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Apoptosis and its Role in Parkinson’s Disease

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Doi: http://dx.doi.org/10.15586/codonpublications.parkinsonsdisease.2018.ch4

Abstract: Parkinson’s disease is one of the most common neurodegenerative diseases in the elderly. The motor symptoms occur predominantly due to substantial dopamine depletion, caused by degeneration of the dopaminergic neurons in substantia nigra pars compacta. Apoptosis has been implicated as the main mechanism of neuronal death in Parkinson’s disease. Apoptosis is mediated by a number of initiator and executioner caspases, and occurs via the intrinsic or extrinsic pathways. Activation of initiator caspase-9 mediates the intrinsic pathway—also called the mitochondria-mediated pathway. Alternatively, activation of initiator caspase-8 mediates the extrinsic apoptotic pathway—the cell death receptor–mediated pathway. Both initiator caspases converge onto a common pathway of executioner caspases, involving caspase-3 and caspase-6. Activation of the executioner caspases leads to the morphological features characteristic of apoptosis, such as DNA cleavage and its subsequent fragmentation. Proapoptotic factors, such as Bax, have been implicated in neuronal cell death in Parkinson’s disease, and there is evidence that both the intrinsic and extrinsic apoptotic pathways may play a role. This chapter provides an overview of apoptosis and its significance in Parkinson’s disease.
Keywords: Apoptosis; Caspases; Neurodegenerative disease; Parkinson’s disease; Programmed cell death

INTRODUCTION

Neuronal death occurs during normal development and in response to a myriad of pathological factors, such as traumatic injury (1), ischemia (2), infectious agents (3), or genetic aberrations (4). The major mechanisms by which neurons may die are apoptosis and necrosis. Apoptosis is the predominant mode of neuronal death in many neurodegenerative diseases (5, 6), including Parkinson’s disease (7). Whilst the pathogenic processes of Parkinson’s disease are not completely understood, convergent mechanisms result in neuronal death through apoptosis, making apoptotic pathways interesting potential therapeutic targets. Apoptotic cell death has been observed in cell culture and animal models of Parkinson’s disease, and also in nigrostriatal regions of the brains of patients with Parkinson’s disease at postmortem (8–10). This chapter provides an overview of apoptosis and its role in Parkinson’s disease.

APOPTOSIS

Apoptosis—the major pathway for programmed cell death—can be initiated by a number of broad classes of death stimuli, including abnormal intracellular calcium concentrations (excitotoxicity) (11), afferent or efferent trophic factor deprivation (12), activation of death receptors (13), and stress (12). Neuronal apoptosis is common during development and maturation, and is essential for shaping of the nervous system and development of appropriate circuitry (14). Apoptosis consists of a sequence of events, which are energy dependent. It is characterized by specific morphological and biochemical changes, including shrinkage of the cell, the chromatin becoming condensed, nuclear DNA fragmentation, and formation of apoptotic bodies, which contain nuclear material. During this process, the cell membrane retains its integrity. Apoptotic bodies are eventually removed by phagocytosis, importantly without a consequent inflammatory response (15, 16). Biochemically, apoptosis is characterized by increased rates of protein degradation (17, 18) and increased caspase activity (19). The biochemical components of the apoptosis pathways were first described in genetic studies on the nematode, Caenorhabditis elegans (20, 21), with subsequent studies identifying the mammalian homologues (22–24). These apoptotic biochemical components are a group of molecules called the B-cell lymphoma (Bcl-2) family, apoptotic peptidase activating factor (Apaf-1), and caspases (25).

Caspases

Caspases constitute a family of at least 14 cysteine proteases that regulate apoptosis (26). Caspases are present in normal cells as inactive zymogens, which are activated in response to apoptotic stimuli. In general, a single peptide precursor is
cleaved, via one or two chronological proteolytic steps, into an active enzyme, which consists of large and small subunits (27). Caspases can be subdivided into three functional categories: (i) inflammatory caspases-1, -4, -5, -11, -12, -13, and -14, are involved in immune responses to microbial pathogens by mediating the proteolytic activation of inflammatory cytokines (28, 29); (ii) apoptotic initiator caspases-2, -8, -9, and -10, have long pro-domains containing a caspase activation and recruitment domain (e.g., caspase -2 and -9), or a death effector domain (e.g., caspase -8 and -10); and (iii) apoptotic executioner caspases-3, -6, and -7, have short pro-domains. Initiator caspases, which are involved in the initiation of apoptosis, are able to carry out auto-cleavage and the cleavage and activation of common downstream executioner caspases (30). Executioner caspases do not have the ability to perform auto-cleavage, so their activation is dependent on this cleavage step. Once activated, the executioner caspases carry out the downstream events of apoptosis by cleaving a number of cellular substrates (30).

