Sex-Related Differences in Neurodegeneration

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Sex hormones and genes on the sex chromosomes are not only key factors in the regulation of sexual differentiation and reproduction but they are also deeply involved in brain homeostasis. Their action is crucial for the development of the brain, which presents different characteristics depending on the sex of individuals. The role of these players in the brain is fundamental in the maintenance of brain function during adulthood as well, thus being important also with respect to age-related neurodegenerative diseases.

Keywords: sex hormones ; sex chromosomes ; neurodegeneration ; Parkinson’s disease

1. Focus on Parkinson’s Disease

Parkinson's Disease (PD) is the second most common neurodegenerative disease (following Alzheimer’s disease), affecting about 3% of the population by the age of 65 and more than 5% of people over 85, and it is caused by the preferential loss of dopaminergic (DA) neurons in the *substantia nigra pars compacta* (*SNpc*) [1][2]. Degeneration of DA neurons leads to the onset of classic motor symptoms, including tremors at rest, bradykinesia, rigidity, and gait disturbances. Other non-motor symptoms may occur either in the early stages of the pathology, such as gastrointestinal disorders and sleep disturbances, or in the later stages when dementia and psychosis may appear [1].

The typical hallmark of this disease is the accumulation of intracellular proteinaceous inclusions in surviving neurons, which are named Lewy bodies and Lewy neurites, and are mainly constituted by the presynaptic protein α-synuclein (α-syn) [3]. The accumulation of α-syn aggregates can hamper autophagic flux and impact on various intracellular processes critical for neuronal functions, such as synaptic vesicle recycling and docking, finally leading to neuron death [4].

Another characteristic of this disease is sustained microglial activation that determines chronic neuroinflammation, thus contributing to neuronal loss [5].

Astrocytes have also been found to be activated in PD brains [6] and, together with microglia, are known to be involved in α-syn aggregate removal [7]. Despite neurons being the most affected cells in PD, alterations in glial cells are known to contribute extensively to PD etiology. Microglial function presents various sex-related features, which may be relevant for the sex-differences observed in PD that will be discussed below. PD is characterized as being sporadic (of unknown etiology) in about 90% of the cases, while it is associated with gene mutations in about 10% of patients. Among its non-genetic forms, PD has been shown to be associated with exposure to pesticides, such as paraquat and rotenone, or to other environmental pollutants [8][9], which are known to impact mitochondrial function and to induce oxidative stress, and that may increase the risk of developing PD.

The impairment of these molecular mechanisms can lead to dyshomeostasis of neuronal cells and ultimately cause neuron death.

Among the different genes linked to PD, mutations or gene duplication and triplication in the *SNCA* gene, which encodes the protein α-syn, are of particular interest. *SNCA* mutations typically lead to an increased α-syn propensity to aggregate or to form oligomers. Moreover, mutations can alter α-syn function at the presynaptic terminal by impairing its ability to bind lipid membranes and other interactors [10], which are crucial for its role in the regulation of synaptic vesicle recycling. Finally, this protein, likely in its aggregated forms, is involved in the spreading of the pathology across the body and different brain regions according to the Braak hypothesis [11], which would explain disease progression. Despite being still under debate, it is clear that monomeric and aggregated α-syn can be transferred between cells through extracellular vesicles and between organs, i.e., from the gut to the brain, thus contributing to inflammatory processes and neuronal damage as determined by different toxicity mechanisms. Besides causative mutations in specific genes, a large set of other mutations have been identified as risk factors for PD. These mutations are located on genes mainly involved in autophagic–lysosomal function and mitochondrial activity, but also in many other intracellular processes [12].

According to existing information, several animal models (reviewed in [13]) have been developed to study the molecular mechanisms associated with this disease and to test novel therapeutic strategies. In particular, most of the studies have relied on the use of either rat or mouse models, which carry mutations in the genes mostly relevant to genetic PD forms, or neurotoxin-based models, which are obtained by administering to the animals toxins that have been linked to PD in environmental studies.

2. Sex-Related Differences in Microglia and Their Implication on Neuroinflammation in PD

The innate and adaptive immune responses, which development is regulated by genes, sex hormones, age, and reproductive state, display sex differences [14]. These differences could also explain, at least in part, the different susceptibility to neuroinflammation and sex prevalence in neurodegenerative diseases.

