruigrok et al., 2014), a pre-natally determined sex difference that includes both grey and white matter, accentuated over adolescence, exists irrespective of body size, but would not translate into any sex difference in mean general cognitive abilities (Raznahan and Disteché, 2021). But the relative contribution of the sex chromosomes, compared to gonadal factors, to the establishment of sex differences in the human brain and body size during gestation, is still debated. Studies in animals (Burgoyne, 1993; Xu et al., 1992) show that male embryos have a higher number of cells than female embryos early in development, before the sexualization of the embryos, which might affect brain size later in development. Evidence for sex-specific growth differences early in pregnancy, before gonadal differentiation at 7 weeks of gestation, are also seen in humans (Alur, 2019), adding to the likely possibility of a gonad-independent contribution of the sex chromosome complement to these differences. A recent publication showed that the sex chromosome complement plays an important role in shaping sex-biased neuroanatomic differences across the sexes (Mallard et al., 2021). In particular, Mallard and others showed that the X chromosome has a disproportionate effect on brain neuroanatomy relatively to its size, compared to autosomes, especially in males (Mallard et al., 2021). These differences might be linked to functional differences between the sexes in decision making, motor control and attention (Mallard et al.,

2021).

Relative to females, higher volumes of grey matter have also been classically reported in male mice in subcortical (bed nucleus of the stria terminalis (BNST), medial amygdala, and medial preoptic area) and cortical brain areas that are known to be involved in reproductive and social behaviors (Liu et al., 2020). In humans, not all studies agree on anatomical brain differences across the sexes (Ritchie et al., 2018) and, to date, very little is known about functional brain differences in males vs females (Tomasí and Volkow, 2012), if any (Jancke, 2018). In human brains, Liu et al. (2020) found that grey matter volume was significantly greater in females than males within the prefrontal cortex (medial, lateral and orbitofrontal), the superior temporal and lateral parietal cortices and the insula, while it was greater in males in the ventral occipitotemporal regions and in a set of subcortical areas that included the hypothalamus, BNST, amygdala, hippocampus, putamen, and cerebellum. One limitation of this study is that it could not directly access the earlier developmental emergence of these sex differences or their transcriptomic matched data. Indeed, it is known that rates of cell differentiation or apoptosis differ in various regions of the male and female brain during development as a consequence of complex regulations from genes to hormones. For example, in early development, the rat male hippocampus has almost twice as many cells as females due to a higher expression of estradiol in males. However, the final volume of the male rat hippocampus is thought to be only marginally larger than that of females, while there is no evidence of different functions between the two sexes (Waddell et al., 2016; Bowers et al., 2010; Isgor and Sengelaub, 1998). In the developing amygdala of rhesus monkey females, more new cells are present in early development, while differences between sexes are smaller to non-existent at adulthood (Franklin et al., 2000) (Fig. 1A). Nonetheless, like in mice, one key point of the study by Liu et al. (2020) was that the sex bias differences found in grey matter volume overlapped with the spatial distribution of functional systems that subserve sex-bias domains of social cognition (face and facial identity processing in humans, a domain in which women are known to outperform men) (Herlitz and Lovén, 2013). Also, some of the regions involved in sex bias differences (mainly areas of the limbic system, such as the amygdala, hippocampus and insula, where Ruigrok et al. (2014) also reported sex differences in volume and tissue density from a meta-analysis of the literature) are well known to show structural alterations in individuals with NPDs, such as in ASC, depression, schizophrenia, or ADHD (Ruigrok et al., 2014). Another aspect of sex bias that was conserved between humans and mice in adulthood was that sex differences in grey matter volumes were associated with regional expression of sex chromosome genes, with those cortical areas showing high expression of sex chromosome genes also showing larger grey matter volumes in males than in females (Liu et al., 2020) – out of the top four genes involved, three are from the protocadherin gene family (which are critical for the development of the central nervous system (CNS) (Frank and Kemler, 2002)) and one gene (*ZNF711*) encodes for a transcription factor involved in X-linked intellectual disability (van der Werf et al., 2017). The authors suggested that genes from the sex chromosomes exert direct effects responsible for shaping and maintaining regional sex differences in brain structure and volume.

For comparing human and mouse data and including both cortical and subcortical areas, Liu et al. (2020) did not look at aspects of brain morphology other than grey matter volume. In comparison, Ritchie and colleagues, using a single scan sample of over 5000 participants from the UK Biobank, found substantial brain structural and functional differences between men and women (age group 44–77, mean = 61.7) not only in volume but also cortical thickness, surface area, and connectivity, with males showing larger raw volumes, raw surface areas, and white matter fractional anisotropy and females thicker cortices and higher white matter tract complexity (Ritchie et al., 2018). The organization of the functional connectome showed stronger connectivity for males in unimodal sensorimotor cortices but stronger connectivity for females in the default mode network. After adjusting for overall brain

size, 11 out of 68 regions were still significantly larger in males and 13 regions were larger in females, 18 regions had larger surface areas in males but 9 were larger in females, and 24 regions were thicker in females but also 25 regions were now largest in males (Fig. 1A, showing only a few representative examples). Females had greater nucleus accumbens volume and amygdala volumes were modestly larger in males, but no sex differences were found in the hippocampus in this study (Fig. 1A). This large scale study provides a solid foundation for exploring in greater depth the links between sex differences and brain structure, along with their likely causes and potential behavioral and medical consequences, which are still unknown but, much like in the Liu et al. (2020) report, the findings may not apply to younger adults. This is important since a recent study not only confirmed the findings by Ritchie et al. (2018) of sex differences in the brain's functional network, but it also reported an age dependency of these differences (Zhang et al., 2021).

Zhang et al. (2021) confirmed that males had stronger functional connectivity than females but small-to-medium effect sizes were seen within the default mode network, salience attention network, and frontoparietal task network in adolescents while effect sizes increased to large in the adult sample. But then, because the functional connectivity decreased more steeply in males than in females after middle age, the signs of sex differences were flipped in older adulthood, with the majority of sex differences seen in other networks following a similar lifespan pattern—the functional connectivity being stronger in males during adolescence and early adulthood but becoming stronger in females in older adulthood. These changes might be influenced by genetics and sex hormones but not only. Many other factors under the umbrella of the so-called “exposome” (Wild, 2012) (the various environmental contexts, experiences, and behaviours encountered, lived, and displayed throughout the lifespan) might also be involved. It is nonetheless important to highlight that not all work published so far suggests the existence of structural or functional differences between the sexes, and therefore this topic is still controversial (Eliot et al., 2021).

#### 2.4. *Sry* models

A particularly interesting case for neurological and NPDs is represented by the study of the *Sry* gene. The *Sry* gene codes for a transcription factor that initiates and regulates not only the male-specific sex differentiation (She and Yang, 2017) but also the activity of many tissues in which it is expressed (Lahr et al., 1995), like the brain (Fig. 1A), where it acts to control male-specific behaviors, such as sexual behavior (Sinclair et al., 1990; Koopman et al., 1990; Rosenfeld, 2017). In the *Sry* knock out model, male mice carrying a deletion of the *Sry* gene develop female gonads. In these animals, different hormones or hormonal receptors can be modulated experimentally in time and concentration using inducible transgenes or drugs. Several studies have revealed that Y-linked genes having a homologous copy on the X chromosome were expressed in the male brain regardless of the levels of testicular hormones (Xu et al., 2002). *In situ* hybridization and immunohistochemistry analyses of *Sry*, and *Sry* and *tyrosine hydroxylase* (*TH*) gene transcript analyses revealed a clear localization of the *Sry* gene products in specific parts of the brain, including the locus coeruleus (the major location of the noradrenergic cell bodies in the brain), dopaminergic cell bodies of the substantia nigra pars compacta and ventral tegmental area, and glutamic acid decarboxylase-positive (GABAergic) neurons of the substantia nigra pars reticulata (Dewing et al., 2006; Czech et al., 2012; Milsted et al., 2004). *Sry* can also regulate *TH* transcription by binding at the promoter region of the *TH* gene, suggesting a mechanism by which *Sry* mediates the regulation of catecholamine biosynthesis in catecholaminergic neurons of the male brain (Koopman et al., 1990; Milsted et al., 2004; Czech et al., 2014) and the adrenal medulla (Milsted et al., 2004). These observations suggest a way by which *Sry* would confer “maleness”, by affecting the development of the function of the catecholaminergic system (Kopsida et al., 2009), and could also suggest the

existence of a potential link between *Sry* dysregulation and the etiology of certain conditions with a known catecholaminergic dysfunction that are more predominantly seen in males, such as ADHD (Pinares-Garcia et al., 2018), substance addiction (Pinares-Garcia et al., 2018), hypertension (Kopsida et al., 2009), and PD (Pilcher, 2006; Johansson et al., 2016) (Fig. 1A). An important note to add is that *Sry* is not the only Y-linked gene to be exclusively expressed in males or to show male-biased expression. Other such genes, like *RBMV*, *TSPY*, or *HSFY* (of which the X chromosome is also carrying a copy, although the function of the X vs Y gene copies might differ (Raznahan and Disteche, 2021)), are expressed in the brain and can be expressed in males at a higher rate than in females, contributing to Y-associated sex differences (Bellott et al., 2014). *PCHD11Y* also has a sex-dimorphic expression and is known to increase male susceptibility to ADHD and ASC (Johansson et al., 2016). Interestingly, *CHD8*, a gene whose deficiency is associated with a cluster of ASC symptoms, seems to directly regulate the expression of the X-inactive specific transcript (*Xist*), a long non-coding RNA that is the master regulator of XCI (Cerase et al., 2021). However, the link with male bias (both in terms of occurrence and phenotypes), as seen in ASC (Hoffmann and Spengler, 2021), is not currently known.