Caspases mediate several intracellular events that are important in apoptosis. These include:

(i) Disabling homeostatic and repair processes, such as DNA repair (31)
(ii) Cessation of cell cycle progression (31)
(iii) Signal amplification and inactivation of apoptosis inhibitors, through cleavage of pro- and antiapoptotic proteins (32)
(iv) Facilitation of nuclear and cytoskeletal disassembly (31)
(v) Marking dying cells for engulfment and disposal (31).

In addition, caspases have been shown to cleave Ca^{2+}-AMPA glutamate receptors, thereby preventing Ca^{2+}-mediated excitotoxicity and subsequent necrosis of neurons (33). Though some studies have suggested that caspases may play a role in necrotic death in some circumstances (34), in general they divert the cell to an apoptotic, rather than necrotic, fate (33, 35).

**Apoptotic pathways**

Caspase activation can be triggered by two well-characterized apoptotic pathways: the mitochondria-mediated (intrinsic) pathway (Figure 1), and the cell surface death receptor (extrinsic) pathway (Figure 2) (36). The intrinsic apoptotic pathway is mediated by members of the Bcl-2 family and the permeability transition pore (PT-pore) (Figure 1) (37). Bcl-2 is a family of proteins that possess either proapoptotic (e.g., Bax) or antiapoptotic (e.g., Bcl-2) properties. Members of this family exist on the cytoplasmic surface of mitochondria as well as many other organelles (38), and act as regulators of the PT-pore (39, 40). Opening of the PT-pore at contact sites between the inner and outer mitochondrial membranes results in depolarized mitochondria, loss of small molecular weight substances from the matrix, and ruptured outer mitochondrial membrane as a result of osmotic mitochondrial enlargement (41). The proapoptotic Bcl-2 family proteins induce outer mitochondrial membrane permeabilization, leading to release of cytochrome c, which normally exists in the mitochondrial intermembranous space (42). When released in the cytosol, it is bound by a protein called Apaf-1 in an ATP-dependent fashion, resulting in the formation of a multimeric Apaf-1/cytochrome c complex. The formation of the Apaf-1/cytochrome c complex is considered the commitment event that makes caspase activation irreversible,
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As this complex recruits procaspase-9, resulting in formation of the apoptosome (43). Procaspase-9 is then activated through proteolysis (42). Once activated, caspase-9 dissociates from this complex and subsequently activates executioner caspases-3, -6, and/or -7 (43). The construction of an Apaf-1/cytochrome c complex sets a relatively high threshold for caspase activation, preventing inadvertent commitment to apoptotic death due to leakage of cytochrome c from the mitochondria (43).

The extrinsic apoptotic pathway is dependent on the activation of cell surface death receptors (Figure 2). These constitute a group of trans-membrane proteins that belong to the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor superfamily. These receptors possess extracellular domains which include a highly conserved cysteine-rich repeat. Structurally associated molecules belonging to the TNF superfamily are the activating ligands for these death receptors (e.g., FAS ligand) (44). Binding of activating ligands to the receptors results in receptor trimerisation and recruitment of specific intracellular receptor-associated proteins, such as procaspase-8. Procaspase-8 is then immediately cleaved into the active form (caspase-8) that comprises two catalytic subunits which are able to activate downstream executioner caspases (45).

