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Unraveling the Role of Peptidyl-Prolyl Isomerases in Neurodegeneration

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Abstract Immunophilins are a family of highly conserved proteins with a peptidyl-prolyl isomerase activity that binds immunosuppressive drugs such as FK506, cyclosporin A, and rapamycin. Immunophilins can be divided into two subfamilies, the cyclophilins, and the FK506 binding proteins (FKBPs). Next to the immunophilins, a third group of peptidyl-prolyl isomerases exist, the parvulins, which do not influence the immune system. The beneficial role of immunophilin ligands in neurodegenerative disease models has been known for more than a decade but remains largely unexplained in terms of molecular mechanisms. In this review, we summarize reported effects of parvulins, immunophilins, and their ligands in the context of neurodegeneration. We focus on the role of FKBP12 in Parkinson's disease and propose it as a novel drug target for therapy of Parkinson's disease.

Keywords FK506 binding proteins · Immunophilins · Immunophilin ligands · Parvulins · Cyclophilins · Neurodegeneration · Parkinson's disease

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Over the past few years, it has become more and more clear that different neurodegenerative diseases (ND) display remarkable similarities. Considerable efforts have focused on protein aggregation in neurodegeneration. Examples of proteins that aggregate in ND are alpha-synuclein (α-SYN) in Parkinson's disease (PD), tau and Aβ in Alzheimer's disease (AD), prion protein in prion diseases, polyglutamine disease proteins in polyglutamine repeat diseases (e.g., huntingtin in Huntington's disease), and SOD1 in amyotrophic lateral sclerosis. All these proteins turned out to be completely or partially unfolded (also called intrinsically disordered (ID)), and to adopt a certain fold upon aggregation, mostly a β-sheet rich amyloid-like fibrillar structure. These neurodegenerative disorders have therefore been classified under the concept of "protein conformational disorders" or "the disorder in disorder concept" (reviewed in [1, 2]). In this review on the role of peptidyl-prolyl isomerases (PPIases) in neurodegeneration, we want to point these out as another potential parallel between different ND.

We first introduce the different families of PPIases (FK506 binding proteins (FKBPs), cyclophilins (CyPs), and parvulins). Next, we discuss α-SYN and PD as an example of a protein conformational disorder. Third, we give an overview of the numerous reports of PPIases and their inhibitors in ND. Finally, we end with a discussion and perspectives on the role of PPIases in ND.

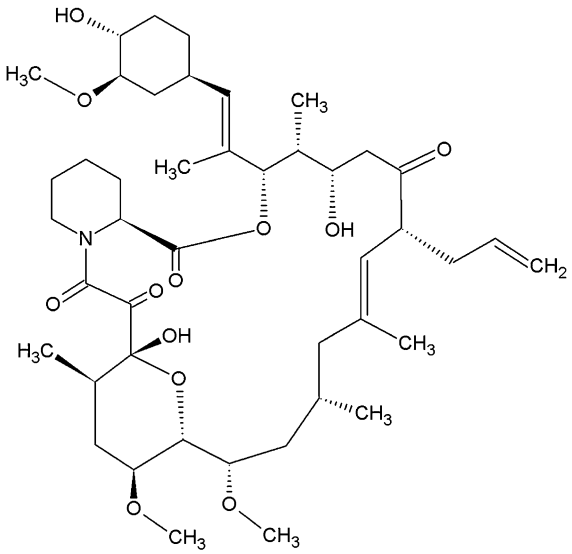
Introduction

FK506 and FK506 Binding Proteins

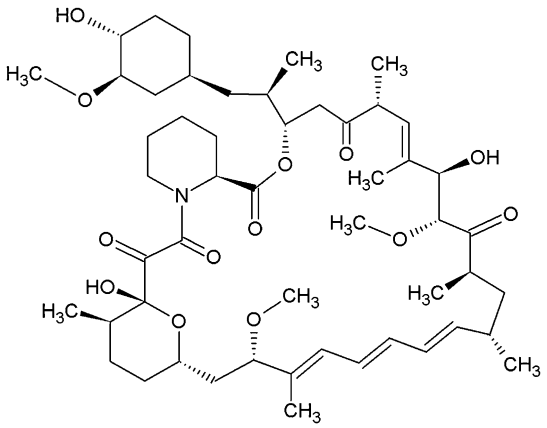
FK506 was originally discovered in 1984 by studying the effects of *Streptomyces* products on lymphocyte prolifera-

tion [3]. A compound from a *Streptomyces* strain found in a soil sample from the city of Tsukuba—therefore named *Streptomyces tsukubaensis*—was found to be strongly immunosuppressive. This molecule, a neutral macrolide lactone with restricted antimicrobial activity [4], was named FK506 (Fig. 1). The immunosuppressive effect of FK506 was found to depend on its interaction with a protein, referred to as FKBP [5]. FK506 (tacrolimus) is now an FDA-approved drug for the prevention of allograft rejection after liver or kidney transplantation.

Since the discovery of the first FKBP, 15 members of the FKBP family have been discovered in humans [6]. All FKBPs display an FK506-inhibitable PPIase activity [7–9] which means that they are able to accelerate the intercon-



FK506



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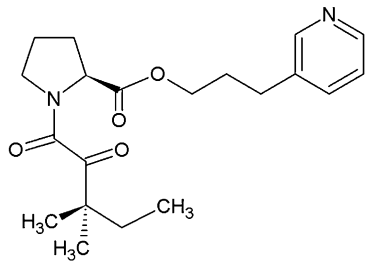

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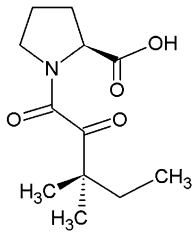

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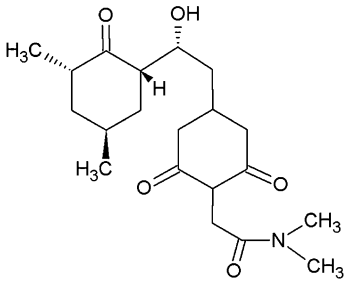