### 3. When X chromosome inactivation “fails”: a key mechanism to the development of female-biased autoimmune and old-age disorders?

#### 3.1. The X chromosome

The X chromosome corresponds to 5 % of the genome in women and men and X-linked genes have a higher expression in the brain compared to other tissues in several mammalian species, which is independent of the sex. More genes are expressed in the brain from the X chromosome than from any autosome (Nguyen and Disteche, 2006; Davis et al., 2021). In humans, the brain-related genes that the X chromosome is enriched in, such as the genes for doublecortin and protocadherins, are genes that are essential to normal brain development and function (Gleeson et al., 1998; Priddle and Crow, 2013), including the development of cognitive abilities (Zechner et al., 2001). The X chromosome is thus thought to play a pivotal role in regulating brain morphology (see above), structure and function and may, in particular, exert a large effect on general cognitive functions in humans. Consequently, it is also a major player in determining the overall sex-related risk of NPDs, causing mental impairment when mutated as well as sex-related differences in reactions to pharmaceutical compounds (Simchovitz-Gesher and Soreq, 2020). Mutations of X-linked genes account for more than 20 % of intellectual disabilities, a substantial fraction of which is linked to ASC (Sahin and Sur, 2015; Vissers et al., 2016). While the Y chromosome contains fewer genes compared to the X chromosome, and while most of them are expressed primarily or exclusively in the testes, a few are also expressed throughout the body, including the brain (Dewing et al., 2006; Arnold, 2004).

The analysis of the impact of X-linked genes on the organism at large and the brain, in particular, and the study of their contribution to sex-biased disorders have been difficult for several reasons. This complexity is linked to the interplay between sex chromosome complement and hormones (Raznahan and Disteche, 2021). What we have learned in humans is thus mostly derived from studies of individuals with an abnormal number of X chromosomes (Raznahan and Disteche, 2021). Either an increase or a decrease of the genetic dose of X-linked genes can enhance sensitivity to disease, including neurological and NPDs. For example, Turner syndrome patients, who are missing one X chromosome (XO), show increased susceptibility to ADHD, ASC, and schizophrenia (Russell et al., 2006; Skuse, 2000). The presence of supernumerary X chromosomes, such as in Klinefelter syndrome (also known as 47,XXY, or XXXY syndrome), can predispose to intellectual disability (Groth et al., 2013), even though supernumerary X chromosomes are XCI-silenced (discussed below).

### 3.2. Biological mechanisms of X chromosome inactivation

One further level of complexity in the study of the gene-function relationship in X-linked genes is due to the existence of interindividual variability in the dosage compensation mechanism that occurs in females for balancing gene expression between the two X chromosomes, the so-called XCI mechanism (Cerese et al., 2015; Robert Finestra and Gribnau, 2017; Mira-Bontenbal and Gribnau, 2016) (Fig. 1C). One of the most striking differences between sexes resides in the fact that one of the X chromosomes is randomly silenced in females as a method of dosage compensation. As a consequence of this phenomenon, females are a living mosaic of cells expressing either the paternal or the maternal X chromosome (Cerese et al., 2015). This aspect of female biology is particularly relevant in the context of autosomal dominant disorders (Franco and Ballabio, 2006). For example, mutations in the X-linked gene *MeCP2* are generally lethal in males, who possess a single X-linked copy of this gene, while it is associated with Rett syndrome in females (Ehinger et al., 2018). It is the presence of two copies of this gene, along with the random nature of XCI, that makes this mutation nonlethal in females (Franco and Ballabio, 2006). Noticeably, the presence of wild-type and mutated alleles in each cell, which are randomly silenced in a 50–50 fashion, offers the possibility of therapeutic intervention by XCI reversal through reactivation of the epigenetically silenced wild-type allele (Lee et al., 2020; Sripathy et al., 2017) (Fig. 1C).

### 3.3. X chromosome inactivation confers protection from X-linked mutations

Another possible explanation behind the evidence that males and females show a different predisposition to disease might rely on the fact that XCI is incomplete and variable between individuals. This results in a high number of genes escaping XCI in the normal human female population (~15 %) (Carrel and Willard, 2005), with tissue-specific variations also playing a role. Through this phenomenon, mutations in X-linked genes may be compensated in females but not in males (Fig. 1A). For example, *Kdm5C* is an X-linked gene encoding a histone demethylase whose mutations are associated with mental retardation but they predominantly affect males, while female carriers are usually either unaffected or show mild intellectual disability (Goncalves et al., 2014). This contrasts with the example of *MePC2* that we mentioned above, whose mutation only affects females while males die at birth or shortly after (Brookes et al., 2015; Connolly and Zhou, 2019). These differences between sexes are easily explained by the gene-specific pattern of expression and the type of inheritance. Mutations in genes that undergo XCI are often dominant, leading to male-lethal disorders, while genes that escape XCI usually show male- or sex-specific predisposition to disease (Franco and Ballabio, 2006; Disteché, 2016; Lushchin, 1973) (Fig. 1A). It follows that genes that escape XCI and have no functional homologs on the Y chromosome or autosomes tend to have a higher level of expression in females over males (Raznahan and Disteché, 2021; Russell et al., 2006; Xu et al., 2005, 2008). In the case of XXXY, individuals have, *de facto*, two additional inactive X chromosomes. The disease phenotype is thought to happen because XCI is incomplete and, therefore, the resulting increased gene dosage stems from XCI escapees (the genes that are not silenced) (Raznahan and Disteché, 2021). In the brain, a partially-compensating supernumerary X chromosome may affect the structure and the size of some grey matter areas (Mauvais-Jarvis, 2015), further highlighting the significant role of the X chromosome in brain structure and function (Hong et al., 2014). Increased expression of *Xist* has also been linked to Alzheimer's disease (AD), both in human samples and mouse models/cell lines (Loring et al., 2001; Wang et al., 2018; Chanda and Mukhopadhyay, 2020). The biological roles of *Xist* upregulation in the pathophysiology of AD are currently unknown, but it is tempting to speculate that increased *Xist* expression might lead to ectopic autosomal silencing (Jachowicz et al., 2022), contributing to the etiology of the disease. Noticeably, X

chromosome aneuploidy is seen in the female brain of AD patients, potentially suggesting a role for XCI in the onset of AD (Yurov et al., 2014) and the higher susceptibility of women to the condition. This is important since a recent study examining differential gene expression profiling of the X chromosome, from an RNA sequencing data set of the dorsolateral prefrontal cortex obtained from autopsied elderly individuals, reported that specific X chromosome genes could contribute risk (but also resilience) to biological pathways of aging and AD (Davis et al., 2021). In addition, the contribution of the X chromosome to the cognitive trajectories of elderly people vs the neuropathological tau burden of their brains was found to be sex-biased. XCI skewing has also been reported in MS (Knudsen et al., 2007a), along with defective XCI, escapee gene expression (Itoh et al., 2019) (see below). Importantly, human-specific XCI regulation might also be a reason for the lack of translatability from animal models to humans, as species may also show intrinsic cell/tissue differences in the XCI process (Peeters et al., 2014). In particular, mice have fewer genes that escape from XCI than many other species, while some genes escape XCI only in primates (Peeters et al., 2014). This is an important aspect to consider when using mouse and rat models in preclinical experiments, as these models may not always recapitulate human sex-specific differences (Naqvi et al., 2019; Arnold, 2009).

### 3.4. X chromosome inactivation, escapee genes, immunity and multiple sclerosis

While females have a wider immune response repertoire compared to males, at the same time they are more prone than males to develop autoimmune disorders (Ngo et al., 2014). This phenomenon is linked to the X chromosome content, which is enriched in many immunity-related genes, (Bianchi et al., 2012) and to XCI (Boeren and Gribnau, 2021; Mousavi et al., 2020). In particular, females have an unusual and incomplete XCI maintenance pattern in T and B cells, leading to biallelic expression of immunity-related genes (Wang et al., 2016).

