



Fishing for synucleinopathy models

Suzita Mohd Noor, Anwar Norazit*

Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

Abstract

Synucleinopathies such as Parkinson's disease (PD) are incurable neurodegenerative conditions characterised by the abnormal aggregation of α -synuclein protein in neuronal cells. In PD, fibrillary synuclein aggregation forms Lewy bodies and Lewy neurites in the substantia nigra and cortex on the brain. Dementia with Lewy bodies and multiple system atrophy are also associated with α -synuclein protein abnormalities. α -synuclein is one of three synuclein proteins, and while its precise function is still unknown, one hypothesis posits that α -synuclein propagates from the enteric nervous system through the vagus nerve and into the brain, resulting in synucleinopathy. Studies on synucleinopathies should thus encompass not only the central nervous system but must necessarily include the gut and microbiome. The zebrafish (*Danio rerio*) is a well-established model for human neuronal pathologies and have been used in studies ranging from genetic models of hereditary disorders to neurotoxin-induced neurodegeneration as well as gut-brain-axis studies. There is significant genetic homology between zebrafish and mammalian vertebrates which is what makes the zebrafish so amenable to modelling human conditions but in the case of synucleinopathies, the zebrafish notably does not possess an α -synuclein homolog. Synuclein orthologs are present in the zebrafish however, and transgenic zebrafish that carry human α -synuclein have been generated. In addition, the zebrafish is a highly advantageous model and ideal replacement for reducing the use of mammalian models. This review discusses the application of the zebrafish as a model for synucleinopathies in efforts to further understand synuclein function and explore therapeutic strategies.

Keywords: Zebrafish, Synucleins, Synucleinopathy, Gut-brain-axis

Introduction

The synucleinopathies are distinct human diseases that share one commonality, the deposition of α -synuclein (SNCA) aggregations in the central nervous system (CNS). Synucleinopathies which include Parkinson's disease (PD), Parkinson disease dementia, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA) feature chronic neurodegeneration of neurons that would have begun years before symptoms arise (Savica et

al., 2018). PD is the most common synucleinopathy characterised by bradykinesia, resting tremor, rigidity, and postural and gait impairment – a collection of symptoms described as parkinsonism (Parkinson, 2002) – which develop due to the loss of dopamine and dopaminergic neurons in the substantia nigra pars compacta (SN) in the brain. Another hallmark feature of PD is the accumulation of SNCA Lewy bodies (LBs) throughout the CNS. As more LB deposits accumulate over time, parkinsonism symptoms worsen alongside progressive cogni-

Received: Oct 15, 2021 Revised: Jan 30, 2022 Accepted: Feb 6, 2022

*Corresponding author: Anwar Norazit

Department of Biomedical Science, Faculty of Medicine, University of Malaya, Kuala Lumpur 50603, Malaysia

Tel: +60-379676604, E-mail: anwar.norazit@um.edu.my

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Copyright © 2022 The Korean Society of Fisheries and Aquatic Science

tive decline which presents as PD dementia. DLB is a type of dementia characterised by progressive and severe cognitive impairment along with parkinsonism, but as these symptoms are highly reminiscent of Alzheimer's Disease and PD, respectively, DLB is often misdiagnosed. Post-mortem confirmation of DLB would reveal widespread LB deposition in the brain. MSA is a rare, sporadic neurodegenerative disease that is characterised not only by parkinsonism but also autonomic failure that is far more severe compared to other synucleinopathies. Detection of glial cytoplasmic inclusions in the brain distinguishes MSA from other synucleinopathies (Coon & Singer, 2020; Dickson et al., 1999). These neurodegenerative diseases are devastating for the patients and their families, primarily impacting patients when they are in their golden years who then find themselves becoming dependent on others for their most basic and even private needs (Lennaerts-Kats et al., 2020). As curative treatments are still unavailable, the economic impact of neurodegenerative diseases is detrimental on societies that are now bearing the cost of long-term palliative care as well as loss of productivity among family members who are forced into caregiver roles (Cantarero-Prieto et al., 2020).