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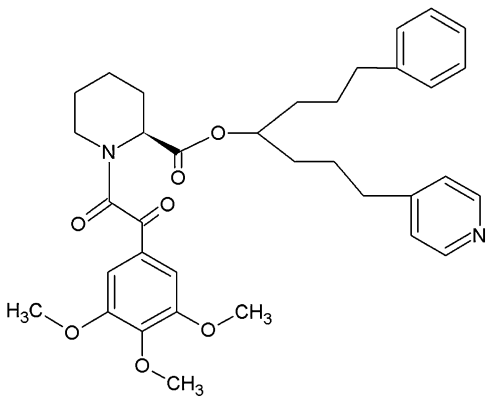
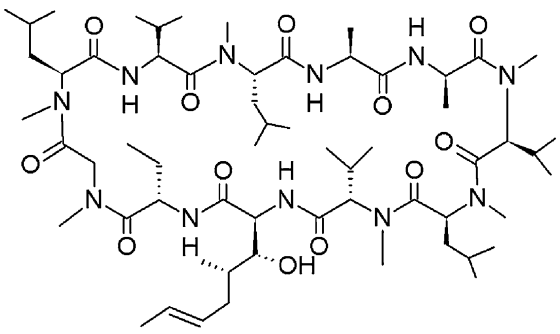


V-10,367



cyclosporin A

Fig. 1 Chemical structure of different immunophilin ligands: FK506 [3], rapamycin [27], GPI-1046 [110], GPI-1485 [159], V-10,367 [160], DM-CHX [107], and cyclosporin A [51]

version of *cis–trans* isomers of X-Pro peptide bonds. This is an energy-demanding step in protein folding (see below).

Within the human FKBP family, four different groups are recognized based on intracellular localization and domain composition (reviewed by Rulten and coworkers [6]). The number describing each FKBP reflects its molecular mass in kilodaltons. The four groups are a cytoplasmic group (FKBP12 and FKBP12.6), a nuclear group (FKBP25 and FKBP135), a tetratricopeptide repeat (TPR)-containing group (FKBP36, FKBP37, FKBP38, FKBP51, and FKBP52), and a secretory pathway group (FKBP13, FKBP19, FKBP22, FKBP23, FKBP60, and FKBP65; Table 1). TPRs are protein–protein interaction domains often found in proteins within multiprotein complexes [10]. FKBP12, the smallest member, is considered as the archetypical protein out of which other FKBPs have evolved by gene duplications and/or changes in the N-terminal and C-terminal domains [6]. The secondary structure of FKBP12 is shown in Fig. 2.

Depending on the FKBP identity and the tissue where it is expressed, FKBPs display a myriad of different activities. These can be PPIase-dependent or PPIase-independent. The most important functions are summarized below and in Table 1. Since the focus of this review is on the effect of immunophilins and their inhibitors (immunophilin ligands (IL)) in ND, the description of FKBP functions will be restricted to those topics that are important to understand the sections below.

Regulation of Ca^{2+} Release in Heart and Skeletal Muscles

Contraction of a muscle is a Ca^{2+}-dependent process. Depolarization of the muscle fiber membrane opens voltage-gated Ca^{2+} channels, permitting a small influx of extracellular Ca^{2+}. Although this is generally insufficient to produce a full contraction, it evokes a rapid and massive release of Ca^{2+} from the sarcoplasmic reticulum. This process is called Ca^{2+}-induced Ca^{2+}-release and allows full contraction of the muscle. Two channels are responsible for Ca^{2+}-release from the sarcoplasmic reticulum; the Ca^{2+}-release channel or ryanodine receptor (RyR), and the IP_3 receptor [11, 12]. These channel proteins release Ca^{2+} rapidly in response to triggering signals and then spontaneously inactivate to prevent depletion of Ca^{2+} in the sarcoplasmic reticulum and excessive intracellular Ca^{2+} concentrations. A sustained high Ca^{2+} concentration has deleterious consequences on virtually every aspect of cell function and should thus be avoided. Two FKBPs are recognized as regulators of Ca^{2+}-release channels: FKBP12 and the closely related FKBP12.6. Concerning RyR, they assist in the inactivation of the channel by stabilizing respectively skeletal and cardiac RyR in the closed conformational state [13–16]. FK506 virtually abolishes the ability of the RyR to close in the presence of high Ca^{2+} levels [17]. However, an interaction between FKBP12(.6) and RyR cannot always be found [18–20]. For IP_3R regulation, the situation is even less clear. Some studies say FKBP12 potentiates IP_3R activity [21]; others claim that FKBP12 inhibits channel activity [22] or that there is no binding between FKBP12 and IP_3R [18, 20, 23]. A possible explanation for all these discrepancies is that the binding and function of FKBP12(.6) on the RyR/IP_3R is highly tissue- and cell-type-dependent [22].

Regulation of the Activation of the Immune System

The immunosuppressive effect of FK506 is the result of an interaction with FKBP12 in T cells [24, 25]. The FK506–FKBP12 complex binds and inhibits the Ca^{2+}-dependent phosphatase calcineurin, thus preventing the dephosphorylation and consecutive nuclear translocation of the transcription factor named nuclear factor of activated T cells (NF-AT) [26]. NF-AT promotes the transcription of the interleukin 2 gene, an important cytokine in the activation and proliferation of immune system cells (Fig. 3).

Rapamycin (Fig. 1), another FKBP inhibitor isolated from *Streptomyces hygroscopicus* [27], also has an immunosuppressive effect that is however mediated through a later event in the interleukin 2-stimulated pathway [28]. The FKBP12–rapamycin complex inhibits proliferation of T cells, through its interaction with the FKBP–rapamycin–associated protein kinase, also known as mammalian target of rapamycin (mTOR) [29, 30]. Of course, rapamycin normally is not present in a cell which excludes a normal physiological role for the FKBP12–rapamycin complex. A recent study, however, identified FKBP38 as the natural inhibitor of mTOR [31], since it was found that FKBP38 bound to mTOR inhibited phosphorylation of downstream targets, eventually inhibiting cell growth. Still, two subsequent reports contradicted the role of FKBP38 as an endogenous regulator of mTOR [32, 33].