The role of the adaptive arm of the immune system in MS has been highlighted from early genetic maps, the disease being historically considered a T cell-mediated immunological and neurological disorder, with the involvement of multiple different T cell subsets (Sawcer et al., 2011). B cells also play an important role in this pathology (van Langelaar et al., 2020), which was confirmed in a recently published genetic and genomic map of MS susceptibility that explains almost half of the disease's heritability. This study also reported enrichment for MS genes in human microglia (but not in astrocytes or neurons), the resident immune cells of the brain (Consortium, 2019). In MS, immune responses are stronger in women than in men and the female to male ratio is about three to one (Smith-Bouvier et al., 2008). The most recent MS genetic map identified the first-ever chromosome X variant in MS (rs2807267) within an enhancer peak area specific for T cells and downstream from the RNA U6 small nuclear 320 pseudogene (*RNU6-320 P*) (Consortium, 2019; Patsopoulos, 2018). The presence of this susceptibility locus on the X chromosome cannot be the only explanation for the strong women bias in the susceptibility to MS but it is an important milestone in the search for the role of the sex chromosomes in MS.

Recent work has identified *Kdm6a* as a likely causal/contributing factor for the higher susceptibility of women to MS (Itoh et al., 2019). This gene codes for a histone demethylase on the X chromosome that shows higher expression in females in humans, mice, and the FCG mouse model and it escapes XCI in CD4<sup>+</sup> T cells. Neuroinflammation signalling pathways are downregulated when *Kdm6a* is deleted in CD4<sup>+</sup> T cells, and in the experimental autoimmune encephalomyelitis (EAE) rodent model of MS the neuropathology appears to be reduced and clinical symptoms improve (Itoh et al., 2019). *Kdm6a* in CD4<sup>+</sup> T cells is immunomodulatory and has a disease-promoting role in EAE, therefore its expression from two alleles in women compared to one in men would logically contribute to the female preponderance seen in MS. By contrast, its downregulation should represent a promising therapeutic

target for MS and other autoimmune conditions predominantly seen in females.

### 3.5. X chromosome inactivation and aging

Research has uncovered a possible link between the XX genotype, neuroprotection and increased longevity and, by contrast, between XCI degeneration and older-age disease (Davis et al., 2019; Wahl et al., 2018; Davies et al., 2007). Male and female brains are thought to age at a different rate due to different brain metabolisms, which are themselves regulated by the sex chromosome complement and gonadal hormones (Xu et al., 2005; Wahl et al., 2018; Kiraly et al., 2016). Studies carried out in various cell populations have also revealed that age-associated disease might be linked to XCI defects. While XCI is random in humans, each chromosome having the same probability to become the inactive chromosome (Xi) (Fig. 1C), this no longer holds during aging. For instance, it was found in women that, with age, blood and buccal biopsies become enriched in cells in which one of the two X chromosomes predominantly becomes the Xi (Gentilini et al., 2012; Knudsen et al., 2007b; Mengel-From et al., 2021). The underlying molecular mechanisms for the development of this age-related bias are currently unknown, but added to this is the interesting observation reported by Schoeftner and colleagues that XCI degenerates in accelerated-aging mouse models. In brief, using a telomerase-deficient system (an “aging” model), these authors have shown that with aging the Xi tended to lose its repressive marks, such as H3K27me3 (Cerase and Tartaglia, 2020), resulting in Xi genes becoming partially reactivated (Schoeftner et al., 2009; Cantone and Fisher, 2017). It is possible to hypothesize that a similar phenomenon happens but at a slower rate during physiologically normal aging; however, there are no *in vivo* studies that have addressed this so far. Transposing these concepts to the brain, escaping XCI would result in a higher expression of affected genes in female than in male brains (Russell et al., 2006; Xu et al., 2005, 2008), making it tempting to propose that the occurrence of female-biased old-age disorders might be associated, to some extent, with XCI degeneration. According to this hypothesis, and depending on which X chromosome is preferentially inactivated, various degrees of skewing (Fig. 1C) would result in various levels of severity of a disease phenotype in women carrying an X-linked dominant mutation, and could also modulate the expression of the phenotype in female X-linked recessive disorders (Franco and Ballabio, 2006).

## 4. The exposome, epigenetic regulation, and sex differences in the vulnerability to neurological and NPDs

### 4.1. It is not all about coding and non-coding genes

Studies in monozygotic twins have shown that genetic influences play an important role during the different phases of brain development, and therefore research has tried to identify genetic variants associated with brain morphology and function as causative factors of NPDs. Haplotype-specific development, including the development of the brain and its “hard wiring”, is particularly variable between individuals and might be an emerging cause of NPDs (Jansen et al., 2015).

Recent studies have found that copy number variations or *de novo* rare mutations in coding genes were significantly correlated with neuropsychiatric phenotypes (Mitchell, 2011). Moreover, common variants are shared to a degree between different neurodevelopmental disorders, such as ADHD and ASC (Martin et al., 2019). However, while it is acknowledged that genes contribute to disease susceptibility, heritable predisposition to NPD due to common variants remains controversial and contradictory results have been published (Traglia et al., 2017; Xia et al., 2021). A large study involving 24,248 patients with schizophrenia and 97,322 controls found that ten genes have disruptive coding variants that conferred risk for schizophrenia (Singh et al., 2022). Traglia et al. did not observe consistent increased genetic load in

the lower-prevalence sex or a disproportionate role for the X chromosome in disease risk, despite sex heterogeneity on the X for several traits (Traglia et al., 2017). On the other hand, Xia et al. found a significant enrichment in sex-associated genes in psychiatric disorder-associated gene sets (Xia et al., 2021). These conflicting results may stem, in part, from the fact that in such heterogeneous disorders it is the cumulative effects of multiple variants that should be studied, by collapsing common and rare variants, rather than focusing on single-variant-based association studies (Kang et al., 2020). It remains that some NPDs are more frequently diagnosed in one sex than in the other, and the picture is complicated by the fact that psychiatric signs and symptoms would manifest differently or at a different time in life in the two sexes (Brand et al., 2022), leading to different interpretations and diagnoses and, therefore, to an unbalanced epidemiology (Hull and Mandy, 2017). For male-predominant diagnoses, such as early-onset schizophrenia, addiction disorders, and ASC, one commonly accepted hypothesis for the sex bias is that females are more protected than males by their hormones (Taylor et al., 2016) (but findings in this regard are mixed (Chen et al., 2017) –Fig. 1A, Fig. 2), or other dimensions of sex-differential biology. However, the picture is different in other disorders. Females are 2–3 times more likely to develop major depressive disorder than males, for instance, with higher symptom severity, greater functional impairment, and a unique set of symptoms (Labonté et al., 2017; Gerhard and Duman, 2018). A recent study that used large scale whole-exome sequencing in an east-Asian sub-population has identified five genetic markers potentially associated with increased risk of depressive disorder in females, three variants mapping to chromosome 19p13.2 and two novel variants mapping to chromosome 17p25.1 (Kang et al., 2020), suggesting that the higher prevalence of depression in women might be attributable to inherited variants. Depressed patients homozygous for these variants showed more severe symptoms and higher suicidality than heterozygote patients and homozygotes for the non-associated allele. Of interest, no male-specific risk variants were identified in this cohort, but these results suggest that a higher genetic burden may be required for men to develop a depressive disorder, which may contribute to the higher resilience against depression that is seen in males.

Analysis of post-mortem human brains has also shown that around 2.5 % of all genes are differentially spliced and expressed between males and females (Trabzuni et al., 2013) and that mRNA expression level differences depend on sex (Nishida et al., 2005) (Fig. 1A). Recent studies have found markedly different transcriptional patterns between sexes in depressed patients, with more divergence than convergence (Labonté et al., 2017; Gerhard and Duman, 2018; Seney et al., 2018; Labonté et al., 2017). For instance, analyzing postmortem microarray data from brain regions known to be involved in major depressive disorder (subgenual anterior cingulate and dorsolateral prefrontal cortices; basolateral amygdala), Seney et al. (2018) showed that 52 genes were not only expressed differentially in men and women but were also expressed in opposite directions. The repertoire of non-coding regulatory microRNAs is also known to differ between males and females with schizophrenia and bipolar disorder, which might reflect different regulatory processes in functionally relevant pathways, such as those involved in acetylcholine-regulated processes (Lobentanzer et al., 2020, 2019). In this case, pathways known to be important in neuronal physiology, including MAPK and calcium signalling and pathways involved in axonal guidance and cholinergic neurotransmission, were found to be enriched in sex-specific coding genes. These pathways have already been suspected of being involved in an increased risk for developing an NPD, such as ASC (Lei et al., 2017), depression (Wefers et al., 2012), or schizophrenia (Berridge, 2014). In major depressive disorder, differentially expressed (down-regulated) genes were enriched for synapse-related pathways for depressed men. For depressed women, they were enriched for antigen-related pathways (while synapse-related genes exhibited transcriptional increases) (Gerhard and Duman, 2018; Seney et al., 2018). Oligodendrocyte- and microglia-related genes were upregulated in men with major depressive disorder but downregulated