The downstream steps in the apoptotic pathways are then mediated by the executioner caspases, which cleave a large number of specific substrates (46). For instance, caspase-3 and caspase-7 inhibit DNA repair by cleaving the nuclear enzyme poly(ADP-ribose) polymerase (PARP), which normally participates in DNA repair (47). Caspase-3 also degrades DNA-dependent protein kinase

![Diagram of the intrinsic apoptotic pathway](image-url)
(DNA-PK), leading to reduced DNA repair capacity of the cell and subsequent promotion of the characteristic DNA cleavage that occurs in apoptosis (48). Furthermore, caspase-3 digests cytoskeletal proteins, such as actin and fodrin inducing cell shrinkage and membrane blebbing (49). Caspase-3 also leads to chromatin condensation and nuclear fragmentation through proteolytic activation of protein kinase C delta (50). Caspase-6 cleaves lamins, the main structural proteins of the nuclear envelope, resulting in nuclear shrinkage and the ultimate formation of apoptotic bodies (51). Morphological features of apoptosis include chromatin condensation, which starts peripherally along the nuclear membrane forming a ring-like structure, internucleosomal fragmentation of double-stranded DNA, and nuclear fragmentation (52). In addition, other morphological characteristics of apoptosis are membrane blebbing (53), cell shrinkage (54), and formation of apoptotic bodies, which are tightly packed with cytoplasmic organelles and nuclear fragments, and are ultimately engulfed by neighboring cells without provoking inflammation (55). The chief molecular components of apoptosis in neurons are the same as those in other nonneuronal cell types (56).
APOPTOSIS IN PARKINSON’S DISEASE

Apoptosis is the main mechanism of neuronal loss in Parkinson’s disease, as evidenced by the identification of DNA fragmentation and apoptotic chromatin changes in dopaminergic neurons of Parkinson’s disease patients in postmortem studies (10). In addition, the role of apoptosis in the pathogenesis of Parkinson’s disease was confirmed in postmortem and in vitro studies that illustrated elevated activity of caspase-3 and increased expression of active caspase-3 in substantia nigra pars compacta (57–59). Furthermore, dopaminergic neuronal death is inhibited by overexpression of anti-apoptotic proteins, such as Bcl-2, in cell models of Parkinson’s disease (60). Caspase inhibitors have also been shown to rescue neurons from death in cell models of Parkinson’s disease, adding further support to the notion that apoptosis is the main mechanism of neuronal death in Parkinson’s disease (61). Elevated levels of proapoptotic proteins, such as Bax, have also been seen in postmortem brain tissue from Parkinson’s disease patients (62).

Whilst there is some suggestion that the extrinsic apoptotic pathway may be active in Parkinson’s disease, its role remains unclear. The predominant mechanism of neuronal death is thought to be the intrinsic apoptotic pathway. Mitochondria-mediated apoptosis has been extensively studied in Parkinson’s disease. It involves a sequence of events including increased generation of reactive oxygen species, cytochrome c release and ATP depletion, as well as caspase-9 and caspase 3 activation (63). It remains unclear as to how the multiple pathogenic processes of PD such as alpha-synuclein (α-synuclein) aggregation and mitochondrial dysfunction, for example, interact with one another to converge toward apoptotic cell death. In the remainder of this section, some of the possible triggers of apoptosis in Parkinson’s disease are discussed. These include the interaction of α-synuclein with the mitochondrial membrane, the presence of nuclear DNA mutations, accumulation of mitochondrial DNA deletions, and mitochondrial dysfunction through other mechanisms (64).

α-synuclein is abundantly expressed in the central nervous system, particularly presynaptically (65). It is prone to fibrillar aggregation forming a major component of the Lewy bodies that are the pathological hallmark of Parkinson’s disease (65). α-synuclein aggregates and inclusions are formed in Parkinson’s disease brains, and rodents and cells treated with mitochondrial toxins (66–68). Accumulation of wild-type α-synuclein in dopaminergic neurons leads to decreased activity of mitochondrial complex I and increased reactive oxygen species generation—an effect which is more pronounced by the expression of the aggregation-prone mutant A53T α-synuclein (69). α-synuclein has also been shown to localize to the mitochondrial membrane in SHSY cells overexpressing A53T mutant or wild-type α-synuclein, and in isolated rat brain mitochondria (70), and this interaction has been suggested to lead to oxidative stress and the release of cytochrome c into the cytosol, in in vitro systems. Subsequent to its release into the cytoplasm, cytochrome c interacts with pro-survival, antiapoptotic proteins, triggering mitochondria-mediated apoptosis (70, 71).