Assistance in Protein Folding and Regulation of Protein Conformation

As mentioned before, FKBP proteins have a PPIase activity. In unfolded proteins, there generally exists equilibrium between the *cis* and the *trans* conformation of X–Pro peptide bonds, whereas proline residues in folded proteins populate solely the *cis* or *trans* conformation depending on their local environment. Around 5% of prolines adopt the *cis* conformation in folded proteins [34]. This is considerably more than other amino acids, which exist almost exclusively in the *trans* conformation. This implies however that several X–Pro peptide bonds

Table 1 Overview of human PPIases

	Protein	Class/localization	PPIase domains	Extra domain (s)	Functions	Reference
	Human FKBPs					
1	FKBP12	Cytoplasmic	1		Ca^{2+} signaling, immune suppression neurodegeneration	[13, 14, 16] [24, 26] [123]
2	FKBP12.6	Cytoplasmic	1		Ca^{2+} signaling	[15]
3	FKBP25	Nuclear	1	DNA binding	Tumor suppression	[162]
4	FKBP135	Nuclear	1	WH1, MT, DNA binding	Endosome transport/cytoskeletal organization	[163]
5	FKBP36	TPR containing	2	TPR, LZ, CaM	GAPDH inhibitor spermatogenesis	[164] [165]
6	FKBP37	TPR containing	2	TPR,LZ,CaM	AH signaling	[166]
					Hepatitis B suppression	[167]
7	FKBP38	TPR containing	2	TPR,LZ,CaM	apoptosis	[129]
8	FKBP51	TPR containing	3	TPR,LZ,CaM	Hormone signaling	[168, 169]
					Cancer	[170]
					Neurodegeneration	[135]
9	FKBP52	TPR containing	3	TPR,LZ,CaM	Hormone signaling	[168–170]
					Neurodegeneration	[123, 133, 134]
					Ca^{2+} signaling	[171]
10	FKBP13	Secretory pathway	1		Complement system	[172]
					Vesicular trafficking	[173]
11	FKBP19	Secretory pathway	1	TM	Unknown	[6]
12	FKBP22	Secretory pathway	1	EF hand	Cancer resistance	[174]
13	FKBP23	Secretory pathway	1	EF hand	Chaperone regulation	[175]
14	FKBP60	Secretory pathway	4	EF hand	Unknown	[176]
15	FKBP65	Secretory pathway	4	EF hand	Osteogenesis	[177]
					Chaperone	[178]
	Human cyclophilins					
1	CyP18a (hCyPA)	Cytoplasmic/ secreted	1		Immune suppression, Cell-related events, HIV-1 and HCV replication	[26] [179, 180] [181, 182]
2	CyP18b	Nuclear	1		Oncogene	[183]
3	CyP18c (hCyPJ)	Nuclear	1		Spliceosome	[184]
4	CyP18ci	Nuclear	1		Spliceosome	[184]
5	CyP18d (CGI-124)	Nuclear	1		Spliceosome, cancer growth	[184, 185]
6	CyP22b/p (hCyPB)	ER/secreted	1		Protein folding, TPRV6 channel-associated	[186] [187]
7	CyP22C/p (hCyPC)	Membrane	1		Unknown	[188]
8	CyP22D/p (hCyPF)	Mitochondrial	1		Mitochondrial pore and function, apoptosis	[54] [149] [189, 190]
9	CyP19 (hCyPH)	Nuclear	1		Spliceosome, pre-RNA splicing	[184] [57]
10	CyP33 (hCyPE)	Nuclear	1	RRM	RNA-binding/spliceosome	[191]
11	CyP35 (hCyP35)	Nuclear	1	RRM	Organism growth	[192]
12	CyP40 (hCyPD)	Cytoplasmic	1	TPR	HSP-steroid receptor	[193]
13	CyP46	Cytoplasmic/nuclear	1	LLR	Signal transduction	[58]
14	CyP54	Nuclear	1	WD40	Cancer marker/spliceosome	[194]
15	CyP57	Nuclear	1	RRM	Spliceosome	[195]
16	CyP58 (hCyP60)	Nuclear	1	RING	Spliceosome	[184]

Table 1 (continued)

	Protein	Class/localization	PPIase domains	Extra domain (s)	Functions	Reference
17	CyP73	Nuclear	1	WD40	Spliceosome	[184]
18	CyP88 (hCyPG)	Nuclear	1	SR	Spliceosome	[196]
19	CyP157	Nuclear	1	SR	Spliceosome	[197]
20	CyP358	Nuclear	1	Nup	Nucleoporin	[198, 199]
	Human parvulins					
1	Pin1	Nuclear	1	WW	Cell cycle regulation	[200, 201] [63]
					Molecular timer	[202]
					Cancer neurodegeneration	[151, 154]
2	Par14	Mitochondrial	1		rRNA processing	[203]
					Cell cycle progression	[204]
					Chromatin remodeling	[205]
3	Par17	Mitochondrial	1		Unknown	[206]

Information on FKBPs has been adapted from [6]; data for the cyclophilins have been adapted from [52]

AH aromatic hydrocarbon, WH1 WASP homology region 1, binds polyproline-containing peptides, *MT* myosin tail, *TPR* tetratricopeptide repeat, *LZ* leucine zipper, *CaM* calmodulin-binding domain, *TM* transmembrane region, *EF hand* Ca^{2+} binding domain, *WD40* Trp-Asp containing β-propeller repeat domain, *RRM* RNA-binding, *LLR* leucin-rich repeat, *Nup* nucleoporin domain, *RING* really interesting new gene domain, *SR* Ser/Arg-rich domain, *WW* double Trp domain interacting with pro-rich sequences

have to switch their conformation during folding; a process with a high activation energy barrier (80–100 kJ mol^{-1}) and therefore often the rate-limiting step in protein folding. PPIase activity facilitates this *cis–trans* conformational interchange, and, although limited, evidence exists that FKBPs can assist in protein folding [35–37]. Based on this property, FKBP proteins have been categorized as chaperone or heat-shock proteins.

Other Functions

Several other functions of FKBPs have also been found, but fall out of the scope of this review: a role in carcinogenesis [38–40], insulin secretion [41], Ca^{2+}-absorption in the kidney [42], hormone receptor signaling [43–47], nitric oxide synthase function [48], and viral replication [49].

Cyclosporin and Cyclophilins

Next to the FKBPs, two other protein families have PPIase activity: the cyclophilins and the parvulins. Cyclophilins are also immunophilins since inhibition by cyclosporin A causes immune suppression. Parvulins, however, are not immunophilins because they are unable to influence the activity of the immune system. The parvulins will be discussed in the next section.