Microglia are considered the macrophages of the CNS: they can release reparative factors and adopt both pro-inflammatory and immunosuppressive phenotypes. Moreover, these cells regulate neural proliferation in early development by secreting pro-proliferative cytokines and further pruning superfluous cells by inducing apoptosis and targeted phagocytosis [15]. During adult neurogenesis, microglia control the plastic remodeling of neuronal circuits [16].

As reviewed by Chowen and Garcia-Segura in 2021, macro- and microglia show marked sexual dimorphism in their physiology and in their ability to react to brain pathology. For this reason, glial cells are recognized for being able to contribute in different ways to brain homeostasis and to the innate immune response which protects the brain against insults [17].

Microglia show different immunoreactive properties between the two sexes, which are initially established during sexual differentiation. The perinatal surge in testosterone and the hormonal milieu shape the morphology of different brain areas, including the microglial phenotype, which is further maintained during adulthood [15][18]. Moreover, the X chromosome contains a large number of genes related to the immune system, thus strongly contributing to the sex-related differences in immune response and inflammation (reviewed in [19]).

Male microglia have a higher density and higher phagocytic capacity, and they have been associated with more neuroinflammatory action, while female microglia are more supportive of neuronal functions, are neuroprotective, promote repair, and inhibit inflammatory response (reviewed in [20]). Interestingly, these abilities are also retained in the adult brain, as demonstrated by Villa and colleagues in 2018, who observed sex-related differences in the gene expression, in the morphology, and in the competence of female microglia to reduce ischemic damage even when transplanted in the male brain [18]. Adult microglial cells show sex-specific transcriptomes and are differentially subjected to the action of sex steroids depending on the hormone receptors they express (reviewed in [21]).

3. Sex Bias in Parkinson’s Disease and the Role of Sex Hormones

3.1. Estrogens

The sex difference in PD incidence points toward the possible protective effects of female steroid hormones, particularly estrogens. This possibility is also suggested by the reduced PD risk in women with longer exposure to endogenous estrogens due to early menarche and/or late menopause [22][23][24]. On the contrary, surgical or premature menopause, especially when occurring at an early age, is associated with an increased risk of PD [25][26]. However, besides these correlations, other studies have found no evidence of a positive effect of endogenous estrogens on the risk of PD [27][28], suggesting that the matter is likely more complicated.

Apart from epidemiological studies on PD incidence and its correlation with sex and female reproductive status, the neuroprotective and anti-inflammatory roles of estrogens have also been explored using model organisms of the disease.

Male rodents treated with the neurotoxin MPTP [29] show a higher depletion of striatal DA neurons and, consequently, of dopamine in the SN compared to females, thus mimicking the sex differences observed in human PD [30][31]. Similar results have recently been obtained using a rotenone animal model of PD [32].

The neuroprotective action of estrogens can also be due to the activation of anti-inflammatory pathways. In fact, estrogens have been found to modulate the response of microglia and astrocytes against oxidative and inflammatory injury in an MPTP rodent model, resulting in DA neuron protection [33]. Moreover, Tripanichkul and co-workers demonstrated that estrogen treatment prevents dopamine reduction, neuronal loss, and glial activation in the striatum of

MPTP-treated animals and determines a decrease in the secretion of pro-inflammatory modulators [34]. A similar outcome was obtained with estradiol benzoate treatment in a MPP+ rat model of PD [35]. The authors also detected an upregulation of the expression of paraoxonase-2 (PON2), a mitochondrial protein working as an antioxidant. Interestingly, this protein is transcriptionally regulated by estrogens and presents higher expression levels in the brain of female mice, thus making female brain cells less sensitive to oxidative stress [36].

In a previous study, the anti-inflammatory action of estrogens was shown to also occur by reducing microglial reactivity in rodents treated with 6-hydroxydopamine (6-OHDA) [37]. In the same model, an induction of the autophagic process was found to be activated by estrogens to prevent degeneration of DA neurons [38].