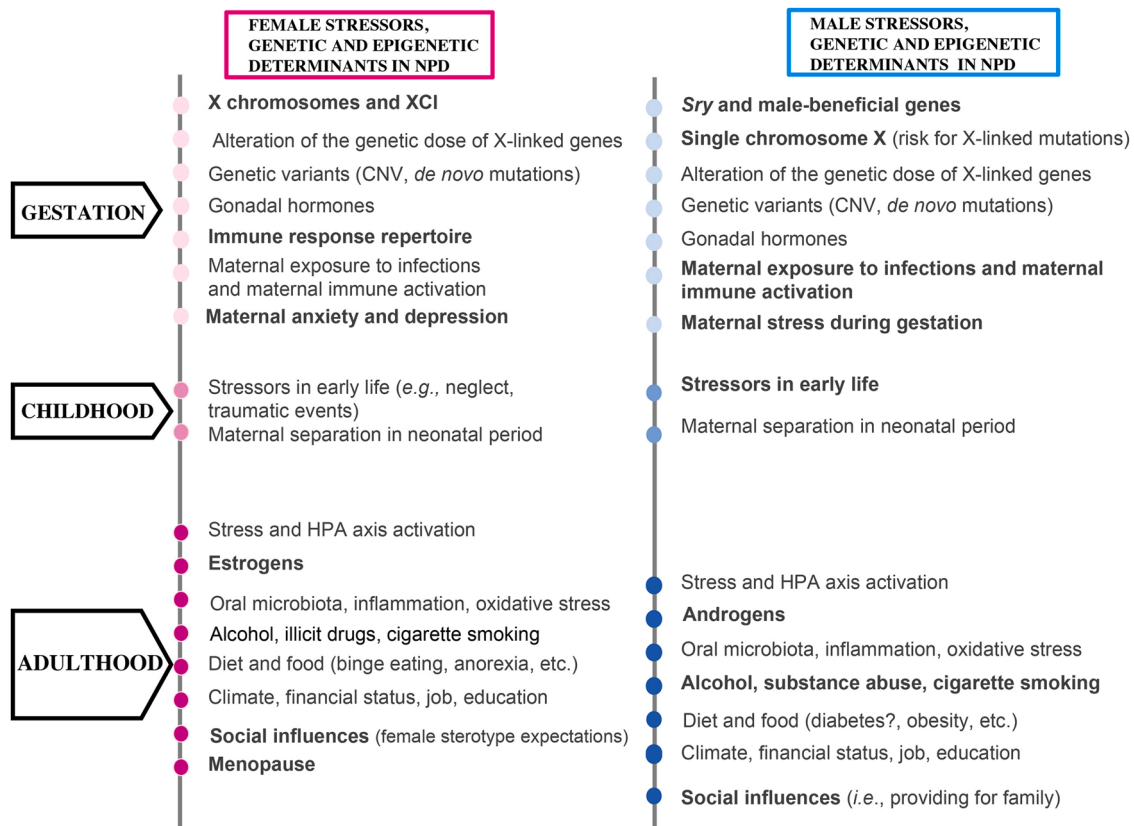


Fig. 2). Female and male stressors biases encountered over the entire lifespan. Three main stages of human life have been selected: gestation, childhood, and adulthood. The stressors that have a higher impact on the general wellbeing and mental health of each of the two sexes are highlighted in bold (see the main text for more details and information).

in women. All in all, these results show a link between perturbation of pathways that are crucial for the brain and genetic dysregulation in unique cell populations, suggesting, for instance, a causal relationship in depressed males between increased microglia and a decreased number of synapses, in relation with the known role of microglia in synaptic pruning.

The differences observed between sexes in genomic transcription and the existence of disease-relevant single-nucleotide polymorphisms in regulatory regions support the likely possibility that the bi-directional relationships between genotype and phenotype depend on sex (Xia et al., 2021). However, the likely additive role of *de novo* rare mutations (often undetected), the presence of developmental variations leading to maladaptive brain wiring, and the influence of the environment (Mitchell, 2011; Mitchell and Porteous, 2011), additionally linked with the fact that altered epigenetic profiling has also been associated with several mental health conditions, including depression, bipolar disorder, and schizophrenia (Akbarian and Nestler, 2013; Chase et al., 2015; Houtepen et al., 2016; Sun et al., 2013), add to this complexity. It follows that a full understanding of the interactions between genes and the occurrence of NPDs calls for considering all other factors potentially involved, in addition to genes.

#### 4.2. Gene-environment interactions

After almost two decades of research, epigenetics, the regulation of gene expression that can occur without any alteration in the genomic sequence, is now considered the intrinsic mechanism of gene-environment interactions (Kubota et al., 2012) (Fig. 2). One of the first reports demonstrating that epigenetic changes can be induced by even short-lived environmental influences was the observation that individual variations in rat comforting maternal behavior (licking and

grooming and arched-back nursing) during the first week of lactation could be associated with profound alterations in the epigenetic status of the glucocorticoid receptor gene promoter in the hippocampus of the progeny. Stable differences in DNA methylation associated with altered histone acetylation status and expression of transcription factor nerve growth factor-inducible protein A (NGFI-A) were seen, leading to variations in the expression of the glucocorticoid receptor and nongenomic transmission of interindividual differences of reactivity to stress to the offspring (Weaver et al., 2004) (Fig. 2). This early-life programming would result in a stable alteration in neuroendocrine and behavioural reactivity, preparing the pups for the environmental conditions in which they will ultimately live as independent adults.

Given the high variability of phenotype penetrance in NPDs, it can be assumed that similar mechanisms involving the influence of secondary environmental factors might contribute to the occurrence of these conditions (Bayer et al., 1999; Daskalakis et al., 2013). Such factors include maternal exposure to infections (Mitchell, 2011; Brown and Patterson, 2011) and early life stressors (Weaver et al., 2004; Szyf, 2011) (Fig. 2), as well as environmental exposure to poisonous substances, which may exert sex-related alterations in the epigenetic regulation of brain-specific genes. The exposome paradigm (Wild, 2012; Vrijheid, 2014) offers a very good representation of the variety of environmental influences that can affect the genome and epigenome (Guloksuz et al., 2018a, 2018b). The exposome corresponds to the complete description of the history of individual environmental exposures and offers a good representation of its complexity (Guloksuz et al., 2018a, 2018b), complementing the genome as the non-genetic lifelong contributor to the phenotypical presentation of “non-communicable” chronic diseases. It includes three different domains of likely influences; for each, we give below examples of factors that are relevant to neurological and NPDs: The internal domain (*e.g.*, oral microbiota (Lozupone et al., 2020), inflammation



(Gumusoglu and Stevens, 2019; Shenhar-Tsarfaty et al., 2014), oxidative stress (Uttara et al., 2009), the sex steroid hormones), the specific external domain (e.g., chemical contaminants and environmental pollutants (Burns et al., 2013; Feat-Vetel et al., 2018), prenatal conditions (Hashimoto-Torii et al., 2014), diet (Brandt, 2019), lifestyle factors such as tobacco and alcohol use (Crocq, 2003)), and the general external domain (e.g., climate (Zammit et al., 2021), level of education/financial status (Ngui et al., 2010; Durkin and Yeargin-Allsopp, 2018), psychological and mental stress (Keynejad et al., 2019)) (Fig. 2). These categories are not exclusive but can overlap and interact with a genetic predisposition to certain diseases (Guloksuz et al., 2018b). We focus below on three examples of environmental challenges from the specific and general external domains that are well known to induce or have the potential to induce different epigenetic changes in one sex compared to the other: psychological stress, gestational exposure to infections/stress, and poor dietary habits. However, it is important to highlight that additional factors from the external domain, such as cultural and societal beliefs, demands, and roles can also impinge on the brain during different stages of development, modulating the sex differences in brain structure and function, along with their phenotypic presentation. For instance, social status (class and power) and social rules (e.g., culturally embedded traditions) may affect access to education or the ability to engage in certain behaviors, driving all other gender differences in behavior (Ngun et al., 2011; Hyde, 2014; Wood and Eagly, 2002). One example is labor division by gender. In societies where the role of taking care of the home and family has traditionally been assigned to women, these experiences may possibly lead women to acquire behaviours that are less dominant in men, such as nurturing behaviors and a facility for relationships (Wood and Eagly, 2002). Another example is the access to illicit drugs, which is easier for males in societies in which men have more freedom in how to spend their time after work than women, who are responsible for the household; this can in turn affect neuro-modulation and the phenotypical presentation of schizophrenia (Brand et al., 2022) - although despite the higher prevalence of substance abuse comorbidity in men diagnosed with schizophrenia, the association between substance use and psychosis risk may actually be stronger in women (Pence et al., 2022). Still, these reports highlight the importance of the social determinants of health (socioeconomic status, level of education, job-related exposures (Teschke et al., 2002)) as additional powerful factors of influence in the emergence of sex bias differences in behavior and susceptibility to NPDs (Thayer and Kuzawa, 2011).