Much alike the FKBPs, cyclophilins were originally discovered as cyclosporin A (CsA) binding proteins [50]

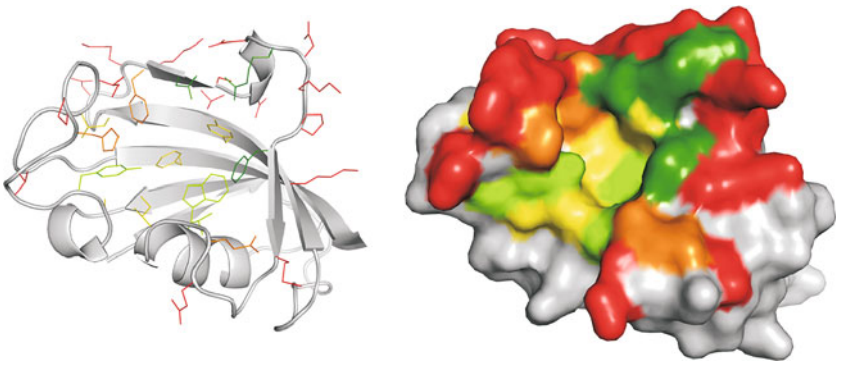


Fig. 2 Structure of FKBP12. FKBP12 is depicted as a cartoon with important residues shown as lines (*left*) or with a Conolly surface (*right*). While the bulk of the protein is *gray*, several residues are color-coded: *yellow*: aa interacting with FK506; *red*: aa interacting with calcineurin; *blue*: aa responsible for catalytic activity; *orange*: aa interacting with FK506 and calcineurin; *light green*: catalytic aa interacting with FK506; *dark green*: catalytic aa interacting with calcineurin and FK506. Images were made in PyMOL (DeLano Scientific LLC, Palo Alto, CA, USA) based on PDB ID: 1TCO [161]

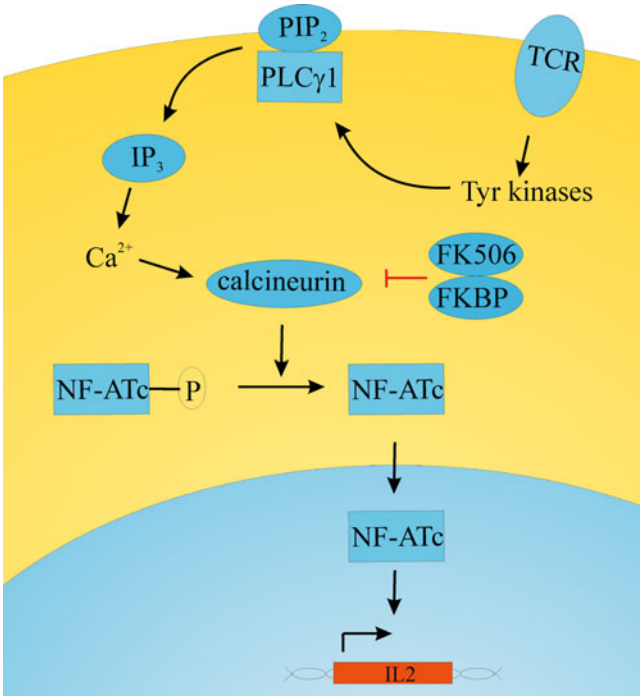


Fig. 3 Immune suppression by the FKBP–FK506–calcineurin complex. When an antigen binds a T cell receptor (TCR), a signal cascade involving IP_3 is initiated, leading to the activation of calcineurin. Calcineurin dephosphorylates NF-AT, after which it translocates to the nucleus and regulates initiation of IL2 transcription. The FKBP–FK506 complex inhibits calcineurin, effectively halting the immune response

with PPIase activity. CsA is a cyclic peptide initially isolated from the fungus *Tolypocladium inflatum* in 1969 [51]. Nowadays, some 20 paralogues of cyclophilin A (CyPA), the first cyclophilin identified, have been found [52] (Table 1). Moreover, many proteins also have cyclophilin-like domains with PPIase activity, although several of those cannot bind CsA. The first and most important use of CsA is immune suppression. Again, like the FKBP12/FK506 complex, the CyPA/CsA complex binds to and inhibits the phosphatase activity of calcineurin which causes immune suppression. Although the sequence diversity of cyclophilins is rather large, the CsA binding domain displays structural homology and also several conserved residues [53]. The cyclophilins can be found in different cellular organelles having diverse biological functions such as assembly of mitochondrial permeability transition pore regulation [54], chaperone functions and assisting in protein folding [55, 56], assembly of mRNA splicing supracomplexes [57], and association to channel proteins [58]. A more complete overview can be found elsewhere [53].

Parvulins

The parvulin family is the most recently identified and to date, smallest subgroup of PPIases. Only three members have been identified in humans: Pin1, Par14, and Par17. Pin1, the most well-known parvulin, can accelerate protein folding in vitro [59]. The discovery of its unique specificity for prolines preceded by phosphorylated Ser or Thr residues (pSer/Thr-Pro) [60, 61] has initiated research on the regulatory functions of Pin1 (reviewed in [62–64]). Briefly, Pin1 regulates phosphorylation signaling by changing the local conformation of proteins around a phosphorylation site. This makes the Ser or Thr less or more accessible for dephosphorylation which affects the availability of the protein for downstream effects of phosphorylation. In this way, Pin1 can act as a molecular timer to initiate or halt signaling cascades at certain time points/events in the life of a cell.

Alpha-Synuclein and Parkinson's Disease, a Protein Conformational Disorder

Named after James Parkinson, a British physician who first described the disease in 1817, PD is a slowly progressing disorder that affects primarily neurons of the substantia nigra (SN). This small nucleus in the midbrain consists of approximately 400,000 nerve cells and is an important regulator in the voluntary movement pathway. Pathologically, PD is characterized by the presence of Lewy bodies (LB) and Lewy neurites [65, 66] and a selective loss of dopaminergic neurons in the SN pars compacta (SNpc). LBs were found to predominantly contain a fibrillar form of the protein α-SYN [67]. Gradual degeneration of SNpc cells induces typical PD-related motor symptoms—resting tremor on one or both sides of the body, general slowness of movement, stiffness of limbs, and gait/balance problems. Non-motor clinical features may also occur including dementia, depression or psychosis, and abnormalities in olfactory and visual perception. Treatment with L-dopa, a precursor in the synthesis of dopamine, usually improves the motor impairments [68]. However, this is a purely symptomatic treatment which aims to restore dopamine levels in the affected brain regions. Current research therefore aims to unravel important pathways leading to disease in order to find a treatment that stops or slows down the course of disease. An important clue to identifying these pathways comes from the familial forms of PD, caused by mutations in an increasing number of genes, determined as PARK genes. Currently, 15 PARK genes have been identified [69]. So far, extensive studies on these genes have suggested an important role in PD for mitochondrial function, synaptic transmission, protein breakdown, axonal transport, and oxidative stress (reviewed in Mizuno et al. [70]). Complementary with genetic studies, epidemiological studies already identified several non-genetic risk factors [71]. These point to oxidative stress as a main trigger for disease onset.