Finally, by binding to the ERβ isoform, estrogens are known to decrease microglial activation [39]. Therefore, it is unsurprising that ovariectomy in mice leads to an increase in neuroinflammation [40].

The neuroprotective role of estrogens was confirmed using knockout mice for the *Cyp19a1* gene, which encodes the aromatase enzyme. These mice, that are unable to synthesize these hormones, were found to show dopaminergic neuron impairment and enhanced vulnerability to MPTP-induced nigrostriatal damage, when compared with both treated WT and even their ovariectomized counterparts. The latter result suggests that estrogens produced locally by the brain may fulfill important neuroprotective functions [41].

Estradiol also exerts its protective role by upregulating *BDNF* expression in the SN, as demonstrated in a 6-OHDA male rat model of PD [42].

Evidence shows that circulating estradiol upregulates the expression of TH, an enzyme responsible for dopamine synthesis [43]. Estradiol treatment, indeed, increases the fiber density of DA neurons in pharmacologically induced PD mouse models. This neuroprotective action seems to be specifically mediated by ERα [44], as confirmed by the higher vulnerability to MPTP in mice in which ERα has been silenced, compared to WT and ERβ KO mice [45][46]. This protective effect of estrogen through ERα in DA neurons requires the activation of membrane-bound G protein-coupled estrogen receptor (GPER) [47].

Membrane-associated estrogen receptor alpha (mERα) can also exert a neuroprotective role by activating rapid non-genomic signaling cascades aimed at counteracting oxidative imbalance and mitochondrial dysfunction, thus promoting cell survival [48].

Finally, estradiol, besides being involved in neuroprotection as previously described, is thought to contribute to the maintaining of lipid rafts present in cell membranes, which provide a proper environment for optimal protein stability and molecular interactions on the cell surface. Aging and menopause-associated reduction in estrogen levels could lead to the loss of essential lipid components of membranes, finally causing neuronal dysfunction [49].

3.2. Progesterone

Progesterone has been found to exert a protective effect on experimental models of PD. This hormone contrasts the DA neuron depletion induced by MPTP in male mice [50] and exerts neuroprotective effects on the striatal neurotransmission systems in a male rat PD model [51]. Interestingly, the myenteric plexus of the enteric nervous system in MPTP-treated mice benefits from progesterone, thus suggesting a role for this hormone in gut myenteric plexus defense and in the prevention of gastrointestinal alterations, which are one of the main non-motor features of PD [52].

Finally, the treatment with the progesterone metabolite, allopregnanolone, has been found to improve motor coordination and increase the expression of TH protein and TH cell number in a MPTP mouse PD model [53].

3.3. Androgens

The role of androgens in neuroinflammation and PD is less clear. PD patients have been reported to present reduced testosterone levels [54], thus suggesting a possible protective role of this steroid. Moreover, testosterone replacement therapy has been found to ameliorate motor symptoms of PD patients [55].

However, this hormone has recently been found to increase neuroinflammation in N27 dopaminergic cells under oxidative stress. This is shown to occur through a putative membrane-associated androgen receptor via the activation of inflammatory pathways (nuclear translocation of NF-κB and activation of COX2 signaling), which induce the apoptosis of DA neurons [56] as well as exerting a suppressive role in the midbrain dopaminergic pathways [57].

In good agreement with these findings, testosterone and DHT are unable to counteract MPTP-induced dopaminergic toxicity [58][59].

In contrast with the results described above, the positive effects of chronic treatment with testosterone propionate, such as improved motor problems and reduced dopamine depletion, have recently been reported in a reserpine-induced progressive rat model of PD [60].

4. Effects of Estrogen-Based Pharmacological Treatments on Parkinson's Disease

Exogenous sex steroid hormone therapies (oral contraceptives, supplemented estrogens and progestogens, and synthetic anabolic steroids) are largely used to treat different conditions, such as testosterone deficiency (hypogonadism) [61], contraception or postmenopausal symptoms [62].

Due to the pleiotropic functions that sex steroid hormones exert on the brain during both development and adult life, the therapeutic use of these steroids can affect the brain in different ways. Therefore, it is not surprising that hormone replacement therapies (HRT) can impact cellular processes and pathologies involving this organ.