#### 4.3. The deleterious impact of psychological stress on mental and neurological health

Stress is a known contributor to neuroaging and the etiology of most psychiatric phenotypes, both through early-life traumas (involved in the building of “vulnerability factors” (Durkin and Yeargin-Allsopp, 2018)) and later-life events (“precipitating factors”) (Keynejad et al., 2019; Park and Yang, 2015), increasing the susceptibility to developing neurological conditions such as AD or PD (McEwen et al., 2015; Alkadhi, 2013), but also potentially worsening the symptoms and structural alterations of a variety of diseases, including mental health and neurological disorders. In humans, a major traumatic life experience can lead to post-traumatic stress disorder, characterized by irritability, depression, and impaired cognitive abilities. This is thought to stem from robust cholinergic stimulation that would facilitate long-lasting changes in the activity of genes involved in acetylcholine metabolism (Kaufer et al., 1998). On the other hand, chronic and sub-chronic stress is believed to influence in the long run the susceptibility to mental and neurological health by activating the hypothalamic-pituitary-adrenal (HPA) axis (Pariante, 2017). Hyperactivation of the HPA axis is present in many NPDs, including, but not limited to, depression (Baumeister et al., 2014), bipolar disorder (Duffy et al., 2012), and psychosis (Brenner et al., 2009). Chronically increased production of cortisol, the main glucocorticoid in humans, from the adrenal cortex (McEwen, 2005), induces a

maladaptive stress response that affects many body organs and functions (Sousa and Almeida, 2012). It affects immunity and metabolism in the periphery and neuronal function in the CNS, where chronic cortisol leads to the generation of a metabolically adverse cellular environment (Alkadhi, 2013; Tran et al., 2011) resulting, for instance, in decreased neurogenesis and hippocampus atrophy.

Findings obtained in humans and rodents suggest that non-genetic factors linked to both childhood and adulthood adversity can affect the development of the brain by leading to dramatic changes in gene expression (Gunnar et al., 2001; Meaney and Szyf, 2005), which might contribute to the development of NPDs with sex bias differences (Pence et al., 2022). It has become increasingly clear that fetal exposure to dysregulation of the maternal HPA axis, excessive glucocorticoids, and inflammation, all produce epigenetic changes at the placental and fetal levels (Kim et al., 2015), although the key mechanism(s) underpinning such outcomes has not been identified yet. Early-life adversities are known to result in inflammation dysregulation, facilitating a persisting systemic and central inflammation (Fig. 2). In the CNS, increased immune activation can become a trigger for other metabolic abnormalities. The evidence that drug-naïve individuals presenting with symptoms of first-episode psychosis show markers of altered metabolic homeostasis (e.g., insulin resistance (Perry et al., 2016), increased blood glucose levels (Perry et al., 2021)) might suggest the presence of a persistent inflammatory dysregulation as the leading cause of the development of psychosis (Nettis and Pariante, 2020). Supporting this notion, individuals presenting with anxiety show elevated plasma levels of C-reactive protein, reflecting elevated inflammation, which would be modulated by cholinesterase-regulated microRNAs in an age- and sex-regulated manner (Shenhar-Tsarfaty et al., 2014; Meydan et al., 2016), and casual and experimental evidence also show a strong link between elevated levels of markers of inflammation and depression (Dantzer, 2019). Stress also predisposes to infectious and inflammatory diseases by promoting unhealthy habits (e.g., smoking, drinking alcohol, illicit drug use, poor sleep and diet, lack of physical activity) (Sinha, 2008) that, in turn, increase the risk for immune system dysfunction and promote immunosenescence, interfering in the long run with the physiological ability to decelerate the immune system’s production of proinflammatory agents, a failure that stems from immune cells becoming insensitive to cortisol (Dantzer et al., 2018; Cohen et al., 2012).

#### 4.4. Sex differences in the psychological stress response and predisposition to disease

That sex differences in the stress response exist is well documented both in the human and animal model literature (Novais et al., 2017; McCarthy, 2019). The effect of an adverse stressful childhood on the quality of symptoms, the severity, and the course of illness between males and females has been particularly well studied in schizophrenia, where physical and sexual abuse in childhood is associated with a younger age of disease onset both in males and females, but this association seems to be stronger in females (Pence et al., 2022; Comacchio et al., 2019; Kocsis-Bogar et al., 2018) – an earlier age at onset translating into a worse outcome in psychotic disease. Another feature that has been investigated is the effect of immigration on the likely development of NPDs in males vs females. Male migrants seem to present a higher risk of developing an NPD compared to female migrants (Cantor-Graae and Pedersen, 2013; Dykxhoorn et al., 2019), but the family structure would influence this risk in a sex-related manner. Social isolation and lack of family support might represent risk factors for women while immigrating with a dependent child might rather represent a risk factor for men (Dykxhoorn et al., 2019). This suggests that the response to stressful environmental factors differs according to sex and may contribute differentially to the expression of psychosis between the two sexes. The clinical manifestations of stress-related NPDs are also known to be epidemiologically different between sexes (Kucharska,

2017; Bale and Epperson, 2015), with men being more likely to develop substance use disorders as a maladaptive response to stress (an aspect that is also seen in patients with schizophrenia and first-episode psychosis (Ochoa et al., 2012)), while women have a higher risk of post-traumatic stress disorder (Ditlevsen and Elklit, 2010; Brezing et al., 2015), depression, and anxiety. More generally, and for both sexes, it is the capability of producing an adaptive response to stress during the entire lifespan, starting as early as the gestational period, that is critical for reducing the risk of developing an NPD (Bale and Epperson, 2015).

Several lines of evidence indicate that stress interacts in a sex-specific manner with the genome and that males and females are differentially vulnerable to specific stressors. For instance, in mice, chronic stress activates the HPA axis in both males and females but, in some stress paradigms, chronic corticosterone triggers negative valence behavior only in males and would be partly responsible for the observed differences between sexes (Dantzer, 2019; Iniguez et al., 2018; Mormede et al., 1990; Moieni et al., 2019). Experiments with dams have shown that the resulting long-term effects of lack of maternal care and prenatal stress on adult behavior are sex-dependent (Fig. 2). Maternal stress during gestation has been shown to predominantly affect male fetuses (Kim et al., 2015), resulting in a four- to eight-time increased risk of being affected by a neurodevelopmental disorder such as ASC, mental disability, stuttering, dyslexia, and ADHD than females (Bale, 2016). Maternal stress induces placental inflammation (Clifton, 2010; Martin et al., 2017; Mueller and Bale, 2008; Lesseur et al., 2014), and the placenta has been proposed as a key determinant in sex-specific vulnerabilities (DiPietro and Voegtline, 2017) to NPDs (Bale and Epperson, 2015). Sex differences in placental biology probably cause sex-specific transplacental signals to be relayed to the developing brain. The increased vulnerability of the male offspring would be mediated by epigenetic modifications, such as DNA CpG methylation (Champagne, 2008; Lux, 2018; Kuehner et al., 2019; Entringer et al., 2011). Various X-linked genes are expressed in greater quantities in the female placenta, playing a key role in neurodevelopmental programming. In particular, a significant increase in the X-linked gene *O-linked N-acetylglucosamine transferase (OGT)* and its control of the transcriptional repression mark H3K27me3 in the trophoblast cells of females, compared to males, might result in the placenta being less responsive to the gestational environment, protecting the developing brain (Bale, 2016). Another explanation for the reason why males would be more susceptible to in-utero developmental perturbations than females (Clifton, 2010; Eriksson et al., 2010) could be because their brain develops more slowly than that of females, placing them at increased vulnerability to adverse challenges over a relatively longer period than females during gestation (Bale, 2016). But, in prenatal stress experiments, the vulnerability outcome between sexes seems to also depend largely on the stressor that the dams have been exposed to. For instance, when dams were exposed to sound stress and forced swimming sessions, only the male offspring showed increased emotional reactivity to sound stress and cognitive deficits in the Morris water maze later – these effects seemed to involve the serotonin 5-HT<sub>1A</sub> receptors and were abolished if dams were given an anxiolytic before the stress sessions (Nishio et al., 2001). Mild maternal restraint stress during gestation also resulted in impaired spatial learning in the male offspring only but females, on the other hand, showed more anxiety and depressive-like behaviors, and both the anxiogenic behavior in females and the learning impairment in males were abolished if the dams were previously adrenalectomised (Zagron and Weinstock, 2006).