The pathophysiology of synucleinopathies, in brief, involves the misfolding of SNCA proteins in the cytoplasm of neuron cells. The affected cells are either unable or fail to eliminate the misfolded proteins and as the misfolded SNCA proteins aggregate into insoluble amyloids and accumulate within the cells, functional disruptions occur (Melki, 2015; Rodriguez et al., 2018). The regions of the CNS where SNCA aggregates proliferate are specific to each disease and the progressive neurodegenerative decline associated with the respective diseases follow divergent but predictable sequences impacting numerous pathways (Mehra et al., 2019). Such divergent pathologies despite the same originating protein error is possibly explained by the prion strain hypothesis, whereby a single SNCA protein sequence could potentially misfold into the distinct disease-causing amyloid structures (Hoppe et al., 2021). The normal function of SNCA is still the subject of numerous studies (Bendor et al., 2013; Kaur & Lee, 2021; Li et al., 2020; Hernández-Vargas et al., 2021), with most recognising a duality in the role of SNCA as either a physiological neuroprotector of synapses or a pathological inducer of neurodegeneration (Bonini & Giasson, 2005).

The zebrafish is a common laboratory animal model, particularly for developmental biology and neuroscience (Kimmel, 1989; Kimmel, 1993; Stewart et al., 2014). However, the zebrafish does not possess an ortholog to the human SNCA. With

SNCA playing such a major role in numerous neurodegenerative pathologies, the question arises whether the zebrafish can be applied as a model for synucleinopathies. This review seeks to catalogue studies reported to date on the use of the zebrafish model in studies pertaining to synucleinopathies. A systematic search of the literature using Scholar Google, PubMed/Medline, and Scopus was done in June – September 2021. The following search strategy was used for each of the three bibliographic databases: Title, abstract, keywords, or topic: (“synuclein” OR “synucleins”) AND (“zebrafish”); (“synucleinopathy” OR “synucleinopathies”) AND (“zebrafish”); (“Lewy body” OR “Lewy bodies”) AND (“zebrafish”); (“aggregates” OR “aggregations”) AND (“zebrafish”); (“gut brain axis” OR “gut-brain-axis”) AND (“zebrafish”). The search was applied for peer-reviewed articles and reviews written in English and published at any time up to 2021.

Synucleins

The synuclein protein was first described in the nuclei and pre-synaptic nerve terminals of the *Torpedo californica*, commonly known as the Pacific electric ray (Maroteaux et al., 1988). The neuron-specific *Torpedo californica* synuclein consisted of 143 amino acids and its gene was expressed only in the nervous system (Burré et al., 2018). The cDNA clone encoding *T. californica* synuclein was used to isolate a 140-amino-acid synuclein protein in rats. Synuclein homologs were then identified in various vertebrates and three distinct synucleins have been described, namely SNCA, β -synuclein (SNCB) and γ -synuclein (SNCG) (George, 2001). The synucleins are small water-soluble proteins that have a highly conserved amino-terminal domain that includes a variable number of 11-residue repeats and a less-conserved carboxy-terminal domain (George, 2001). Each synuclein is between 127 to 140 amino acids in length, sharing 55% to 62% sequence similarity (Goedert & Spillantini, 2012). The protein homology between SNCA and SNCB have a similarity of 62% and are co-localised within presynaptic nerve terminals in the CNS, whereas SNCG is primarily expressed in the peripheral nervous system (Goedert, 2001; George, 2001; Jakes et al., 1994).

