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Is Parkinson's Disease a Neurodevelopmental Disorder and Will Brain Organoids Help Us to Understand It?

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Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease. The incidence of PD cases increases with age, accordingly classically PD is considered to be an age-associated neurodegenerative disease. In this review, the hypothesis that PD is actually a neurodevelopmental disorder that is compensated for a long time will be discussed. However, patients who suffer from PD typically do not show symptoms early in their lives. This implies that, if the hypothesis that PD has a significant neurodevelopmental component is correct, the developmental defects are compensated for a long time. Furthermore, these developmental defects might not causally lead to the disease but increase the susceptibility for disease onset after a ''second hit.'' In this logic, deregulated developmental processes might represent the ''first hit.'' Even a minor developmental defect could lead to a reduced compensatory capacity or reduced fault tolerance of the entire system. In such a case of an already imbalanced system one or more additional hits could perturb the entire system sufficiently to bring it out of balance and lead to the pathology and symptoms which we classify as PD. However, if the developmental hypothesis and the ''multiple hit'' hypothesis are correct, an early diagnosis of these developmental defects might allow the start of a therapy for at-risk individuals before disease pathology becomes severe and before symptoms occur. Modern stem cell technologies, including the generation of personalized brain organoids, might play an important role in these strategies.

Keywords: Parkinson's disease, induced pluripotent stem cells, development, organoids

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease (AD). Currently, around 1% of the population over an age of 65 years is suffering from PD. More precisely PD should be seen as an umbrella term that summarizes several disease forms that eventually converge on a similar late-stage clinical manifestation [1,2].

PD is a progressive disorder with a wide spectrum of clinical features [3]. Generally, these can be classified as nonmotor symptoms and motor symptoms. The nonmotor symptoms often precede the motor symptoms by years or even decades; they include hyposmia, sleep disorders, hallucinations, and depression. Well-described motor symptoms are bradykinesia, rigidity, and tremor. Particularly the motor symptoms are caused by a reduction in dopamine levels in the striatum, which are caused by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta [4]. This degeneration of dopaminergic neurons is also the first main histopathological hallmark of PD [5]. The second major hallmark is the appearance of protein aggregate structures, containing misfolded proteins. The major component of these structures, which are called Lewy bodies or Lewy neurites, is the protein ALPHA-SYNUCLEIN. Importantly, the motor symptoms only appear after 50%–60% of the substantia nigra dopaminergic neurons are degenerated and the level of dopamine release in the striatum is reduced even further [6]. Thus, the disease must have originated long before the appearance of the motor symptoms.

Genetic as well as idiopathic forms of PD have been described. Most (>90%) of the genetic cases have a familial history while the rest is of sporadic nature [7]. In total, probably 5%–10% of all PD cases are clearly genetic. Currently, 34 chromosomal loci are associated to genetic forms of PD [8]. These include 11 Mendelian inherited genes causing autosomal dominant or recessive PD, and several more common genetic variants that represent risk loci. However, there are indications that also at least some idiopathic forms of PD have a probably rather complex genetic origin (Sanchez-Danes et al. [9]). Some genes with PD-associated mutations are involved in mitochondria biogenesis and quality control (LRRK2, PARKIN, PINK1, DJ-1, ALPHA-SYNUCLEIN) the autophagy-lysosome pathway (VPS35, ATP13A2, LRRK2, ALPHA-SYNUCLEIN) and the endosome-lysosome pathway (VPS35, DNAJC6, ALPHA-SYNUCLEIN); also a multitude of other cellular functions have been described for PD-associated genes. Among the nongenetic factors, the exposure to toxins, herbicides, pesticides, and heavy metals has been associated with the risk to suffer from PD [10]. But also infection, chronic

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inflammation, or psychosocial factors might contribute to the risk to suffer from PD [11–13].

So far no cure or neuroprotective treatment for PD is available. Only symptomatic therapies are efficient in a subset of affected individuals for a restricted time window [14]. The symptomatic treatments include the administration of Dopamine analogs as well as deep brain stimulation. Importantly, the chronic treatment with dopamine supplements often leads to serious side effects, such as abnormal involuntary movements and dyskinesias [15]. Furthermore, neither dopamine supplementation nor deep brain stimulation do ameliorate the actual neuropathological processes that are associated to PD.

The incidence of PD cases increases with age, accordingly classically PD is considered to be an age-associated neurodegenerative disease. In this review, I will take a different view point and address the question whether PD has a neurodevelopmental component.