Regarding postmenopausal hormone therapies, their use has been reported to lead to a reduced risk of developing PD or a diminished disease severity together with an improvement in DA activity and dopamine transporter density [25][63]. Still, the results reported in different studies regarding PD incidence and the possible positive effects of HRT on this disease remain controversial, likely because of the different factors (dosage, duration, and composition of the replacement therapies used) that have been considered in each study [64][65][66].

In different neurotoxin animal models of PD, protective effects have been obtained when treating the animals with estrogens, as well as ER modulators (SERMs) or ER agonists (reviewed in [67]), thus suggesting that a potential repositioning of steroids, particularly estrogens, may improve the life quality of PD patients [66]. Unfortunately, estrogen replacement therapies can also be associated with detrimental peripheral side effects, such as uterine and breast stimulation, and consequent increased risk of cancer in these organs, stroke, coronary heart disease, and vascular problems [68]. These complications can be overcome by using estrogens designed to act only in the brain, where they can positively affect neurological diseases and menopausal symptoms, such as hot flushes, depression, and cognitive impairment, while sparing the adverse peripheral side effects.

Recently, Prokai and co-workers [69] synthesized and characterized a small-molecule bio-precursor prodrug, the "10b,17b-dihydroxyestra-1,4-dien-3-one", named DHED. After its systemic administration, DHED is rapidly converted to 17β-estradiol, preferentially in the rat brain, where it stimulates the same gene expression and neuroprotective effects obtained with 17β-estradiol treatment [70].

In vitro metabolic studies have demonstrated that DHED conversion to active estrogens does not occur in estrogen-sensitive peripheral tissues. Interestingly, this molecule reduces symptoms associated with the loss of brain estrogens and results in neuroprotective effects in rats [69][70]. In addition, this molecule cannot bind to ERs and, due to its physicochemical characteristics (increased water solubility and reduced binding to plasma proteins compared to 17β-estradiol), it crosses the blood–brain barrier more easily, thus showing a better uptake in the brain [70].

This prodrug has been tested in a double-transgenic mouse model of Alzheimer's disease in a side-by-side comparison with 17β-estradiol [71][72]. The authors reported that the DHED therapy achieved the same positive neuro-biochemical effects and behavioral improvement obtained with the 17β-estradiol treatment.

The potential of this prodrug has also been analyzed in PD models. In a symptomatic α-syn mouse model carrying the E46K human PD mutation in the *SNCA* gene, the brain increase in estrogens after DHED administration reduced PD-like neuropathy and improved behavioral effects in both female and male mice [73]. In a MPTP-induced PD mouse model, DHED treatments reduced the behavioral impairment and degeneration of DA neurons in the striatum and in the SN. Moreover, the authors measured a decrease in α-syn monomer accumulation and aggregation and a reduction in the levels of oxidative and inflammatory markers [74].

Although requiring more investigations, the lack of peripheral side effects and its brain-specific estrogenic activity suggest that this prodrug could represent an attractive potential therapeutic approach for neuroprotection and treatment of neurodegenerative disorders, including PD.

5. Contribution of Genes Located on the Sex Chromosomes to Parkinson's Disease Etiology

Other possible sex-related contributors to the etiology of PD, which may explain the prevalence of male PD patients, could be the genes expressed on the X and Y chromosomes.

Besides the obvious absence of Y-chromosome genes in XX individuals, it is well-established that the expression levels of X-linked genes can vary between males and females due to epigenetic marks and because some of these genes can be expressed from both gene copies in XX cells, leading to a higher genetic load compared to the XY genotype [75]. In fact, the X chromosome inactivation (XCI) can occur, but it may not always be able to level the differences. Interestingly, the X chromosome contains a six-fold greater number of genes involved in neurodevelopmental and neurophysiological processes than autosomes [75]. Moreover, in XY individuals, the maternal inheritance of the X chromosome leads to a single possible imprinting of X-linked genes, which cannot be balanced as it happens for females. This evidence further suggests that the differential expression of some of the X- or Y-linked genes can occur. Finally, mutations in X-linked genes have been associated with different disorders that are often characterized by intellectual disability, such as the Rett syndrome, the fragile X syndrome, and the Börjeson–Forssman–Lehmann syndrome [75].