Human SNCA is located on chromosome 4q21, encoding 140 amino acids and producing a 14 kDa protein (Bendor et al., 2013; Lee & Trojanowski, 2006). The SNCA amino acid sequence is divided into 3 regions with each region possessing different functions and characteristics (Beyer, 2006). The highly conserved N-terminal domain acquires an α -helical structure

through binding to phospholipids which suggests SNCA could have a role in lipid membrane binding (Leong et al., 2009). The central hydrophobic non-beta amyloid component (NAC) region is responsible for the aggregation process which is essential for SNCA toxicity. The third, a strong negative charged acidic carboxyl-terminal domain is important for the chaperone-like activity of SNCA, which could aid in proper folding of proteins and inhibit irreversible aggregation of proteins (Periquet et al., 2007; Recchia et al., 2004). In normal conditions, SNCA is expressed abundantly in the presynaptic nerve terminals (Bendor et al., 2013; Stefanis, 2012) of the brain striatum, substantia nigra, hippocampus, neocortex, cerebellum and thalamus (Ahmad et al., 2007). The normal function of SNCA has been reported to be as an essential presynaptic, activity-dependent negative regulator of dopamine neurotransmission (Abeliovich et al., 2000). However, the absence of SNCA does not appear to be detrimental, as observed in an inbred mouse strain C57BL/6S that had a spontaneous deletion of the SNCA locus (Specht & Schoepfer, 2001).

Under pathological conditions, the aggregated form of SNCA is the main constituent of LBs and Lewy neurites (Spillantini et al., 1997). It is still up for debate whether the increased expression of SNCA is causative in nature or a defence mechanism towards encroaching disease. It remains unclear whether the abnormally aggregated SNCA protein confers neuroprotection or directs neurotoxicity towards neuronal cells (Luo et al., 2007). In PD pathogenesis, SNCA has been implicated in ubiquitination (Anderson et al., 2006), phospholipid binding (Jo et al., 2000), tyrosine hydroxylase regulation (Perez et al., 2002), and chaperone function (Ostrerova et al., 1999). Point mutations of the SNCA gene have been discovered (Appel-Cresswell et al., 2013; Conway et al., 1998; Krüger et al., 1998; Lesage et al., 2013; Polymeropoulos et al., 1997; Proukakis et al., 2013; Zarranz et al., 2004) that lead to early onset forms of α -synucleinopathy (Houlden & Singleton, 2012). Two of these mutations promote the increased formation of large protofibrils which are intermediate aggregates, and induce membrane permeabilization via lipid membrane binding which in turn increases neurotoxicity (Conway et al., 1998; Nuytemans et al., 2010). As reviewed by Ingelsson (Ingelsson, 2016), SNCA's neurotoxicity can compromise cell membrane integrity, synaptic toxicity, impairment in intracellular degradation, mitochondrial toxicity, dysfunction of the endoplasmic reticulum, inflammatory response, and cell-to-cell propagation.

Alternatively, SNCA expression correlates with neuropro-

tection. *In vitro* studies showed that neurons expressing SNCA after oxidative stress induction were more resistant to apoptotic changes compared to non-expressing neurons (Quilty et al., 2006). This neuroprotective effect was further supported by Chong et al. (Choong & Say, 2011), who reported that wild type SNCA was able to rescue chronic rotenone-exposed SH-SY5Y cells from acute hydrogen peroxide insult. It was also suggested that the increase in SNCA released from neurons could trigger astrocytes to provide a neuroprotective environment (Lee et al., 2010). Further studies supported a 'two hit' hypothesis whereby mild stress to the neurons would initiate a protective up-regulation of SNCA, while an increased stressed environment would intensify SNCA accumulation together with ubiquitin which is central to proteasome-mediated protein degeneration (Muggeridge et al., 2011).

Human SNCB consists of a 134 amino acid protein encoded by the SNCB gene on chromosome 5q35 (Beyer et al., 2011). While SNCB and SNCA have similar expression patterns and localization in the brain (George, 2001), the structural difference between the two synucleins is that SNCB lacks the central NAC region compared to SNCA (Ahmad et al., 2007). Where SNCA has been implicated in neurotoxicity, SNCB has been proposed to play a neuroprotective role in impeding further aggregation of SNCA protofibrils (Hashimoto et al., 2001; Park & Lansbury, 2003) via its non-amyloidogenic properties (Ahmad et al., 2007). Increased SNCB protein levels have also been reported to reduce SNCA protein aggregation and expression, improving motor performance and survival rates of SNCB transgenic mice (Fan et al., 2006).