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Indications for a Neurodevelopmental Contribution to PD

Patients who suffer from PD typically do not show symptoms early in their lives. This implies that, if the hypothesis that PD has a significant neurodevelopmental component is correct, the developmental defects are compensated for a long time. Furthermore, these developmental defects might not causally lead to the disease, but increase the susceptibility for disease onset after a ''second hit'' [16]. In this logic, deregulated developmental processes might represent the ''first hit.'' Even a minor developmental defect could lead to a reduced compensatory capacity or reduced fault tolerance of the entire system. In such a case of an already imbalanced system, one or more additional hits could perturb the entire system sufficiently to bring it out of balance and lead to the pathology and symptoms which are classified as PD. This second hit can be genetic (including somatic mutations), environmental (toxins and infections), psychosocial (stress), or even a combination of these. However, if the developmental hypothesis and the ''multiple hit'' hypothesis are correct, an early diagnosis of these developmental defects might allow the start of a therapy for at-risk individuals before disease pathology becomes severe and before symptoms occur. Modern stem cell technologies might play an important role in these strategies.

But what might be the nature of the actual ''first hit'' that leads to the developmental defects? Obviously mutations in the known PD-associated genes and risk factors are very likely candidates for this. Currently, mutations in 17 genes (8 autosomal dominant and 11 autosomal recessive) are known to be causative for PD [17]. Additionally, there are numerous risk factors. And further investigations, including GWAS studies and whole-genome sequencing approaches keep revealing new mutations with an increasing pace. However, besides clear single mutations, also complex combinations of single nucleotide polymorphisms (SNPs), which alone probably do not have a significant impact, might be sufficient to cause developmental alterations, which increase the susceptibility to develop PD later in life. Besides genetic causes, nongenetic events might also contribute. Which indications are currently available that support this neurodevelopmental hypothesis?

Indications from animal models

First, there are some indications from animal models, particularly mice. In a study from 2013, Garcia-Reitboeck et al. [18] have shown that the development of dopaminergic neurons of the substantia nigra is affected in mice embryos that are knockouts for the PD-associated gene ALPHA-SYNUCLEIN. Mice embryos lacking ALPHA-SYNUCLEIN develop less substantia nigra dopaminergic neurons than their wild-type siblings. Interestingly, PD seems to be caused by an ALPHA-SYNUCLEIN gain-of-function and not a loss-of-function, for example, a triplication of the ALPHA-SYNUCLEIN gene is clearly linked to the development of PD. Obviously it is not possible to directly conclude that, if in a knockout model less dopaminergic neurons are specified, that in a gain-of-function model more dopaminergic neurons than in a wild-type control would be specified. Nevertheless, these data clearly show that the PD-associated gene, ALPHA-SYNUCLEIN, plays an important role in the specification of the major cell type that is affected in PD.

So far, for the PD-associated gene, VPS35, no regulation of the specification of dopaminergic neurons has been described, but VPS35 regulates hippocampal neurogenesis. Particularly, a function during neurite outgrowth and maturation has been demonstrated [19]. Another PD-associated gene, PINK1, has been described to be expressed in the mouse brain at the main period of neurogenesis [20]. Also in zebrafish, a function of PINK1 during brain development has been shown [21].

Similarly, also LRRK2 is significantly expressed during embryonic neurogenesis [22] and it has been speculated that similarly to the function of VPS35, LRRK2 might play a role during neurite outgrowth [23]. Additionally, work with mouse neural stem cells that either expresses the PD-associated mutation R1441C in the LRRK2 gene or that are knockouts for this gene, further support a function for PD-associated genes during neuronal differentiation [24]. LRRK2 knockout cells were more efficient in neuronal differentiation, whereas this process was impaired in LRRK2-R1441C mutant neural stem cells. Very interesting from the developmental point of view is the role of LRRK2 in the WNT signaling pathway, which is one of the most important signal transduction pathways during brain development and neuronal identity specification. LRRK2 binds to WNT signaling proteins of the DISHEVELLED (DVL) family [25]. The interaction between LRRK2 and DVL is even stronger if PD-associated mutations of LRRK2 (eg, G2019S) are expressed. It was proposed that DVL could influence the activity of LRRK2 as a GTPase and kinase [25]. Further work demonstrated an additional binding of LRRK2 to the β-CATENIN destruction complex, a localization to the membrane, and an interaction with the WNT coreceptor LRP6, upon activation of WNT signaling. Based on these data, a scaffold function of LRRK2, as a bridge between membrane components and cytosolic components of the WNT signaling pathway, has been proposed [26]. In this context it also has been demonstrated that pharmacological inhibition of the LRRK2 kinase activity as well as expression of PD-associated mutations of LRRK2 disrupted WNT signaling [26]. Seemingly, both increased kinase activity (PD-associated mutation) as well as decreased kinase activity (pharmacological inhibition) lead to similar outcomes in the modulation of the WNT signaling activity. Likewise, proper embryonic brain development needs an exact and well-balanced activity of all