As stated above, LBs primarily contain the protein α-SYN, a protein of 140 aa that is ubiquitously expressed in the brain and accounts for up to 0.1% of total brain protein [72]. It was first discovered as a vesicle-associated synaptic protein in the electric organ of the *Torpedo californica* (electric eel) [73] and was also proposed to influence song learning in birds [74]. α-SYN belongs to the family of the synucleins together with two closely related members; β- and γ-synuclein [75]. In 1997, α-SYN was the first protein to be linked to PD: PARK1 encodes α-SYN in which an A53T missense mutation was identified as a cause of autosomal dominant familial PD [76]. Later, the protein was implicated in many other ND, now commonly described as the synucleinopathies [77]. Examples hereof are AD, multiple system atrophy, Hallervorden-Spatz disease, amyotrophic lateral sclerosis, and LB dementia. α-SYN is believed to play a central role in PD based on several observations. First, LBs are predominantly composed of aggregated α-SYN [67]. Second, point mutations in α-SYN have been discovered in three autosomal dominant familial forms of PD [76, 78, 79]. The mutant forms of α-SYN were shown to have different aggregation properties than the wild-type (WT) protein [80, 81]: they accelerate the formation of oligomeric/protofibrillar species and/or fibrillar species [80–86]. Third, a dose-dependency of the pathogenic effect of α-SYN was elegantly illustrated by the fact that locus duplication [87, 88] or triplication [89] of the WT α-SYN gene also causes familial PD. This is confirmed by other studies indicating that α-SYN transcriptional control is deregulated in PD [90–93]. Fourth and finally, overexpression of both WT and mutant forms of α-SYN in *Caenorhabditis elegans*, *Drosophila*, rodents, and primates lead to neuronal inclusions and/or pathological symptoms resembling those observed in PD [94–97].

In solution, α-SYN exists as a naturally unfolded or ID protein that is unable to form a specific 3D–structure under physiological conditions (for an excellent review on ID proteins, see [98]). As already mentioned above, a significant number of proteins involved in protein deposition diseases (in which a normally soluble polypeptide becomes insoluble, frequently depositing in the form of amyloid fibrils) have been categorized as ID proteins. The disordered nature of α-SYN is primarily caused by the properties of the C-terminal part of the protein (aa 96→140). This part of α-SYN is enriched in hydrophilic and acidic residues (ten Glu and five Asp), ensuring a low hydrophobicity of the protein. This leads to extensive hydration and a high negative charge which reduces intramolecular and intermolecular contacts. Another factor contributing to the unfolded nature of α-SYN is that the C terminus contains all five Pro residues found in the protein. Pro has a high tendency to be found in turn or coil regions because of the steric hindrance it causes when placed in an α-helix or β-sheet. A logical consequence of these properties is an important regulatory function of the C terminus in α-SYN aggregation [99, 100]. We propose that FKBPs can bind to one or more Pro in the C terminus, thereby accelerating α-SYN aggregation, as will be discussed below.

Peptidyl-Prolyl Isomerases and Neurodegeneration

FKBPs in Neurodegeneration

Lately, it has become clear that FKBPs also have important functions in the brain. Not only are FKBP levels ten times higher in the brain compared with the immune system [101], but neuroregenerative and neuroprotective properties have also been ascribed to FK506 in animal models of neurodegeneration [102–105]. In Fig. 2, the structure of FKBP12, its catalytic residues and those interacting with FK506 and calcineurin are shown. The neurological effects of FK506 could arise from the interaction of the FKBP–FK506 complex with calcineurin (Fig. 3). One report on the effect of FK506 on cellular and animal models of Huntington's disease indeed demonstrated calcineurin inhibition by complex formation with FKBP12 resulting in increased huntingtin S421 phosphorylation, which is a neuroprotective event [106]. However, most reported neuroprotective or neuroregenerative effects are independent of calcineurin activity. This was evidenced by the development of several synthetic ligands without affinity for calcineurin (the so-called non-immunosuppressive immunophilin ligands (NI-IL)) such as GPI-1046, GPI-1485, and V10,367 (Fig. 1). In these compounds, the functional groups of FK506 that interact with both FKBP12 and calcineurin (yellow and orange aa in Fig. 2) were omitted. Most, although not all, studies claim that these NI-IL display equal or even stronger neuroprotective and neuroregenerative effects [107–111] (reviewed in [112]). Since the interaction with calcineurin is excluded, the therapeutic properties of NI-IL more than likely originate from the interaction with FKBP proteins. In the search to identify the FKBPs enabling the NI-IL effects, most reports concentrate on those FKBPs which are highly expressed in the human brain: FKBP12, FKBP38, FKBP52, or FKBP65 [101, 113–116]. We will discuss the potential role of these different FKBPs in neurodegeneration. A summary can be found in Fig. 4.

FKBP12

FKBP12 has been put forward several times as the mediator of the neuroprotective effects of IL. One study, for example, showed that FK506 could limit damage induced by oxygen-