Indeed, mitochondrial dysfunction may be an early occurrence in humans and in animal models of Parkinson’s disease (72–74). A defect in the activity of mitochondrial complex I has been observed in substantia nigra of Parkinson’s disease
patients (75). Dopamine metabolism leads to the generation of reactive oxygen species, which may lower the threshold for apoptotic cell death (76–78). Dopamine is enzymatically metabolized by monoamine oxidase (MAO), leading to the production of $\text{H}_2\text{O}_2$, which subsequently yields reactive oxygen species (76–78). Degradation products of dopamine undergo autoxidation, leading to increased reactive oxygen species generation (76–78). Hence, nigral dopaminergic neurons are particularly susceptible to dysfunction of mitochondrial complex I (79), which is believed to be one of the principal sources of reactive oxygen species in Parkinson’s disease. Reactive oxygen species production may therefore represent a potential important mechanism contributing to dopaminergic neuronal death through apoptosis (80). Defects in the activity of mitochondrial complex I are proposed to increase the susceptibility of dopaminergic neurons for degeneration, through lowering of the threshold for activation of the intrinsic apoptotic pathway (62, 81–83).

A number of mitochondrial toxins result in selective degeneration of dopaminergic nigral neurons through apoptosis, lending support to the idea that these neurons are particularly susceptible to mitochondrial dysfunction. These include 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), rotenone, and 6-hydroxydopamine (6-OHDA), which inhibit mitochondrial complex I causing mitochondrial dysfunction and generation of reactive oxygen species (8, 84, 85).

Dopamine itself is suggested to inhibit mitochondrial complex I, resulting in mitochondrial dysfunction (86). It undergoes autoxidation causing the excessive production of toxic metabolites that lead to oxidative stress and mitochondrial swelling and subsequent opening of the mitochondrial transition pore, which results in the release of anti- and proapoptotic factors (87, 88). Hence, cytochrome c is released into the cytosol, where it induces the intrinsic apoptotic pathway (87, 89, 90). It is also associated with significant increase in p53 phosphorylation, which is suggested to induce apoptosis (91, 92). Addition of antioxidants inhibits the activation of caspase-9 and caspase-3 and prevents apoptosis in response to dopamine exposure, supporting the fact that reactive oxygen species are important in dopamine-induced apoptosis (87, 90). Furthermore, overexpression of the antiapoptotic factor Bcl2 can partially attenuate dopamine-induced apoptosis (93).

MPTP is a neurotoxin that is selective to dopaminergic neurons of the substantia nigra pars compacta (94). MPTP is a lipophilic substance that actively crosses the blood–brain barrier to enter the central nervous system, where it is transformed to its active metabolite called MPP+ (1-methyl-4-phenylpyridinium) (95). This conversion is carried out by MAO that is present in the glial cells (95). Following its reuptake by dopamine transporter, MPP+ builds up in the mitochondria of dopaminergic neurons inhibiting the mitochondrial complex I, leading to ATP depletion and increased generation of reactive oxygen species (96, 97). As a consequence, nigrostriatal dopaminergic neurons die via apoptotic pathways involving caspases (98). MPTP-induced apoptosis is characterized by reactive oxygen species generation, cytochrome c release, p53 expression, cleavage of caspase-3, and DNA fragmentation, as well as by other morphological features characteristic for apoptosis (59, 99). MPTP-induced apoptosis is attenuated by overexpressed Bcl-2 levels (100, 101).

Similarly, rotenone inhibits mitochondrial complex I, resulting in the overproduction of reactive oxygen species and oxidative stress (102). Consequently, depletion in
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ATP levels occurs resulting in selective nigrostriatal dopaminergic degeneration via mitochondria-mediated caspase-dependent apoptosis (102).

6-OHDA inhibits mitochondrial complex I, induces Bax, and causes activation of caspase-3 and caspase-9 (103). 6-OHDA-induced dopaminergic neuronal degeneration is attenuated by caspase inhibitors (104). 6-OHDA also induces apoptosis that occurs via a mitochondria-dependent pathway (85). Whilst these nigral toxin models are not necessarily truly reflective of the pathogenic mechanisms that are occurring in patients, they offer insight into the susceptibility of nigral neurons to mitochondria-mediated apoptosis.