the critical components. The examples described in this study, highlight that PD-associated genes are among these components. Finally, the expression and function of PD-associated genes during embryonic and adult neurogenesis has been reviewed recently [27]. Nevertheless, it is important to also consider that these PD-associated genes might also be involved in temporarily different cellular and molecular processes. Hence, the interpretation of the results presented here needs to be done with great care.

Indications directly from human patients

Other indications for the hypothesis that PD has a strong neurodevelopmental component come directly from patient postmortem brain samples. Obviously, the amount of dopaminergic neurons is reduced in postmortem samples of the substantia nigra. However, during development, dopaminergic neurons are not only specified in the midbrain, but also in the olfactory bulb. Interestingly, it has been shown that the amount of dopaminergic neurons is increased in the olfactory bulb of PD patients [28]. Although the olfactory bulb is a region of active adult neurogenesis in mice [29], in humans, adult neurogenesis for the olfactory bulb is relevant only until the first 18 postnatal months [30,31]. Hence, an overproduction of dopaminergic neurons in the olfactory bulb is clearly a developmental effect. One could argue that dopaminergic neurons in the olfactory bulb are significantly different from the dopaminergic neurons in the substantia nigra and this argument is fully correct. Nevertheless, these data indicate that developmental neurogenesis is affected in PD patients. Furthermore, it is worth mentioning that hyposmia, which is certainly closely associated to olfactory bulb function, is one of the best described PD-associated nonmotor symptoms [32,33].

Additionally, the developmental contribution hypothesis is supported by the occurrence of juvenile PD cases. The first description of juvenile PD has been made already in 1954 [34]. In the meantime, juvenile PD has been reported to be caused by recessive mutations in the genes ATP13A2, PLA2G6, FBXO7, DNAJC6, and SYNJ1 [35]. Additionally, juvenile forms of PD have been reported for patients with mutations in PINK1 or PARKIN [36,37]. For example, there are case reports for two Turkish patients with a mutation in the FBXO7 gene, who showed first motor symptoms at the age of 14 years [38]. Even more extreme, a female Italian patient, also with a mutation in the FBXO7 gene, has been described with an age of onset of just 10 years [39]. Mutations in PINK1 and PARKIN have been described in patients from Japan and Hong Kong. For these patients, the age of disease onset was between 12 and 30 years [36,37]. Finally, mutations in ATP13A2 have been reported in patients from China, Jordan, Afghanistan, Pakistan, and Chile. In these cases, the age of onset was ranging from 10 to 18 years [40]. Obviously these are severe and rare cases. Nevertheless, they clearly underscore that PD is not necessarily an age-associated disease. It is tempting to speculate that in these juvenile cases, additionally to the described mutations, other genetic or nongenetic factors were contributing to the early onset.

A special look on the transcription factor NURR1

Beyond species differences, the transcription factor NURR1 (NR4A2) is a very interesting candidate connecting neurodevelopment and PD. NURR1 is a steroid/thyroid hormone nuclear receptor that regulates the expression of proteins involved in dopamine biosynthesis; these include TYROSINE HYDROXYLASE (TH), GTP CYCLOHYDROLASE I (GCH1), and TETRAHYDROBIOPTERIN (BH4) [41–45]. Furthermore, NURR1 is expressed during development in differentiating substantia nigra dopaminergic neurons as well as in the adult in mature dopaminergic neurons. Additionally, it is long established that NURR1 is important for the differentiation itself as well as for the survival of dopaminergic neurons [41–43]. Strikingly, new-born NURR1-deficient mice lack dopaminergic neurons in their midbrain [41,42]. Hence, a neurodevelopmental function for NURR1 is very clear [46]. However, what are the indications that NURR1 is of relevance for PD? In this context the first insights are that mice, which are heterozygous for NURR1, show a higher degree of vulnerability toward toxins, such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which is known to cause PD [47]. Additionally, NURR1 knockout mice show a progressive dopaminergic dysfunction [48]. These and other mouse genetics date clearly support the concept that absence of NURR1 leads to the appearance of PD-like characteristics in mice.