Lastly, human SNCG, also known as the breast cancer-specific gene 1 (*BCSG1*) is located at chromosome 10q23, encoding 127 amino acids (Lavedan et al., 1998). The SNCG protein (SNCG) is mainly expressed at the axons and cell bodies of primary sensory neurons, peripheral nervous system motor neurons and sympathetic neurons (Ahmad et al., 2007). In the brain, SNCG plays a role in dopamine neurotransmission (Senior et al., 2008). In amyotrophic lateral sclerosis patients, SNCG aggregation has been implicated in the pathogenesis of disease (Peters et al., 2015). Overexpression of SNCG has also been observed in a wide range of cancers from advanced infiltrating breast carcinoma, ovarian tumours and cervical cancer to liver and lung cancers which suggest its association with malignancy, tumour progression and metastasis (Ahmad et al., 2007; Lavedan et al., 1998; Zou et al., 2012).

Zebrafish

The zebrafish (*Danio rerio*) is a teleost fish from the minnow family originating from South Asia which has become established as a laboratory animal in addition to being a common household pet for aquarists (Aleström et al., 2020; Engeszer et al., 2007; Varga, 2018). Teleosts are ray-finned fish that make up 96% of all fishes and are believed to have emerged 260 million years ago. As teleosts are vertebrates, this makes them a more relatable model for human conditions as compared to other popular non-mammalian models, namely *Drosophila melanogaster* and *Caenorhabditis elegans*. Indeed, fishes are the oldest and the most diverse class of vertebrates and all extant vertebrates are said to be descendants of an ancestral fish (Elgar, 2004; Volf, 2005). Fish underwent large-scale gene expansion early in their evolutionary history (Ravi & Venkatesh, 2018). Two rounds of whole genome duplications resulted in four times as many genes in vertebrates as there are in invertebrates (Dehal & Boore, 2005), thus establishing the incredible gene diversity that vertebrates possess. Over time, many of these genes disappeared but there were also many that were retained in teleosts. The teleosts, especially zebrafish, then experienced more recent lineage-specific duplication events that gave rise to species-specific duplicate genes (Lu et al., 2012).

The zebrafish genome has been fully sequenced, revealing zebrafish as possessing 26,206 protein-coding genes; for comparison, humans are reported as having 19,116 protein-coding genes (Howe et al., 2013; Piovesan et al., 2019). Of the human genes, 71.4% have at least one zebrafish ortholog, of which 47% of such human genes have a one-to-one relationship with a zebrafish ortholog. For the human genes that do not have matching zebrafish orthologs, the functions encoded by such genes may instead be performed by functionally similar zebrafish proteins (Barbazuk et al., 2000; Howe et al., 2013). Genetic sequences may have diverged significantly between species (Howe, 2020; Siddiqui et al., 2016) but conservation of functional properties can be uncovered. Hence, while the *SNCA* gene itself is absent in the zebrafish genome, its function very likely is conserved in the zebrafish. Zebrafish are already being used as animal models for neurodegenerative diseases that include synucleinopathies such as PD (Makhija & Jagtap, 2014; Najib et al., 2020; Wang et al., 2017) and tauopathies in Alzheimer's Disease (Bai & Burton, 2011; Ding et al., 2019). In the case of the latter, zebrafish have been shown to possess the *mapt* and *maptb* duplicate orthologs of the microtubule-associated protein tau (*MAPT*) implicated in the formation of neurofibrillary

tangles in the brains of patients with Alzheimer's disease (Chen et al., 2009a) and observable tau changes were reported in zebrafish adults exposed to hypoxia to mimic stroke or cerebral ischaemia in human patients (Moussavi Nik et al., 2014).