Hormones play a key role in brain functionality. They can regulate the programming of certain brain regions (those implicated in the HPA axis notably), leading to differences in response to stress between males and females across the lifespan. Both androgens and estrogens, along with their metabolites, can modify the stress response by acting on their receptors located inside and around the paraventricular nucleus of the hypothalamus (PVN) to modulate the release of corticotropin-releasing hormone (CRH) (Bale and Epperson, 2015). Significant differences between sexes in the

PVN/HPA axis and their regulatory mechanisms would be driven by gonadal hormones during development, resulting in sex and gender differences in responsiveness and vulnerability to stress (Handa et al., 1994; Goel and Bale, 2008; Walker et al., 1986) (Fig. 2). In humans, puberty marks a critical time point after which significant differences emerge between sexes in terms of physiological and pathological responses to stress. From this point in time, in men, the HPA axis becomes blunted during the progression through puberty by the rise of testosterone (which influences the GABAergic inhibitory tone of the PVN), while an increased susceptibility to stress is seen in women during the peripubertal and pubertal period, which are important for the programming of long-term risk for stress-related affective disorders (Bale and Epperson, 2015). There is thus a timing difference between males and females, the former showing an increased vulnerability to prenatal adverse events while females would be more susceptible at the time of puberty and then again during menopause, where hormonal dysregulation can trigger novel episodes of depression and psychosis (Seeman, 1997). In humans, girls that had been exposed in utero to maternal anxiety and depression have an increased risk of depression post-puberty (Heim et al., 2009) (Fig. 2). In rats, females are less sensitive to the effects of chronic prenatal stress but they show a more reactive HPA axis when they are re-exposed to chronic stressors later in life (Garcia-Caceres et al., 2010). Interestingly, when experimenters looked at the long-term effects of chronic prenatal stress on the reactivity to stress of the adult offspring, many of the hypothalamic structural rearrangements (pituitary adrenocorticotropic hormone (ACTH) content, CRH mRNA levels, cell turnover and cell death levels, astrogliosis, levels of pre- and post-synaptic proteins) involved in the long-term endocrine outcomes of chronic stress in the adult were different (and sometimes opposite) between males and females, suggesting that these sex-dependent hypothalamic structural changes could underlie the sex differences in the long-term sequelae of chronic stress, including the future reactivity to stress (Garcia-Caceres et al., 2010). This supports the idea that “the womb may be as important as the home” (Kim et al., 2015), with the risk of developing an NPD depending on a complex interaction between prenatal programming, later hormonal changes, and subsequent adverse life events.

Later in life, aging also affects the reactivity to stress of men and women differently, with women showing an enhanced cortisol response to physiological, psychological or pharmacological challenges compared to men of the same age (Otte et al., 2005). On the other hand, the post-menopausal period sees an equal distribution of the prevalence of affective disorders among sexes, further demonstrating the role of hormonal regulation in stress response and the onset of NPDs (Freeman et al., 2004) (Fig. 2).

#### 4.5. Studying gestational exposure to infections in the laboratory: the Maternal Immune Activation (MIA) model

A series of epidemiological studies pointed to a correlation between maternal infections during pregnancy and the onset of several NPDs, such as ASC (Atladóttir et al., 2010; Atladóttir et al., 2012), schizophrenia, mood disorders, epilepsy, and cerebral palsy (Atladóttir et al., 2010; Brown et al., 2004; Brown and Derkits, 2010; Mednick et al., 1988; Jiang et al., 2016; Coiro and Pollak, 2019). Original observations were based on epidemiological evidence in the human population looking at the association between viral infections and the risk of developing schizophrenia (the so-called “psychoses of influenza”) (Kepinska et al., 2020), leading to the hypothesis that immune activation in early life, for instance in response to a maternal viral or bacterial infection, would result in long-term effects on cerebral development (Fig. 2). As mentioned above, the placenta plays a significant role in regulating the interactions between the immune system and the development of the brain, through the maternal-fetal interface (Hsiao and Patterson, 2012). Several Maternal Immune Activation (MIA) animal models have been developed to replicate in a controlled manner the data

obtained from clinical epidemiological studies (Gumusoglu and Stevens, 2019).

In MIA studies, treatment with a double-stranded RNA synthetic analogue, polyinosinic:polycytidylic acid (Poly(I:C)) (Reisinger et al., 2015), which activates the Toll-like receptor 3 pathway, or with the bacterial endotoxin lipopolysaccharide (LPS) (Oskvig et al., 2012), are used to mimic viral and bacterial infection, respectively, during pregnancy (Coiro and Pollak, 2019), as they trigger the production of several pro-inflammatory cytokines that activate the immune response. The offspring of Poly(I:C)-exposed MIA rats mimic the phenotypical manifestations of psychiatric symptoms, which can be reversed by the use of antipsychotics. ASC-like behavior, expressed as inappropriate social interactions and repetitive behavior, is seen in the offspring of rhesus macaques exposed to MIA (Bauman et al., 2014). Anxiety-like behaviors can also be induced in MIA models that use LPS, and then reduced by the use of a selective serotonin reuptake inhibitor drug, such as the antidepressant fluoxetine (Lin et al., 2012). Rather than being due to direct damage from the infectious agent itself, the association of MIA with a specific neuropsychiatric predisposition in animal models would stem from an altered immune balance between the mother and fetus environments, the affected milieu of the developing CNS facilitating the emergence of aberrant brain structures and functions (Coiro and Pollak, 2019). Several hypotheses have been made to shed light on the pathophysiological effect of MIA on the development of the fetus' brain (Schwartz et al., 2013; Stolp, 2013). On one hand, inflammation-induced disruption of synaptic pruning (Gilmore et al., 2004) and neuronal survival (Paolicelli and Ferretti, 2017; Paolicelli and Gross, 2011), through overly active microglia releasing pro-inflammatory cytokines in excess, could contribute to the reduction in brain cortical thickness that is commonly found in patients with schizophrenia (Goldman et al., 2009). On the other hand, increased activation and density of astroglia and microglia, which have been found in post-mortem individuals diagnosed with depression, schizophrenia (Goetzl et al., 2020, 2021), and ASC (Frick et al., 2013), could induce synaptic disruption that would lead to altered synaptic transmission and connectivity (Fernández de Cossío et al., 2017), resulting in loss of local circuits and cortico-cortical connections (Goldman et al., 2009). Interestingly, recent transcriptomic profiling of the cerebral cortex in five major NPDs (ASC, schizophrenia, bipolar disorder, major depressive disorder and alcoholism) (Gandal et al., 2018) suggested synaptic dysfunction in ASC, schizophrenia, and bipolar disorder, supported by down-regulation of synaptic genes and up-regulation of astroglial genes. ASC being an early-onset disorder, it is remarkable that the condition showed a distinct upregulated microglial signature, following the proposed role of microglia in the regulation of synaptic connectivity during neurodevelopment (Salter and Stevens, 2017) (samples from patients with major depressive disorder showed neither the synaptic nor astroglial pathology but dysregulation of the HPA-axis and hormonal signalling, which was not seen in the other sampled disorders).

#### 4.6. Sex differences in the impact of gestational exposure to infections

The mechanistic underpinnings of sex differences in MIA models are still to be elucidated. In the vast majority of articles published so far, the outcome of MIA has been largely analyzed in the male offspring (Coiro and Pollak, 2019) and results might also vary across animal models, species, and experimental protocols. For example, in mouse offspring that has been exposed to MIA, only males show increased depression-like behaviors (Khan et al., 2014), while these are seen in both sexes in the rat offspring (Lin and Wang, 2014). In the offspring of MIA-exposed rats, the resulting adverse neurodevelopmental outcomes, such as reduced brain volume and alterations in the cerebral vasculature, were delayed in females relative to males (Piontkewitz et al., 2011). In mice, a prenatal immune challenge with Poly(I:C) resulted in an increased microglial reactivity in the hippocampus of the male

offspring compared to females, accompanied by increased expression of inflammation-related genes in the cerebral cortex and the hippocampus, and sensorimotor deficits (Hui et al., 2018). In another study, prenatal LPS induced a reduction in the expression of the fractalkine microglial receptor (CX3CR1) in the hippocampus, a receptor involved in mediating the pruning process in the offspring, but only in the male mouse progeny, leading to a significant increase in the number of spines in the granule cells of the dentate gyrus in males only (Fernández de Cossío et al., 2017). Others have described the presence of ASC-like repetitive behaviors only in MIA-exposed male mice, not in the female offspring (Xuan and Hampson, 2014). Therefore, while being exposed to inflammation during gestation is a known contributor to the risk of developmental NPD, being male seems to be another major risk factor, but the mechanisms underlying both risk factors, and how they relate to each other, are still poorly understood (McCarthy, 2019).