In humans it has been reported that the expression of NURR1 is downregulated (in postmortem brain samples) in cases of sporadic PD [49–51]. Although, so far, studies identifying NURR1 mutations as risk factors for PD several polymorphisms/variants have been reported in PD patients:

- Exon 1; −253C>T, −223C>T, −309C>T
- 5′-untranslated region; −291delT, −245T>G
- Intron 6; 7048–7049insG
- Intron 7; IVS7 + 33C>T
- Exon 3; 709C>G

Interestingly, only the 709C>G mutation is in the coding region of the NURR1 gene. Nevertheless, for most of these variants a reduction in the levels of NURR1 has been reported.

Finally, there are several reports showing that a pharmacological activation of NURR1 could be beneficial for PD treatment [52,53]. In summary, all these data indicate that NURR1 is not only an important factor for the specification of midbrain dopaminergic neuron identity during development, but also that reduced levels of NURR1, potentially caused through genetic variants, are associate to PD. This further supports the concept that deregulated developmental processes contribute to the susceptibility for PD.

Stem Cell Technologies for In Vitro Disease Modeling, Organoid Generation, and Drug Development

Usage of induced pluripotent stem cells for in vitro disease modeling

The ability to reprogram somatic cells into a pluripotent state revolutionized several areas of biology and biomedical research. The group of Shinya Yamanaka first achieved the reprogramming of terminally differentiated mouse cells into pluripotent stem cells that were very similar to embryonic stem cells; this new cell type was named induced pluripotent stem cells (iPSCs) [54]. This reprogramming was achieved by the overexpression of four key transcription factors. Just 1 year later also human skin fibroblasts were reprogrammed

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into iPSCs [55,56]. Importantly, iPSC generation is a rejuvenation process during which iPSCs lose most, if not all, age-associated marks [57]. With the availability and rapid development of the iPSC technology it became possible to generate PD patient-specific in vitro disease models. Intriguingly, iPSC models are a priori personalized models.

Over the last couple of years, several patient-specific iPSC-derived in vitro models for PD have been published. Obviously, most studies aimed at deriving dopaminergic neurons from iPSCs and to identify cellular phenotypes in these neurons. In the following, a few examples for in vitro disease modeling with PD patient-specific iPSCs are given, since the amount of articles on this topic is too large to be covered here, only a selection of articles will be discussed hereunder, whereas certainly others also would be worth mentioning.

Importantly, all results obtained with in vitro systems include the risk of in vitro artefacts. This limitation needs to be always taken into account when interpreting the results for such in vitro experiments.

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Using iPSC-derived dopaminergic neurons expressing the PD-associated mutation G2019S in LRRK2, together with isogenic control cells, allowed the detection of cellular phenotypes such as reduced neurite complexity and enhanced sensitivity to toxin treatment [58,59]. Particularly the LRRK2-G2019S mutation-induced reduced neurite complexity has also been confirmed in other studies [60,61]. Another study compared cellular phenotypes in iPSC-derived neurons from idiopathic patients to phenotypes in iPSC-derived neurons from patients with the LRRK2-G2019S mutation [9]. Also this study revealed a patient-specific reduced complexity of dopaminergic neurons. Furthermore, autophagy-related phenotypes are described. Strikingly, the phenotypes in neurons from patients with the LRRK2-G2019S mutation were comparable to those obtained in neurons derived from idiopathic PD patients. Since it is extremely unlikely that environmental (nongenetic) factors that a patient might have been exposed to persist after the complex process of iPSC derivation and dopaminergic neuron generation, the most straightforward explanation is that also these idiopathic cases are actually genetic. Most probably they are not monogenetic, but represent a complex combination of mutations or SNPs. It would be exciting to follow-up on these results and reveal the actual genetics underlying these phenotypes.

iPSC-derived dopaminergic neurons with mutations in GBA1 share some of the already-described phenotypes in this study. These include defects in autophagy processes as well as increased vulnerability to stress [62]. Interestingly, these GBA1 mutant neurons also have increased levels of ALPHA-SYNUCLEIN. Also for iPSC-derived dopaminergic neurons with alterations in ALPHA-SYNUCLEIN itself multiple reports exist. The majority of these articles are focusing on lines derived from patients with a triplication of the ALPHA-SYNUCLEIN gene. For these lines, several phenotypes, including higher levels of ALPHA-SYNUCLEIN and increased stress susceptibility are described [63]. Dopaminergic neurons derived from iPSCs with PD-associated mutations in the two genes, PINK1 and PARKIN, show, as expected, not only phenotypes in their mitochondrial functionality but also increased vulnerability and increased levels of ALPHA-SYNUCLEIN [64].