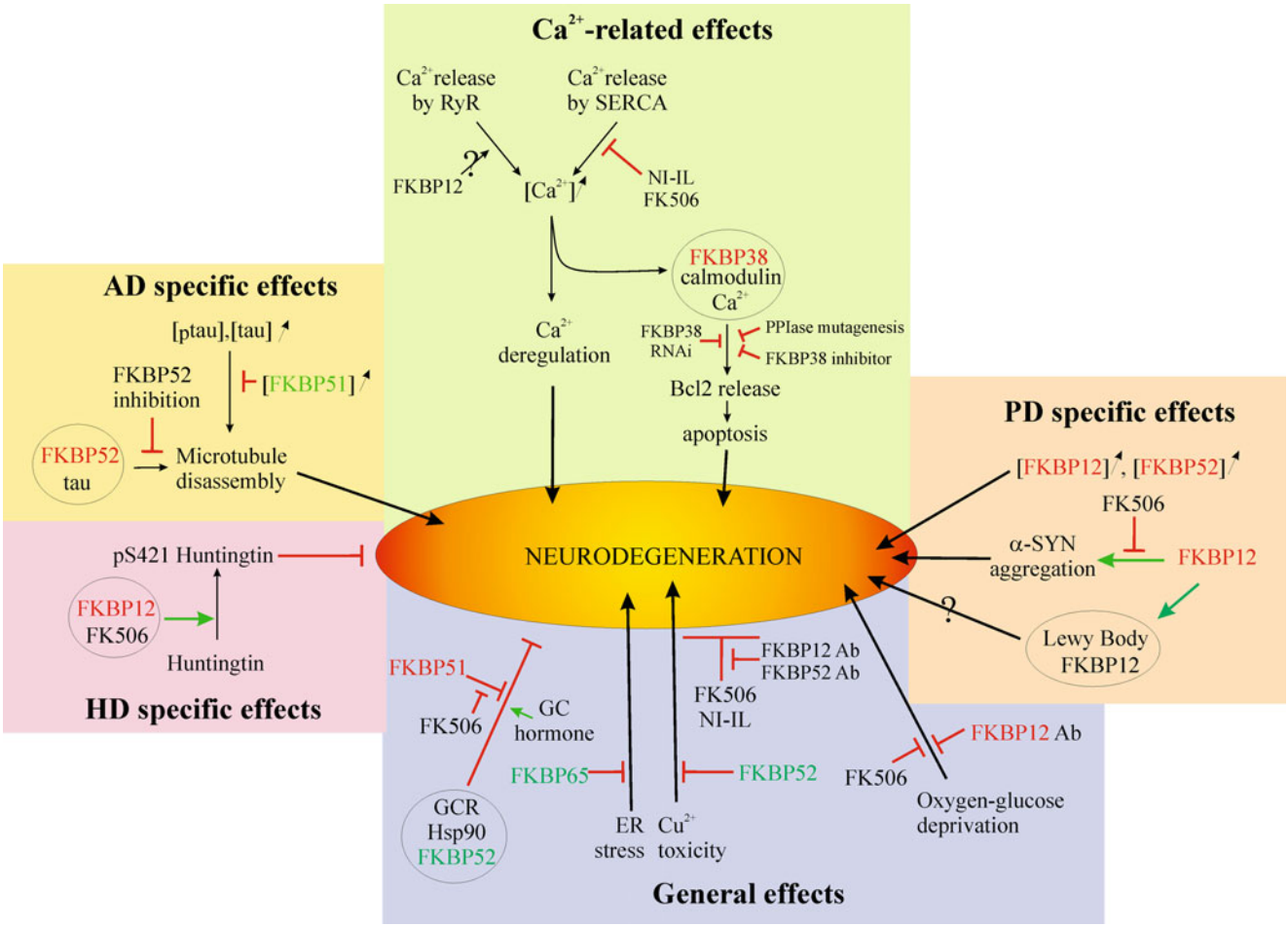


Fig. 4 Overview of potential roles of FKBPs in neurodegeneration: Stimulatory effects are visualized with a *green arrow*, inhibitory effects with a *red arrow*. Reported complexes are *encircled*. When a beneficial role has been reported for the FKBP, it is marked in *green*, when it has a detrimental role, it is marked in *red*

glucose deprivation in primary neurons when added during or after the insult [117]. This effect was not present when an anti-FKBP12 antibody or the competitive inhibitor rapamycin was added, which suggests that the binding of FK506 to FKBP12 is crucial for the neuroprotective effect. Another study showed upregulation of both FKBP12 and FKBP52 in surviving neurons following brain damage in rats [118, 119], suggesting a role for one or both proteins in neurodegeneration. Interestingly, postmortem analyses of patients with neurodegenerative diseases provided important supporting information. Avramut et al. found that the expression of FKBP12 increased in the brain of patients with PD, AD, and dementia with LB [116]. In synucleinopathies, FKBP12 co-localizes with α-SYN in LBs and Lewy neurites [116], and in AD, FKBP12 accumulates in neurofibrillary tangles [120]. The latter also suggests a binding between FKBP12 and tau, the main component of neurofibrillary tangles in AD. Next to this putative interaction, binding between FKBP12 and amyloid precursor protein, was confirmed via yeast-two-hybrid and coimmunoprecipitation and could be blocked dose-dependently by FK506 [120].

We were the first to show a direct link between FKBP12 and α-SYN aggregation, a central event in PD, as explained above. We noticed by serendipity that an impurity found in a recombinant α-SYN preparation increased its aggregation kinetics [121]. This impurity was unambiguously identified as SlyD, an FKBP-type *Escherichia coli* PPIase. To verify the physiological relevance of this finding, we tested the human archetypical FKBP, FKBP12, in the same assay. FKBP12 clearly accelerated aggregation of recombinant α-SYN in vitro, an effect that was inhibited by FK506 [121, 122]. Next, we showed that FK506 dose-dependently inhibits α-SYN aggregation and neuronal cell death in a cellular synucleinopathy model [123]. Using high-content analysis, we examined the role of FKBPs in α-SYN aggregation and apoptotic cell death. Both inhibition by FK506 or RNAi-based knockdown of FKBP12 or FKBP52 reduced the number of α-SYN aggregates and apoptotic cells, while overexpression of FKBP12 or FKBP52

accelerated both α-SYN aggregation and cell death. The effect of FKBP12 on the synucleinopathy phenotype was more pronounced than that of FKBP52. Moreover, after viral vector-mediated overexpression of α-SYN in adult mouse brain, the oral administration of FK506 significantly reduced α-SYN aggregate formation and neuronal cell death. In an ongoing follow-up study, we have examined the specificity of this effect by testing a whole range of PPIases [124]. Among the PPIases that are highly expressed in the brain, until now, FKBP12 proved to be the most potent stimulator of α-SYN aggregation and related cell death. We are currently trying to validate FKBP12 as an accelerator of α-SYN pathology in an animal model by knocking down FKBP12. The above studies all suggest that FKBP12 has a role, in most cases determined as harmful, in PD and other ND.

Considering the important role of FKBP12 in the regulation of Ca^{2+}-waves in skeletal muscle, this raises the question of a possible similar role in neurons. It is known that FKBP12 binds to both the RyR1 and RyR3 [23]. The role of RyRs in cell signaling pathways in the CNS is poorly understood. Nevertheless, RyR2 is an abundant cerebral isoform while RyR3 is highly expressed in the whole CNS. In a recent review regarding Ca^{2+}-deregulation and AD, it was stated that elevated Ca^{2+}–release from RyRs appears to contribute significantly to cell death and vulnerability in several models of neurotoxicity [125], pointing to a possible regulatory function of FKBP12 in the CNS. However, since FK506 and related ligands are neuroprotective in the brain but deleterious to Ca^{2+}-regulation in muscle cells, it is not straightforward to draw the parallel between the function of FKBP12 in Ca^{2+}-regulation in the skeletal muscle and in the brain. Therefore, further research is required.