In the PD framework, X-linked forms of parkinsonism have been reported and are associated with mutations in different genes. One example is the *RAB39B* gene, which encodes the small GTPase RAB39B and is located on the X chromosome. A variety of *RAB39B* mutations have been found, which mainly cause protein truncation, destabilization, or mislocalization, and are linked to early onset PD with intellectual disability [76][77]. This is believed to be associated with defects in the autophagic pathway, which were found to impact on synapse formation and function in *Rab39B* knockout mice [78].

Along the same line, it has been shown that about 39% of the male carriers of the expanded CGG repeats of the fragile X mental retardation gene (*FMR1*) present fragile X-associated tremor/ataxia syndrome (FXTAS) [79]. FXTAS patients often present PD or parkinsonism, suggesting a possible link between the two diseases [80]. The occurrence of PD pathological hallmarks, such as the occurrence of protein inclusions in the brain, in FXTAS patients suggests that *FMR1* can actually be considered a PD-associated gene [81].

Mutations in the *TAF1* gene, which is located on the X chromosome, have been associated with X-linked dystonia-parkinsonism (XDP) [82]. TAF1 protein is involved in the regulation of the initiation of transcription by RNA polymerase II, and it is still unclear how it participates in the development of XDP. Nevertheless, XDP is primarily observed in males [83], while no association with *TAF1* variants and female PD patients has been identified, which may be caused by the pattern of XCI previously mentioned [82].

Another interesting example is represented by a rare polymorphism in the X-linked gene *GLUD2*, which encodes a mitochondrial glutamate dehydrogenase specifically expressed in the brain. Gain-of-function mutations in this gene result in an earlier age of onset in PD, which does not occur in heterozygous female patients with PD, likely because estrogens are known to suppress its enzymatic activity thus compensating for the aberrant enzymatic activity caused by the mutations [84]. This estrogen effect on GLUD2 activity would further suggest that the interplay between genetic sex and sex hormones also needs to be taken into account when investigating molecular mechanisms associated with PD in males and females.

In all existing cases, limited information is available to understand how X-linked mutations in parkinsonism-associated genes may differentially contribute to the molecular mechanisms underlying the etiology of the disease. In an attempt to further verify the specific contribution of mutations on genes located on the X chromosome, X-chromosome-wide association studies (XWAS) would be recommended. Nevertheless, even if the X chromosome accounts for about 5% of the human genome and would provide interesting information on the matter discussed in this part of the review, it is usually excluded from most GWAS studies because of technical difficulties, thus hampering the access to this information [85]. The first report providing a XWAS on PD patients was obtained through a meta-analysis that included all available PD cohorts with data on the X chromosome, with the goal of identifying possible sex-specific risk factors that were not observed before [85].

When considering other X-linked genes within this subgroup, two additional genes reveal interesting hints for their possible link with PD development, i.e., *USP9X* encoding the protein ubiquitin-specific peptidase 9 X-linked and *OGT* encoding OGlcNAc transferase, both of which have been associated with intellectual disabilities [86][87].

In the PD framework, these proteins are particularly interesting because both ubiquitination and O-GlcNAcylation, together with phosphorylation and oxidation, are key posttranslational modifications of α-syn that allow the maintenance of its balance and homeostasis in cells [10][88]. USP9X was shown to deubiquitinate α-syn, but USP9X levels were significantly lower in the cytosolic fractions of the SN of PD patients compared to controls [89]. According to the authors, this may contribute to the accumulation of monoubiquitinated α-syn in Lewy bodies, which also occur in vitro when *USP9X* is downregulated.

In females, *USP9X* expression could be higher compared to males because *USP9X* is a gene known to escape X inactivation [90], providing a possible protective effect that reduces α-syn aggregation and, thus, the PD incidence in the female population.

Something similar may occur for the *OGT* gene: a higher expression in females may increase O-GlcNAcylation levels of α-syn. Since α-syn O-GlcNAcylation is shown to have site-specific inhibitory effects on α-syn aggregation and toxicity in vitro and in cell models [91] and in adenovirus α-syn mouse models for PD [92], *OGT* expression levels may be protective in XX individuals compared to XY individuals. It is worth noting that O-GlcNAcylation is generally crucial in neuronal development and signaling. Specifically, increased O-GlcNAcylation improves synaptic function in DA neurons [92], likely by reducing α-syn toxicity. However, other effects supporting DA neuronal function cannot be excluded.