Gonadal hormones may play a critical role in the outcome of MIA (Wolstenholme et al., 2013; Keever et al., 2020) as regulators of neurotransmitters that are important in stress responsivity, not only CRH, GABA, and steroids, but also serotonin, norepinephrine, acetylcholine (Kaufer et al., 1998) and thyroid hormones (Bale and Epperson, 2015). Gonadal hormones modulate the expression of these neurotransmitters at almost any level, from transcription to microRNA-mediated arrest of translation to degradation (Soreq, 2015; Madrer and Soreq, 2020). Epigenetics might also play a critical role in determining sex differences in early-life programming, and thus vulnerability to developmental NPD (McCarthy, 2019). In rats, the development of the brain is affected by a set of genes involved in immune regulation that are normally strongly suppressed by epigenetic modifications, but this suppression is less important in males, as shown by the upregulation of immune cells and inflammatory mediators seen in certain brain areas of the developing male brain, such as the preoptic area (McCarthy, 2019). This makes for a critical sensitive period to inflammation, resulting in increased vulnerability to MIA in males, which overlaps with the temporal window for the "masculinization" of the brain and which might contribute, along with the influence of other likely factors (steroids from the fetal gonads, genes on the X and Y chromosomes, and a more active maternal immune system against male than female fetuses), to the differences observed between sexes in the risk for developing NPDs (Fig. 2).

#### 4.7. Sex-biased impact of eating behavior and dietary habits on brain health

Food is critical for the brain (Fig. 1A) and the quality/composition of food intake affects all cerebral activities, from brain metabolism to neuronal plasticity (Gomez-Pinilla and Tyagi, 2013; Briguglio et al., 2018); it is thus a key factor to consider in the phenotypical presentation of NPDs.

Overall, binge eating and irregular eating behaviors do not differ significantly between sexes (Forrester-Knauss and Zemp Stutz, 2012). However, sex-biased eating disorders are heavily influenced by societal expectations and cultural differences between sexes (reviewed in Jacka et al., 2010; Lojko et al., 2019; Spence et al., 2016) and, therefore, outcomes are often country-specific. Men are also too often underrepresented in clinical studies of subjects seeking treatment for binge eating disorder (Franko et al., 2012), while they are more affected by obesity than females (Rajan and Menon, 2017). But few sex differences are seen in the behavioral and psychosocial correlates of binge eating disorder in obese patients (Udo et al., 2013) and, while there is a bidirectional association between obesity and depression, this association is stronger in women (Rajan and Menon, 2017). However, treatment-seeking obese men are almost three times more likely than women to meet the criteria for metabolic syndrome (Udo et al., 2013). This latter sex difference has major clinical implications since binge eating disorder increases the risk for developing a metabolic syndrome over and above the risk that can be attributed to obesity (Udo et al., 2013; Hudson et al., 2010). This is important, as increasing reports alert on an actual higher prevalence

rate of eating disorders in adolescent boys than girls (Udo et al., 2013). If left untreated, binge eating disorder and obesity may result in the development of severe chronic conditions, such as cardiovascular disease and type-II diabetes, particularly in men (Hudson et al., 2010). Several bidirectional links exist between cardiovascular disease and mental illness (De Hert et al., 2018). In males, but not in females, with ASC, unhealthy habits relate to an excess risk of cardiovascular conditions (Mohammad et al., 2022; Weir et al., 2021). Mid-life presence of cardiovascular risk factors or mid-life obesity (Tolppanen et al., 2014) increase the risk of subsequent dementia (Dye et al., 2017). A bidirectional association between NPDs and diabetes also exists, NPDs being both a risk factor for and a complication of diabetes (Kota et al., 2012), which often causes a variety of neuropsychiatric symptoms (Ducat et al., 2014) and is a key independent risk factor for the development of cardiovascular disease (Martin-Timon et al., 2014) and the occurrence of neuropsychiatric symptoms in early AD (Shi et al., 2020).

The case of obesity is a powerful illustration of how poor dietary habits might have different indirect consequences on brain health between sexes in the long run. An inappropriate diet can also have a direct impact on brain function (McGrattan et al., 2019; Cai, 2013) with likely different outcomes between men and women. Neurons use glucose as their main energy source for supporting their activities, but a high glucose intake is commonly associated with an increased risk of depression (O'Neil et al., 2014) or cognitive decline (Pistollato et al., 2018), and acute consumption of a high-fat diet can prime the hippocampus to produce a potentiated neuroinflammatory response to a mild immune challenge that results in memory deficits (Spencer et al., 2017). Early-life adversity, poor early-life diet, overeating, acute high-fat diet consumption, and obesity can all produce an inflammatory response in peripheral immune cells and centrally, within brain structures mediating cognition (hippocampus) and emotion (e.g., hypothalamus, amygdala, and prefrontal cortex) (Spencer et al., 2017). Amplified inflammation in these regions results in impaired optimal brain functioning, leading to memory disturbances and/or depressive-like behaviors (Spencer et al., 2017). Neuroinflammation and the accumulation of senescent cells in the CNS, as well as overall cognitive decline, are associated with NPDs (McGrattan et al., 2019). On the basis that female brains are more sensitive to the detrimental effects of immune mediators, the higher prevalence of major depressive disorder seen in women, compared to men, may stem from a more severe inflammatory response experienced in response to psychosocial stressors (Dantzer, 2019). It is tempting to propose that an unhealthy lifestyle promoted by stress, including a poor diet, might participate in this. A western diet of processed or fried foods, refined grains, and sugary products provides an excessive ratio of omega-6 over omega-3 polyunsaturated fatty acids (PUFAs), leading to an unbalanced body composition between these two types of fatty acids, with potential consequences for cardiovascular and brain health. Such diet has been associated with depression and anxiety in women (Jacka et al., 2010).

An overlap exists in the neural circuitries of food intake, which are associated with reward mechanisms, mood and emotions (Spence et al., 2016; Singh, 2014), and stress. This may reinforce the link between stress and feeding behavior through elevated glucocorticoids and a dysfunctional HPA axis, which are common to both depression and obesity (Singh, 2014). Sex differences exist in the neural sensitivity to reward and are revealed when individuals are subjected experimentally to a bout of inflammation -the inflammation-induced decrease in ventral striatum activation in response to expected reward is more marked in tested females than in males (Moieni et al., 2019). This could represent a biological substrate for a higher risk of developing depression in women when experiencing inflammation, a phenomenon that would be exaggerated in those individuals who are already chronically inflamed and whose innate immune system has been primed by adverse social determinants of health. Note that in adolescents with an eating disorder it is the boys, who often have an obesity history and a longer eating disorder duration than girls, that are more likely to present with comorbid

depression (Dantzer, 2019; Moieni et al., 2019; Ridout et al., 2021).

Higher arachidonic acid and docosahexaenoic acid (DHA) levels, the main omega-6 and omega-3 PUFAs found in the brain, are seen in women compared to men, and these sex-related differences could be linked to sex hormones, estrogens stimulating and testosterone inhibiting the conversion of the respective precursors of omega-3 and omega-6 PUFAs (Spencer et al., 2017). It is not known if these sex-biased differences in PUFA levels have a role in brain diseases that have a sex component, but considering the pivotal roles of arachidonic acid and DHA in brain physiology, including the regulation of neuroinflammation and neurobiological processes involved in cognition and mood, this is an interesting area for future research (Spencer et al., 2017). Very few data are currently available –but see this pre-clinical study by Wahl et al. (2018). Like other environmental factors, the diet can modify the epigenome, and there is a known association between epigenetic measures of age acceleration and the diet (Bacalini et al., 2014; Quach et al., 2017). A recent study found sex-specific correlations between certain Mediterranean diet compositions and later age of PD onset (Metcalf-Roach et al., 2021). The pathophysiological mechanisms relating nutrition to sex-biased prevalence rates of PD are unknown, but these results suggest that sex-specific nutritional strategies may be a very effective tool to delay the onset of PD and, by and large, other NPDs. Of interest, a five-year nutritional intervention based on the Mediterranean diet was found to regulate the DNA methylation levels of eight inflammation-related genes (Arpon et al., 2016). Targeting chronic neuroinflammation is a promising strategy for PD and other neurodegenerative diseases (Wang et al., 2015). Sex-specific nutritional interventions may also be of particular importance in conditions where the establishment of a healthy lifestyle is challenging, e.g., in females with ASC (Weir et al., 2021), and to support healthy aging. Since increasing age is likely to significantly contribute to cognitive decline and incidence of dementia (Dye et al., 2017), the potential of implementing gender-specific diet (Gensous et al., 2020) and lifestyles (Wang et al., 2020) during midlife and late life for improving late-life outcomes warrants further exploration. Metcalfe-Roach et al. (2021) insist that healthy dietary habits should be promoted from an early age since prodromal features of various NPDs can manifest decades before diagnosis, and also because it is currently unknown if there are particular critical time windows in which dietary and other lifestyle habits are influential on brain health (Metcalf-Roach et al., 2021).