One study specifically discussed the modulating properties of NI-IL on Ca^{2+}–release channels in neurons in a model system mimicking the neurotoxic effects of HIV-1 infection [126]. Neuroprotective properties of the NI-IL GPI-1046 (Fig. 1) originated from a reduced IP_3- and ryanodine-sensitive toxic release of Ca^{2+} from the ER in response to cell stress. This was a consequence of GPI-1046 inhibition of the sarcoplasmic/endoplasmic reticulum Ca^{2+}/Mg^{2+}-ATPase (SERCA) pump. Since SERCA pumps normally transport Ca^{2+} against a concentration gradient from the cytosol into the ER, GPI-1046 inhibition reduced total ER Ca^{2+} load, explaining the reduced Ca^{2+} release in stress conditions. However, it was not specified whether the authors believe this effect of GPI-1046 on SERCA pumps to be a direct one or an effect via inhibition of, e.g., FKBP12. Analogous to a previously described inhibitory effect of FK506 on SERCA pumps [127], this could be a direct but rather unspecific FKBP12-independent effect of compounds with a macrocyclic lactone ring structure.

FKBP38

FKBP38, localized in mitochondria, is the only FKBP which can bind to and inhibit calcineurin in the absence of FK506 [128]. Its enzymatic activity is dependent on interaction with Ca^{2+}-bound calmodulin, unlike any other FKBP [129]. When the intracellular Ca^{2+}-concentration increases, the FKBP38/calmodulin/Ca^{2+}–complex is formed. This allows association of the PPIase domain of FKBP38 with Bcl-2, a protein involved in apoptosis regulation. Consequently, Bcl-2 is released from the mitochondria which induce apoptosis [129]. Functional inhibition of FKBP38 via site-directed mutagenesis or RNA interference protected against this pro-apoptotic function. The same group described a specific FKBP38 inhibitor DM-CHX (*N*-(*N*_,*N*_-dimethylcarboxamidomethyl)cycloheximide; Fig. 1) with neuroregenerative properties [107]. They suggest that the inhibition of apoptosis via the FKBP38–Bcl-2 pathway is the primary cause for an observed reduction in cell death after cerebral ischemia in rats. Remarkably, the NI-IL GPI-1046 inhibits FKBP38 much stronger (30×) than other FKBPs, arguing that the neuroprotective and neuroregenerative effects of GPI-1046 are mainly mediated via FKBP38. Another study though, ascribed anti-apoptotic properties to FKBP38, suggesting that it inhibits apoptosis by anchoring Bcl-2 to mitochondria. In their hands, FKBP38 overexpression protected against apoptosis while functional inhibition induced apoptosis [128]. This interpretation however fails to explain the general neuroprotective effects of IL.

FKBP52

The first publications on neuroprotective or neuroregenerative effects of IL generally assumed that FKBP12 was the main FKBP mediating the IL effect. In a mechanical injury model (nerve crush or transection), the group of Gold et al. observed that FKBP12 knockout mice still responded to FK506 treatment [104, 130], pointing to other FKBPs having an effect on neuroregeneration. Moreover, FK506-stimulated neurite outgrowth in SHSY5Y cells could be blocked using an FKBP52 antibody. Their conclusion was that FK506 causes neurotrophic effects via its interaction with FKBP52. According to the authors, FK506 disrupts glucocorticoid receptor–Hsp90–FKBP52–hormone complexes, causing an increased activation of the steroid receptor and consequently, an increased expression of genes responsible for neurotrophic effects [130]. In a follow-up study, they also included FKBP51 in their reasoning, an FKBP that is present in low amounts in the CNS [131]. However, the relevance of these cellular studies was contradicted by the generation of FKBP52- and FKBP51-deficient mice [132]. Contrary to expectations,

especially for the FKBP52-deficient mice, no alterations in glucocorticoid receptor-regulated physiology were observed. Instead, the primary phenotype of FKBP52 ablation was infertility, pointing to a role of FKBP52 in androgen receptor signaling. This turned out to be a direct effect on gene expression instead of altered androgen receptor hormone binding and/or nuclear translocation functions. FKBP51 was not essential to androgen receptor signaling in vivo.

Another hint to a role of FKBP52 in neurodegeneration was the interaction with Atox1, a copper-binding metallochaperone functioning in copper efflux [133]. Overexpression of FKBP52 increased rapid copper efflux in ^{64}Cu-loaded cells, suggesting that FKBP52 protects neurons against copper toxicity. This finding may be of therapeutic value since alterations in metal homeostasis are thought to contribute to AD, amyotrophic lateral sclerosis, PD, and prion diseases. But again, this function does not explain the neuroprotective and neuroregenerative properties of IL.

Recently, a role for FKBP52 in tau protein function and therefore also in AD was discovered [134]. FKBP52 binds directly and specifically to tau. The affinity of the interaction increased with increased phosphorylation of tau. FKBP52 inhibited the promotion of microtubule assembly by tau. According to the authors, FKBP52 inhibition helps to restore tau function, which further explains neuroprotective functions of (NI-)IL. However, this property of FKBP52 awaits testing in a disease model for AD. Next to FKBP52, FKBP51 was also recently implied in AD [135]. As a consequence of age-related changes in cellular chaperones, FKBP51 expression was shown to be increased in AD, to prevent tau clearance and to negatively regulate its phosphorylation status. The latter was dependent on its PPIase activity and resulted in an increased stability of microtubules. In this context, FKBP51 would have a beneficial role in AD.

Another recent study linked FKBP12 and FKBP52 with a function in Ca^{2+}-signaling in the brain. It demonstrates a novel physiological function of FKBP12 and FKBP52 in chemotropic nerve guidance through transient receptor potential cation channel, subfamily C, member 1 (TRPC1)-gating. TRPC1 is a channel that mediates Ca^{2+} influx in response to netrin-1, a chemotropic protein [136]. These Ca^{2+} influxes ensure proper axon guidance towards high netrin-1 concentrations. The study reported that FKBP52 regulates agonist-dependent opening of TRPC1 channels whereas FKBP12 stimulated spontaneous opening, both in a PPIase-dependent fashion. Using GPI-1046, it was shown that FKBP52 activity was required for growth cone guidance, but not for general axon growth. The authors raised concern that the use of FK506 and other IL may therefore disturb fetal nervous system development [137] but acknowledge that the use of specific inhibitors may have therapeutic relevance.