The zebrafish laboratory animal model has many advantages compared to the more common vertebrate rodent and nonhuman primate models. Zebrafish adults are small in size, measuring 4–5 cm in length, and can be housed in aquaculture systems designed to hold hundreds or even thousands of animals within controlled parameters in a single research facility (Aleström et al., 2020). Zebrafish achieve sexual maturity by 3 months of age, breed all year without seasonal variation, and are highly fecund, with a fertile pair producing hundreds of embryos in a week that can be raised and accurately staged by age of development in Petri dishes (Kimmel et al., 1995; Parichy et al., 2009). The embryos develop rapidly and are transparent, allowing for easy stereomicroscopic observation of their internal organs and systems during development, and easily visualised for *in vivo* live analyses with reporter transgenes or immunolabelled *in situ* with RNA probes or antibodies (Godoy et al., 2020; Kuil et al., 2021; Kumar et al., 2019). In addition, the Principles of the 3Rs (Tannenbaum & Bennett, 2015) are important considerations to encourage the use of zebrafish as an alternative vertebrate model that relatively replace the use of mammalian models (Madden et al., 2012). The matter of refinement is also crucial, as in recent years it has become recognised that zebrafish are capable of pain perception and discomfort (Ohnesorge et al., 2021). Just as one would when using mammalian models, suitable analgesia and anaesthesia protocols should be approved by the institution's ethics of animal care and use committee if experiments utilise adult zebrafish (Martins et al., 2016) while early embryo studies can proceed without regulation prior to their free-feeding stage of life (Strähle et al., 2012).

The zebrafish is a highly tractable organism and experiments done on zebrafish can be as simple as exposing the animal to compounds that are dissolved in its water column or by delivering the compound materials into the fish's body via injections or gavage; or can involve forward or reverse genetics via mutagenesis studies, transient knockdowns, knockouts (KOs), and transgenesis (Kalueff et al., 2014; Stewart et al., 2014; Wyatt et al., 2015). In addition to genetic and functional homology, zebrafish are homologous for human cells and organs with the exception of the lungs, and exhibit behaviours that can be monitored and correlated with human disorders (Flinn et al., 2008).

The zebrafish nervous system is simple but well characterised, and most importantly, it is a vertebrate nervous system (Blader & Strähle, 2000). All life stages of zebrafish can be used to model various human pathologies, including neuronal conditions in order to delineate the mechanisms of disease and to discover new therapies (Barnhill et al., 2020; Choi et al., 2021; Fontana et al., 2018; Keller & Keller, 2018; Maximino et al., 2010; Stewart et al., 2014; Thawkar & Kaur, 2021).

Zebrafish synucleins

The SNCA is highly conserved gene. A check on the NCBI page of SNCA orthologs displays 265 species (NLM, 2016). Notably, one vertebrate missing from the list is *Danio rerio*, the zebrafish. Zebrafish do possess synuclein genes, namely *snbc* and two γ -synuclein paralogs, γ -synuclein A (*sncga*) and γ -synuclein B (*sncgb*), but the absence of SNCA is intriguing as the gene is present in other teleosts (Toni & Cioni, 2015). Table 1 shows the similarity between the zebrafish synucleins and SNCAs of several other species. Blastn algorithm was performed to compare somewhat similar sequences between zebrafish *snbc* and *sncga* query sequences aligned the subject sequences of other species' SNCA genes. Mostly dissimilar sequences (discontiguous mega-

blast) was used to compare zebrafish *sncgb* query sequences with the other species' SNCA subject sequences. Protein-protein BLAST (blastp) was used for all zebrafish synuclein amino acid query sequences aligned with subject sequences of the other species' SNCA proteins.