A number of inherited forms of Parkinson’s disease occur due to mutations in genes related to mitochondrial health and function. These include mutations in Parkin, LRRK2, PINK1, and DJ-1, for example (105). Whilst these mutations are rare within the Parkinson’s disease population, they offer some supportive evidence to the fact that nigral neurons are susceptible to mitochondrial damage and mitochondria-mediated apoptosis, and that these processes may be relevant in idiopathic Parkinson’s disease.

Parkin deficiency results in mitochondrial dysfunction in mice (106). Parkin has many roles that are potentially relevant in Parkinson’s disease pathogenesis. For example, it can promote mitochondrial biogenesis, mtDNA replication, and transcription of mitochondrial genes (107). Thus, Parkin is vital for mitochondrial respiration and function (107). In addition, Parkin acts as an E3 ubiquitin protein ligase that targets particular substrates for degradation via the ubiquitin-proteasome system, including the glycosylated form of α-synuclein (108). The loss of Parkin activity is thought to contribute to the buildup of toxic protein aggregates causing Parkinson’s disease (108). Interestingly, Parkin acts downstream of one of the other aforementioned genes—PINK1—a mitochondrial kinase in which mutations can cause an autosomal recessive familial form of early onset Parkinson’s disease. This is demonstrated by the fact that Parkin overexpression can compensate for mutations in PINK1 (109, 110). Whilst the mechanisms by which these mutations precipitate Parkinson’s disease pathology are unclear, there is some evidence that the PINK1-Parkin pathway may play a role in susceptibility to mitochondria-mediated apoptosis. For example, upregulation of wild-type PINK1 reduces cytochrome c release and caspase activation (111, 112).

Mutations in DJ1, which is present in the mitochondrial matrix and intermembranous space, can cause early onset Parkinson’s disease (113). Lack of DJ1 increases susceptibility to free radical-associated injury (114), whilst overexpression of wild-type DJ1 can be protective (115). Mutations in DJ1 result in increased oxidative stress. In addition, mutant DJ-1 binds very tightly to mitochondrial Bcl-XL, which is an antiapoptotic protein, resulting in dissociating Bax from Bcl-XL and its subsequent enrichment in the outer mitochondrial membrane, leading to the dopaminergic neuronal degeneration via mitochondria-mediated apoptosis (116).

In vitro studies have suggested a toxic gain of function brought about by LRRK2 mutations that cause Parkinson’s disease (117). LRRK2 mutation can lead to defective mitochondrial morphology and dynamics and increase generation of reactive oxygen species in cells (118). LRRK2 mutations have been suggested to cause dopaminergic neuronal death by mitochondria-mediated apoptosis subsequent to mitochondrial dysfunction. Apoptosis can be induced in vitro by the
overexpression of mutant LRRK2 with cell death being prevented by caspase inhibitors and genetic ablation of Apaf1 (61).

Mitochondrial DNA deletions have been observed in nigrostriatal dopaminergic neurons in aging and Parkinson's disease, possibly increasing their susceptibility to mitochondria-mediated apoptosis (119, 120). Mechanisms underlying mitochondrial DNA deletions are unknown with the possible involvement of oxidative stress (121). Combination of mitochondrial DNA depletion and deletion (without any alteration in the overall mitochondrial mass) results in reduced mitochondrial function and integrity, which increases the risk of cytochrome c release and apoptosis (122, 123). In addition, a rare form of inherited PD may occur due to mutations in the nuclear gene encoding DNA polymerase G (POLG), which plays an important role in the expression of a number of the genes encoded in mitochondrial DNA (124, 125).

**THERAPEUTIC IMPLICATIONS**

Given that the end-point of the Parkinson's disease pathogenic pathway is apoptotic neuronal death, treatments that target the molecular and biochemical events that allow progression of apoptosis may protect against the loss of dopaminergic neurons. As has been discussed, apoptosis is dependent on caspase activation (126). Thus, caspase inhibition has been considered as a novel therapeutic approach in neurodegenerative diseases occurring via apoptosis (126). Indeed, caspase inhibition prevents cell death of dopaminergic substantia nigra pars compacta neurons induced by MPTP or its active metabolite MPP+ in vitro and in vivo (127). However, although the dopaminergic neurons could be rescued, the nigrostriatal terminals were disrupted, suggesting that this approach may simply allow for the survival of dysfunctional neurons, suggesting that inhibition of apoptosis alone may in fact be detrimental (127). However, concomitant administration of glial cell line-derived neurotrophic factor (GDNF) circumvented this problem, allowing for restoration of striatal dopamine concentrations (127). It may therefore be feasible that caspase inhibition in combination with specific growth factors could play a role in future treatment of Parkinson's disease.