#### 4.8. Emerging perspectives: considerations of sex and gender identity for future medicine and research

The understanding of the complex interactions between genetics, the structure of the brain, the psychological factors, and the many environmental influences that the brain is exposed to during the lifespan is critical to apprehending the development of the vulnerability to neurological and mental health disorders. According to Paquin et al. (2021), a comprehensive model of risk for identifying key targets for the prevention of NPDs should replace risk factors within every individual's ecosystem. Applied to the development of schizophrenia, for instance, an array of factors, ecological and urban (social fragmentation, access to perinatal care, food, nature, and recreation), psychosocial and lifestyle (social support, physical activity, sleep hygiene, dietary habits), perinatal health (prenatal and early postnatal infections and/or stress, obstetrical complications), and biological (immune activation, stress hormones, perinatal hypoxia-ischemia), could influence human neurodevelopment and the mental health outcome through main actors (epigenetic alterations, oxidative stress, glial cells, neural growth, myelination, neurotransmitters, connectivity) that work in tandem with genetic susceptibilities, sex, and the timing of prenatal and postnatal exposure to biological insults. The outcome on mental health would be moderated by later-life exposure and the ecosystem would itself be reciprocally transformed throughout life by human action. Within this "ecosystem approach" to human health, it is becoming evident that the

sex variable alone has a considerable impact (Goyal et al., 2019; Naqvi et al., 2019; Li et al., 2019; Norheim et al., 2019). Still, the current knowledge on sex differences in the vulnerability to neurological and NPDs is very limited.

Sex is a parameter that receives relatively little consideration in the clinic (Ferrara and Srihari, 2020) and is often mostly disregarded in basic research, which in return perpetuates the vicious circle of lack of translation into the clinical practice. An improved understanding of the many differences between sexes will help to inform novel therapies and tailored treatments, but the sex variable also needs to be considered from a biopsychosocial point of view. At a time when the numbers of individuals identifying as transgender or seeking care in gender dysphoria clinics are increasing rapidly (Nolan et al., 2019; Marchiano, 2017) (a 240 % increase in referrals in gender dysphoria clinics between 2013 and 2018 (Torjesen, 2018)), particularly among young people, with an average age falling from just under 30 in 2014 to just over 20 four years later, and while some claim that biology plays a role (Luders et al., 2009), what contributes to our sense of gender is still unknown. The transgender population could help researchers to get an invaluable insight into the understanding of the determinants of gender identity. In a population showing higher rates of suicide, self-harm, smoking, self-medication, alcohol and drug abuse, and depression, a greater risk of long-term conditions and possibly a shorter life span compared to their peers (Torjesen, 2018), getting a more in-depth understanding of sex- and gender-dependent differences in the susceptibility to NPDs has never been more urgent. This is in the context of the recent emergence of a neuroimaging concept of brain androgyny (Zhang et al., 2021), with clear advantages attributed to such a brain in terms of mental health and well-being (Luo and Sahakian, 2021).

Zhang et al. (2021) used those differences in resting-state connectivity between brain areas that were seen between men and women (see section 2.3.) to build multivariate classifiers that have been trained to classify the sex of the brain. On a large cohort of participants (Luo and Sahakian, 2021), they achieved a 78% classification accuracy. This suggested that the functional architecture of the brains that could not be classified according to the dichotomy male/female may present with characteristics of both sexes. From a psychological point of view, some studies had already considered that certain individuals could display “mixed” male/female personality traits. At the level of the population, most people likely fall somewhere on a spectrum between the classic stereotypes of what is considered to be either male or female (Luo and Sahakian, 2021; Reis and Carothers, 2014). Next, instead of binarizing the output of their classifier into male and female, they created a brain male/female continuum, with the biological information they gathered about sex defining the ends of this continuum (either male or female) and the centre of the continuum defining androgyny. They found that those participants who mapped at the centre of the continuum had fewer internalizing or externalizing symptoms than those who mapped at the two extreme ends. These results offer an exciting avenue for future research. They highlight the importance of studying the influence of sex in the development of NPDs and other disorders not as a binary discriminant but as a continuous variable between the two extreme phenotypes of masculinity and femininity.

## 5. Conclusion

We have brought to light the role of sex as a modifier of the risk of developing an NPD with a focus on the contribution of genetic and epigenetic factors and the exposome. We have described a male excess in early-onset disorders that involve neurodevelopmental impairments and a female excess in adolescent-onset emotional disorders (Rutter et al., 2003). From a genetic point of view, inherited variants and multiple transcriptional changes, some going in opposite directions between men and women, might be involved in the higher prevalence of depressive disorders seen in women and the different clinical presentations of the disease between men and women, with different cell populations and

neural pathways affected between the two sexes. This gives rise to the exciting possibility of sex-tailored treatment for depression (Gerhard and Duman, 2018; Seney et al., 2018), which may assist in solving the apparent differences in the dynamics and efficacy of treatment between males and females with mental disorders (Simchovitz-Gesher and Soreq, 2020). X inactivation and immune gene escapees might help to explain the higher susceptibility of women to immune-related disorders, including depression (Dantzer, 2019; Leonard, 2010), and need to be factored in for basic and clinical research. Epigenetic and non-coding RNA-mediated changes under the influence of factors and/or challenges from the three domains of the exposome are heavily sex-dependent throughout the lifespan, opening new avenues for specific treatments and preventative strategies. Considering that gender's attributes are more likely to differ along a continuum rather than being simply categorical, another exciting avenue would be to encourage, from a young age, every individual to develop by offering them opportunities that are not restricted by their biological sex, as a simple tractable means for shaping brain and mind towards optimal performance and well-being throughout life, as recently suggested by Luo and Sahakian (Luo and Sahakian, 2021). “Let Toys Be Toys” (<https://www.lettoysbetoys.org.uk/>), a campaign directed towards the toy and publishing industries to stop limiting children's interests by advertising some toys and books as only for girls or boys, for instance, proposes ten ways to challenge gender stereotypes in the classroom.

Since the early 2000 s, calls and initiatives for implementing sex and gender into biomedical and health research have emerged across different levels of policy and research in the US and the EU (Powers et al., 2017). An NIH Policy on the Inclusion of Women in Clinical Research has been enforced, and women now account for around half of all subjects included in clinical trials supported by the NIH (guidelines available at: <https://grants.nih.gov/policy/inclusion/women-and-minorities/guidelines.htm>). This was followed by an NIH policy on *Sex as A biological Variable*, which was implemented in January 2016. In both pre-clinical and clinical research, the NIH now expects that sex is factored into the designs, analyses, and reporting, an effort that is supported by their “4 Cs of Studying Sex to Strengthen Science” (Consider; Collect; Characterize; Communicate). A comprehensive roadmap toward similar goals was established by the European Gender Medicine project (EUGenMed, 2013–2020). The actions, requirements and recommendations put forward by the European Commission are guiding the national funding bodies and research councils in the implementation of policies regarding gender equality and sex and gender analysis in research. As a result of this EU-wide effort to improve the value of biomedical science and translational research, by 2021, eleven national funding agencies (Austria, Cyprus, France, Germany, Ireland, Norway, Spain, Sweden, Switzerland, the Netherlands, and the UK) had implemented requirements for sex and gender considerations in knowledge production. Later this year and from 2023, the UK Medical Research Council will require from grant applications that sex is specified in all experimental designs involving animals, and human and animal tissues and cells. According to their new policy, the use of both sexes will be the default, and single-sex studies will need justification and become the exception.

However, while the funders of biomedical research are actively pushing for the move, in a concerted effort that needs to involve scientists and clinicians, patients, drug developers and policymakers, the call to action is still on (Arnegard et al., 2020) and the enforcement of sound policies will be crucial to implement a tangible and long-lasting change in the face of likely resistance at three main levels –cultural, institutional, and individual, as highlighted by Karp and Reavey (2019). We hope that this review will contribute to the change and encourage researchers from all biomedical and medical fields to consider sex as a crucial variable in their experiments and always relate to male/female balanced cohorts to analyse the outcome of the two data sets separately, standards that should be endorsed by pre-clinical scientists and researchers involved in clinical trials alike. We also encourage journal

editors and peer reviewers to require, or request, that sex is systematically considered as a biological variable in study design and analysis. Precision medicine will not progress without such a critical inclusion paradigm.

### Author contributions

AC conceived the idea of this review and chose the main topics with PNP. AC focused on the genetic and epigenetics aspects, including XCI and chromosome sex complement and related topics. PNP focused on stress, inflammation, dietary habits, on the organization of the article, and edited the final version. MF and FR wrote the parts about the genes and environment, including inflammation. HS added the RNA regulators concept, MTF helped with the writing and provided expert support. The final result is the fruit of teamwork.

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### Conflict of Interest statement

Maria Teresa Ferretti is the co-founder and Chief Scientific Officer of the non-profit organization Women's Brain Project. In the past two years, she has received personal fees from Eli Lilly, Lundbeck, GW and Roche for projects not directly related to the present review.

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