Not only phenotypes in neurons have been described, but also some reports describe phenotypes even in developmentally earlier cell types such as neural progenitor cells. It has been reported that neural stem cells, derived from patient-specific iPSCs, with the LRRK2-G2019S mutation, show increased susceptibility to proteasomal stress. Additionally, depending on the passage, defects in nuclear envelope organization and neuronal differentiation became apparent [65]. The passage dependency might indicate that defects would occur rather later in development. In another study in neural progenitor cells, first the expression of ALPHA-SYNUCLEIN has been demonstrated. Afterward it has been shown that these neural progenitor cells, when they express the PD-associated mutations, A53T or A30P in ALPHA-SYNUCLEIN, display deficiencies in the functionality of their mitochondria [66]. Importantly, this study is based on isogenic pairs of cells that only differ in the investigated mutation. Hence, it is safe to conclude that indeed a PD-associated mutation causes significant phenotypes in neural progenitor cells.

iPSCs themselves are comparable to embryonic stem cells. Also, iPSC-derived cells, including neurons, typically have any immature identity. A recently conducted single-cell sequencing approach confirmed that human stem cell-derived neurons typically resemble human fetal dopaminergic neurons [67]. That raises the question what the actual meaning and relevance of phenotypes in patient-specific iPSC-derived cells is. Does the appearance of these phenotypes support the neurodevelopmental contribution hypothesis for PD? The in vitro culture conditions might represent a stressor that accentuates phenotypes, which in vivo would be compensated. In line with this it seems conceivable that these phenotypes are detectable in in vitro models because they do not have the capacity for compensation like the full brain has. But still, phenotypes that are caused by PD-associated mutations in neurons that in most of their characteristics resemble dopaminergic neurons from the developing fetal human brain, are a strong argument for the concept that altered processes during brain development that contribute to the susceptibility of developing PD at later stages in life. Nevertheless, a strong drawback of all of these iPSC-derived models is that they do not convincingly recapitulate the cardinal features of PD, which are the appearance of ALPHA-SYNUCLEIN-containing protein aggregates, and most importantly, the strong degeneration of substantia nigra pars compacta dopaminergic neurons. A similar lack of an adequate in vitro model that recapitulates key disease-associated features existed in AD. However, recently the three-dimensional (3D) culture of human neural stem cell-derived neurons, expressing AD-associated FAD mutations, allowed for the first time the recapitulation of the two main pathological hallmarks of AD, which are the formation of amyloid-β plaques and neurofibrillary tangles [68]. Interestingly, it was even possible to use this in vitro model for first drug testing approaches. Probably similar approaches could be used in PD in vitro disease modeling.

Three-dimensional models, brain organoids, and their translational potential

Beyond the almost classical two-dimensional iPSC-derived dopaminergic neuron models in the last years, advanced 3D cell culture system has started to emerge. Currently, various approaches and platforms, with different

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capabilities are used by different groups. As an example, a scalable, 3D biomaterial platform for the generation of dopaminergic neurons from pluripotent stem cells has been described [69]. The major advantage of this approach seems to be that cell cultures in this 3D environment show a better survival after in vivo transplantation. In a different approach, neural progenitor cells were differentiated in a 3D matrix of Matrigel into functional dopaminergic neurons [70]. Among other features these neurons showed pacemaker activity, which is characteristic for substantia nigra dopaminergic neurons. Interestingly, in this particular approach, the neurons are differentiated in 3D in microfluidic plates that have the format of standard microtiter plates. This degree of standardization allows the utilization of robotics and automation, which is essential for high-throughput phenotyping and drug testing [71]. Even more complex than these 3D neuronal network cultures are pluripotent stem cell-derived brain organoids, collectively called mini-brains. By definition, organoids should contain several cell types that are organized similarly to the way they are organized in the organ they model [72]. Taking into account that some structures strongly differ in their development between human and animals or are even absent in common laboratory animals, organoids provide an extremely valuable tool to model the complexity of the human brain. Particularly for the brain, these culture approaches have been pioneered for the so-called cerebral organoids [73]. These cerebral organoids in principle should model the complete brain and they are particularly well suited to study cortex development. Following this pioneering work, other brain organoid systems have been described; these are in particular cerebellar [74] and forebrain organoids [75].