Interfering with events in the induction phase of apoptosis upstream to activation of caspases was regarded as strategy to prevent death of dopaminergic neurons and restore their function (128–132). For instance, Bax is upregulated in dopaminergic neurons subsequent to MPTP treatment (128). In addition, genetic deletion of Bax prevented dopaminergic neurodegeneration in the MPTP mouse model of nigrostriatal degeneration (128). Furthermore, Bax inhibition could decrease the loss of the nigral dopaminergic neurons that was caused by intrastriatal administration of 6-OHDA, suggesting Bax-inhibiting peptides as possible therapeutic avenue for Parkinson's disease (129).

The propargylamine derivative CGP 3466 (dibenzob,f]oxepin-10-ylmethyl-methyl-prop-2-ynyl-amine) has been shown to possess neuro-rescuing and anti-apoptotic characteristics (130). CGP3466B suppresses neuronal apoptosis by upregulating protein-L-isoaspartate (D-aspartate) O-methyltransferase (PCMT1), which is an enzyme that repairs damaged L-isoaspartyl residues in intracellular proteins. Upregulated PCMT1 leads to overexpression of the antiapoptotic Bcl-2
and underexpression of the proapoptotic Bax and active-caspase3, and thus inhibiting mitochondria-dependent apoptosis (133). Concomitantly, it prevents dopaminergic cell death both in vitro and in rodent models of Parkinson’s disease, and it consequently inhibits the development of MPTP- and 6-OHDA-induced motor symptoms (131, 132). Consequently, CGP 3466 may be promising in inhibiting dopaminergic neuron degeneration and the consequent progression of the neurodegenerative process in patients with Parkinson’s disease (131, 132). Thus, treatments that interfere with the apoptotic pathways may represent promising therapeutic strategies in the protection against the loss of dopaminergic neurons and the subsequent pathogenesis of Parkinson’s disease in the patients.

Having discussed these approaches, it must be acknowledged that there are concerns regarding the targeting of apoptosis in neurodegenerative disease. As has been discussed in this chapter, apoptosis in PD is thought to be triggered by a number of intracellular pathologies, with mitochondrial dysfunction being particularly important. Inhibition of apoptosis, therefore, may prevent the programmed removal of dysfunctional, nonviable neurons, which may ultimately lead to necrosis and a potential inflammatory response. In cell culture models of Parkinson’s disease, treatment with caspase inhibitors did indeed trigger a switch from neuronal apoptosis to necrosis (134). In addition, although genetic deletion of Bax inhibited dopaminergic neuronal death in response to 6-OHDA in transgenic mice, it could not improve behavioral deficits that were associated with Parkinson’s disease, and the surviving dopaminergic neurons displayed marked neuronal atrophy (135). Furthermore, systemic administration of an antiapoptotic compound may allow for the prolonged survival and accumulation of dysfunctional and potentially neoplastic cells in many tissues, which would clearly be detrimental. Thus, although apoptosis is the final step in the pathogenic pathway in PD, it remains to be seen whether or not inhibition of apoptosis in Parkinson’s disease can be effective and safe, and cautious evaluation is necessary.

CONCLUSION

Apoptotic cell death is suggested to be involved in the pathogenesis of Parkinson’s disease based on in vitro, in vivo, and human postmortem studies. Elucidation of the triggers of the apoptotic process in Parkinson’s disease can lead to a better understanding of the sequence of events that result in programmed cell death in Parkinson’s disease. Consequently, it would be possible to identify the potential factors that can be targeted therapeutically to stop or slow the progression of the disease and to recognize the individuals who are susceptible to developing Parkinson’s disease at early and preclinical stages.

Conflict of interest: The authors declare no potential conflict of interest with respect to research, authorship, and/or publication of this chapter.

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