Of note, male-specific genetics could also contribute to males' higher susceptibility to PD. For example, the *SRY* gene is expressed in male nigrostriatal dopaminergic neurons, which are more numerous in males than females, and they are known to be involved in dopamine synthesis and metabolism [93]. It has been reported that misregulation of this protein could contribute to PD development. Czech and colleagues in 2012 and 2014 demonstrated an increase in *SRY* expression in response to toxin-induced insults in male DA neurons and in a male neuroblastoma cell line [94][95], whereas Lee et al. (2019) correlated the reduction in SRY expression in toxin-induced rat models for PD (6-ODHA and rotenone models) with a decreased loss of DA neurons and with ameliorated motor defects. This occurred through an improvement in mitochondrial defects and inflammation induced by the toxin administration and, thus, suggested that *SRY* inhibition might be a male-specific therapeutic strategy in PD. According to the authors, these seemingly contrasting results can be explained by the in vitro system that lacks the cellular network between DA neurons and microglia and by the different time course of *SRY* upregulation [96].

6. Sex Differences in Immortalized and Primary Cells Relevant to Parkinson's Disease Research

As shown in different reports, the sex of the animals used as models in research is often omitted, and a strong bias is observed toward the use of males. Similarly, the genetic sex of cells is stated only in a minority of the published journal articles [97][98]. This situation has started changing in recent years, with an increase in the percentage of papers that comment on the sex of the model used. Kim and collaborators in 2018 reported that, among relevant manuscripts published in *AJP-Cell Physiology*, 50% indicated the sex of the cells used: a promising trend if compared with what was observed by Shah and collaborators in 2013, when the same journal could not boast more than 25% of papers assessing the sex of the cells used. However, between 2021 and 2022, this percentage dropped again (as reviewed in [99]). Investigators analyzing other neuroscience journals found less than satisfying rates of sex reporting (for a more detailed description of this phenomenon refer to [97][98][99][100][101]), and most of the existing studies failed to consider sex as a relevant biological variable even when both male and female models were used. This approach leads to an underestimation of possible sex differences due to different responses to drug treatments or genetic manipulations [102][103]. Interestingly, studies dealing with primary human cells were less precise on their model sex compared with those on non-human animal cells, and among the latter studies, cells isolated by researchers were better described than the purchased ones [97].

Cells do have biological sex that is intrinsically determined by the genetic complement of genes encoded by the X and Y sexual chromosomes and by the regulation of epigenetic mechanisms, all independent from the action of hormones, as evidenced, for example, by the morphological and functional differences observed in DA neurons derived and cultured from rats at embryonic day 14 (E14), while gonadal release of testosterone only starts from E15 onward [104].

Sex-related differences have been reported in cells of almost all tissues, including the brain [15][17][98]. Other than gene expression, male- and female-derived neuronal cells differ in morphology; growth rate and maturation; neurotransmitter metabolism; and response to insults, such as starvation, oxidative stress, and inflammation [17][98][105].

This is again of pivotal importance when dealing with the study of diseases characterized by a sex bias, as it happens in many neurodegenerative diseases, including PD.

The sex of cells and cell lines could be determined based on an analysis of the expression profile of the amelogenin gene, which is encoded by both X and Y chromosomes (*AMELX* and *AMELY*, respectively) and can be easily distinguished by using PCR because they present different lengths [106]. However, the sex of cells may change over time or after several passages in culture, even in established cell lines [99]. Cells derived from female donors may present chromosome Y fragments, while male-derived cells can completely lose their Y chromosome; thus, they can no longer be univocally assessed for their sex [98][107]. Moreover, different cell stocks, laboratory protocols, and culture conditions may contribute to the genetic variability and diversification among cell lines [99].