In the context of PD research, particularly the recently described midbrain organoid systems are of interest [75–78]. All of these contain dopaminergic neurons, but the different systems have different other capabilities. In summary, besides the differentiation into dopaminergic neurons some of these systems show differentiation into astrocytes and oligodendrocytes, also synapse formation, and neuronal functionality has been demonstrated. In one of the approaches, it is even possible to distinguish between A9 and A10 dopaminergic neurons [78]. Importantly, two of these midbrain organoid systems reported the presence of Neuromelanin, which is a feature typically absent in standard cell culture approaches as well as in mouse models [77,78]. Neuromelanin is thought to be a neuroprotective substance that is a side product of the high metabolic activity of substantia nigra-specific dopaminergic neurons [79].

Brain organoids that mimic several parts of the brain (eg, cerebral organoids) can have the drawback that the variability between individual organoids can be very high. However, approaches that describe the generation of more regional specific organoids seem to have less variability between individual organoids. All of the so far reported brain organoid systems lack a vascular system as well as microglia. Particularly, the addition of microglia would be an important future development, since these cells are believed to be critical for the PD pathogenesis [80]. However, with the recently published protocols for the derivation of microglia from human iPSCs [81–83], this goal seems to be in reach. Already previously, brain organoids have been proven to be excellent tools to study neurodevelopment diseases, such as Lissencephaly [73]. However, so far none of the brain organoid systems has been used to describe PD-specific phenotypes. Generally two approaches seem conceivable. First, it would be interesting to investigate whether it is possible to induce degeneration of dopaminergic neurons by treatment with toxins (eg, Rotenone or Paraquat) like it is currently done in animal models for PD. Second, it would be of outstanding relevance to find out whether midbrain organoids that are derived from patient-specific iPSCs show disease-relevant phenotypes. It should be addressed whether degeneration of dopaminergic neurons is detectable and whether ALPHA-SYNUCLEIN-containing aggregates occur. Particularly, for the midbrain organoid systems where it is possible to distinguish between A9 and A10 dopaminergic neurons even approaches where the selective vulnerability of the A9 dopaminergic neurons is addressed are conceivable. These new organoid systems open exciting possibilities and certainly will strongly contribute to PD research in the future.

How will the new stem cell-based technologies affect future medical approaches for PD?

Taking into consideration current efforts in research and development on the academic as well as the industry side it seems conceivable that in the next years a stratification of PD into several subgroups will be possible [84]. Already, now this is possible at least for the monogenetic forms [85]. With the usage of iPSC technologies, in principle already now it is possible to derive fully personalized disease models.

Additionally, several publications describe compounds that are effective for one or the other form of PD and several compounds are in the various stages of clinical trials. Examples for compounds are:

- BRF110 a selective agonist of the NURR1:RXRα complex. For this compound a neuroprotective role in animal models as well as in patient-specific neurons has been shown [53].
- Metformin, which originally has been described as a diabetes drug, seems to be effective in specific forms of PD where mitochondria are affected [86].
- Baicalein seems to inhibit ALPHA-SYNUCLEIN aggregation [87].

More of these examples could be presented and accordingly, there are reasons to hope that in the next future compounds, at least for several sub-forms of PD, will be available. It seems conceivable that in the future, persons at risk, for example, with a family background or after an early diagnosis of more or less subtle developmental defects, are treated by using these novel approaches. Patients might leave a sample (eg, skin, blood, or even urine) with their medical doctor. In clinics or specialized biotech companies this sample will be used for generation of iPSCs and their further differentiation in brain organoids. Brain organoids in turn might be first used for analysis and phenotyping to diagnose and stratify the individual into subgroups. For the different subgroups several compounds might be available. In contrast to current practice, where a drug is prescribed and the effectiveness is followed-up in the patient, the availability of personalized brain organoid models with disease-relevant phenotypes, will allow a pretesting of the compounds in these models. The patient will only receive the drug that showed the best effect in his own in vitro

model. Future developments, aiming at deriving organoids for each organ and coculturing them on specific microdevices, summarized as human-on-a-chip approaches, will even go one step beyond. It will not only be possible to test the effectiveness of a treatment, but will also investigate potential side effects at the same time, all fully personalized.

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Author Disclosure Statement

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