FKBP65

FKBP65, the only member of the FKBP family that contains four PPIase domains, is primarily localized within the ER lumen [138]. Importantly, FK506 was shown to inhibit FKBP65 by no more than 25%, suggesting that only one of the four FKBP domains is sensitive to FK506 [139]. FKBP65 is thought to regulate proper protein folding in the ER via its PPIase activity [140]. Loss of this function could contribute to ER stress, improper protein folding, and subsequent aggregation, as has also been suggested for prion protein [141]. FKBP65 has been assigned possible neuroprotective properties through its association with Hsp90 and c-Raf-1 in a heterocomplex [115].

Overall FKBP Activity

One group also directly linked PPIase activity to the neuroprotective effects of FK506 [119]. They showed that the total FKBP enzymatic activity increased after ischemia in rat brain. This activity was blocked dose-dependently by FK506. Expression of FKBP12, FKBP52, and FKBP65 was selectively upregulated by cerebral ischemia, but FK506 treatment did not influence the expression pattern. Protection by FK506 could thus be specifically associated with suppression of the cerebral PPIase activity of FKBPs.

FK506 and the NI-IL analog V-10,367 (Fig. 1) were also shown to mediate neuroprotection by the heat-shock response [142]. In a calcineurin-independent way, both FK506 and V-10,367 induced rapid expression of Hsp27 and Hsp70. However, the mechanism by which the NI-IL exert these effects was not investigated. It remains unclear which FKBP mediates this effect.

Cyclophilins in Neurodegeneration

There are numerous reports of a neuroprotective and/or neuroregenerative role of CsA, mainly in ischemia models [143–145]. The generally accepted hypothesis in these models is that CsA inhibits cyclophilin D (CyPD), a part of the mitochondrial permeability transition pore [146–148]. When this pore is formed, cytochrome C leaks out of the mitochondria, inducing apoptosis. Inhibition of cyclophilin D would prevent formation of the pore.

A direct link between CyPD and AD was made recently [149]. A specific interaction of CyPD with mitochondrially localized amyloid β (Aβ) potentiated mitochondrial, neuronal, and synaptic stress. CyPD deficiency on the other hand improved learning, memory, and synaptic function in an AD mouse model.

In a completely different way, cyclophilins have also been linked to prion diseases [150]. In the search for chaperones assisting in normal folding of the prion protein,

the attention was drawn to PPIases because of proline substitutions in the prion protein giving rise to a familial form of the disease. Indeed, inhibition of cyclophilins with CsA resulted in the accumulation of aggresomes containing the abnormal conformer of prion protein. Mutation of two prolines in prion protein mimicked some of the effects of CsA treatment. This suggests that cyclophilins have a beneficial role in the maintenance of prion protein in its correct conformation and that their inhibition is detrimental for the cell. In short, there are two (sets of) reports that claim CyPs worsen ND phenotypes while there is one in favor of a beneficial role for CyPs in ND.

Parvulins in Neurodegeneration

Given its involvement in numerous signaling pathways, it is maybe not surprising that Pin1 pops up as well in the neurodegeneration field. In AD, phosphorylation of tau or amyloid precursor protein affects respectively tangle and Aβ formation. Pin1 can regulate the phosphorylation of both proteins [151, 152] and thus also the production of harmful aggregates. In both cases, Pin1 function is protective in AD models. This is consistent with the fact that Pin1 is downregulated or inhibited in AD [153].

In contrast to its effect in AD, Pin1 was found in LBs of PD patients and found to facilitate α-SYN aggregation in a PD model [154]. According to the authors Pin1 interacts indirectly with α-SYN through synphilin-1, an α-SYN binding partner. The acceleration of α-SYN aggregation is attributed to an increased interaction between synphilin-1 and α-SYN as well as a decreased degradation of α-SYN.

Conclusions and Discussion

Overall, we can conclude that IL have beneficial effects in animal models for neurodegeneration. However, the drug target and the mechanism of action remain unclear or very disease-dependent. Regarding FKBPs, there seems to be a consensus: in almost every case where an effect of IL was reported, FKBP knockout, knockdown, or mutation could partially or completely mimic the effect of the IL. So, IL most likely exert their neuroprotective or neuroregenerative effect via the inhibition of FKBP(s). One exception on this rule is the inhibition of the SERCA pump, which is however a rather specific effect dependent on the macrocyclic lactone structure of the compound. When trying to pinpoint the exact FKBP responsible for the IL effect, contradictions between research results commonly occur, most probably because the functions of the different FKBPs are so diverse and context-dependent. Moreover, some of the earlier IL such as FK506 target multiple FKBPs and the phenotype may result from direct and/or indirect effects (e.g., as a result of the immune suppression). Of note, based on the neuroregenerative and neuroprotective properties of FKBP inhibitors, phase II clinical trials have been performed with GPI-1485, a NI-IL from the former Guilford Pharmaceutics (taken over by MGI Pharma in 2005), in mild to moderate PD patients. However, neither in a 6-month nor in a 2-year trial with GPI-1485 was an important improvement seen in motor symptoms or DA neurotransmitter levels [155, 156]. Still, in the first study, there was a trend towards delay of dopamine transporter loss which did not reach statistical significance. Several explanations are possible for this disappointing result. First, if we take into account our results describing the stimulatory effect of FKBPs on α-SYN aggregation, GPI-1485 was not selected based on inhibitory potency against α-SYN aggregation or specific FKBP12 activity. Second, the selection of patients may have been more appropriate for a drug promoting neuroregeneration than for a drug preventing early-stage α-SYN aggregation and neurodegeneration. Therefore, early-stage patients diagnosed with sensitive new techniques [157, 158] would in our opinion probably profit better from this novel therapeutic strategy.

On the other hand, one cannot deny the many reports of a beneficial role of FKBP(s) in neurodegeneration, which is in contrast with the neuroprotective and neuroregenerative properties of IL. In light of the pleiotropic role of FKBPs in cell metabolism, it is not surprising that some FKBP members exert positive and others negative effects on neuron function. Depending on the extent of inhibition by an IL of a particular FKBP and depending on the expression and type of activity of that FKBP, it is possible that the general outcome of IL treatment is beneficial to the cell despite some negative effects following inhibition of favorable FKBPs. Therefore, it is essential to identify and validate FKBPs/PPIases as targets in different disease models such as already (partially) performed for PD (FKBP12 and FKBP52) or AD (FKBP52). After identification of the PPIases/FKBPs responsible for pathogenesis in a particular disease model, specific inhibitors should be developed or chosen amongst the already developed NI-IL. If such inhibitors can be found, they may be used to validate the drug target and hopefully to treat the specific disease.

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