Beyond the intrinsic, chromosome-dependent sex, it is important to consider the hormonal environment where cells are grown. Cell culture media can indeed influence the hormonal milieu with estrogen-like compounds, which may differentially influence male and female cells. Serum contains all biomolecules and growth factors necessary for cell growth, cell metabolism, and cell attachment and proliferation, but it is usually rich also in hormones, including sex steroids. Phenol red, which is a common pH indicator present in most commercially available culture media, has been shown to bind and activate ERs, leading to estrogen-like effects in different cell types, including neurons, and even plastic materials could have weak estrogenic outcomes from leached phenolic compounds [101][107].

Therefore, the sex of cells is a matter that deserves attention and must be considered to provide as much complete information as possible, thus taking a first step toward considering and including sex as a biological variable. This is especially important from the perspective of tailored medicine that can address the differences observed between male and female patients.

As PD is one of the most frequent neurodegenerative diseases that affects the elderly population worldwide, with substantial human and social costs, it is necessary to address the molecular causes underlying its occurrence and the mechanisms of its progression to develop new strategies to counteract the neuronal degeneration and introduce new therapies. To do so, researchers are prompted to develop the most suitable models that better recapitulate the many features characterizing this complex disorder. As previously reported, PD shows a strong sex bias, affecting primarily males. Sex contribution, therefore, should be considered in the model used and monitored as a fundamental variable of disease outcome and progression.

The main cellular lines used to study PD are the well-established human neuroblastoma cell line SH-SY5Y and the lesser-characterized BE(2)-M17 [108].

The first one is a subline of the SK-N-SH cell line derived from the bone marrow biopsy of a metastatic neuroblastoma in a 4-year-old female. It was cultured in 1970 and underwent three rounds of clonal selection [109]. BE(2)-M17 cells, instead, were derived from a SK-N-BE(2) neuroblastoma cell line established in 1972 from the bone marrow tumor of a 2-year-old male patient.

They can both differentiate into neuron-like cells upon differentiation protocols with the administration of retinoic acid (RA). However, it has been reported that while RA is suited for obtaining valuable BE(2)-M17 neuronal morphology and functionality, in the SH-SY5Y cell line the most evident effects are induced after treatment with staurosporine [108][110].

It has been observed that these two cell lines show different features at the catecholaminergic level, with the BE(2)-M17 cell line having a more prominent dopaminergic phenotype, compared with the SH-SY5Y cell line, which expresses a more noradrenergic phenotype [108][110].

BE(2)-M17 cells show a more pronounced expression of TH and other markers associated with dopamine metabolism, such as DAT, a specific dopamine transporter, and VMAT2, a monoamine transporter that loads dopamine, serotonin, and other neurotransmitters into synaptic vesicles. Upon differentiation, all of these dopaminergic markers increase their expression. SH-SY5Y cells instead mildly express TH only when differentiated with staurosporine and present an increase in dopamine-ß-hydroxylase [111], which converts dopamine to noradrenaline and is specific of noradrenergic neurons [110][111]. Moreover, differentiation induces an increase in VMAT2 and SERT, a specific serotonin transporter, as it would be expected by serotonergic neurons [108]. Therefore, as it has been reported in different papers [108][110][111], the use of the BE(2)-M17 cell line could be more suitable to characterize the molecular mechanisms of PD that directly involve dopamine metabolism.

The genetic sex of these cells could provide other valuable information on the disease. Czech and collaborators verified that male BE(2)-M17 cells treated with toxin 6-OHDA, a product of dopamine metabolism that is involved in the generation of ROS and consequent DNA damage, present an increase in the expression of *SRY* mRNA and SRY protein transcription. This upregulation occurs rapidly and has a protective role in reducing reactive oxidative species and apoptosis [95]. However, SRY expression is reduced upon prolonged treatment and chronic neuronal injury, and its defensive role is, thus, ablated. Intriguingly, knockdown of endogenous SRY expression in BE(2)-M17 cells leads to increased cell damage and oxidative stress, whereas ectopic over-expression of *SRY* in treated feminine SH-SY5Y cells appears to be protective [95]. Human immortalized cell lines are valuable tools to dissect the molecular mechanisms that characterize PD, but they bring disadvantages, such as their oncogenic origin, which prevents them from fully recapitulating some key functional neuronal properties [112].

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