When first identified, zebrafish *Snbc* was described as being 70% identical and 82% similar with human SNCB (Sun & Gitler, 2008). Another report placed the similarities for zSyn- β , zSyn- γ 1, and zSyn- γ 2 proteins at 69%, 47%, and 50% identity to human SNCB and SNCG, respectively; these three synuclein genes were then named *snbc*, *sncga* and *sncgb* according to standard nomenclature conventions (Chen et al., 2009b). Whole-mount *in situ* hybridization (WISH) with RNA probes showed *snbc* is the earliest expressed of the three zebrafish synuclein genes. From the 8-somite to 16-somite stage, *snbc* was detected only in the trigeminal placode. *snbc* expression then expanded to the ventral diencephalon, olfactory placode, ventral tegmentum, and spinal cord neurons before eventually becoming restricted to the brain and retina by 45 hours post fertilisation (hpf) (Sun & Gitler, 2008).

Meanwhile, synteny inference suggested that *sncga* and *sncgb* were paralogs derived from duplication of the same an-

Table 1. BLAST (Altschul et al., 1990) of nucleotide mRNA and amino acid percent identity between zebrafish synucleins and α -synucleins across selected species: human, nonhuman primate, rodents, and a teleost fish

Zebrafish <i>Danio rerio</i>	Human <i>Homo sapiens</i>	Rhesus monkey <i>Macaca mulatta</i>	Rat <i>Rattus norvegicus</i>	Mouse <i>Mus musculus</i>	Common carp <i>Cyprinus carpio</i>
	SNCA NM_000345	Predicted SNCA transcript variant X1 XM_015138783	<i>Snca</i> NM_019169	<i>Snca</i> NM_001042451	SNCA GQ169720.1
<i>snbc</i> NM_200969	67.14	67.57	66.97	66.97	65.17
<i>sncga</i> NM_001017567	74.31	74.31	76.39	75.69	74.42
<i>sncgb</i> NM_001020652	70.79	70.79	72.07	72.07	71.08
	SNCA NP_000336	SNCA isoform 1 XP_014994269	<i>Snca</i> NP_062042	<i>Snca</i> NP_001035916	SNCA ACS68572.1
<i>scnb</i> NP_957263	54.05	53.38	54.73	54.73	51.54
<i>sncga</i> NP_001017567	58.33	57.29	58.33	58.33	59.34
<i>sncgb</i> NP_001018488	63.54	62.50	58.77	58.77	63.04
References	Linnertz et al., 2009; Spillantini et al., 1995	Chu & Kordower, 2007; Shi et al., 2017	Jiang et al., 2014; Wang et al., 2016	Musgrove et al., 2014; Touchman et al., 2001	Vaccaro et al., 2015

snbc, β -synuclein; *sncga*, γ -synuclein A; *sncgb*, γ -synuclein B; SNCA, α -synuclein.

central locus that diverged to become the human SNCG (Milanese et al., 2012). *sncga* expression was not detected during early embryogenesis but from 26 hpf onwards, *sncga* could be detected in the spinal cord and hindbrain neurons. By 3 days post fertilisation (dpf) *sncga* expression was restricted to the brain and retina. As for *sncgb*, it was seen to be expressed within the notochord early in embryogenesis but not elsewhere. After 2 dpf, *sncgb* transcripts were not detectable by WISH (Sun & Gitler, 2008) and in temporal expression RT-PCR profiles, the intensity of *sncgb* bands were weaker compared to the other synucleins. In adult tissues, moderate *sncgb* mRNA expression was detected in the testis, kidney and brain, whereas high levels of both *sncb* and *sncga* were seen most abundantly in the brain followed by the eyes (Chen et al., 2009b). Indeed, all three zebrafish synucleins were detected in the adult brain, wherein *sncga* had the strongest expression and *sncgb* the weakest. *sncb* and *sncga* were distributed throughout the gray matter of the brain and spinal cord, with *sncb* being more prominent rostrally and *sncga* more prominently expressed caudally. In the brain, both *sncb* and *sncga* were shown to be co-expressed in catecholaminergic neurons (Milanese et al., 2012) which comprise the dopaminergic system and noradrenergic system. It is the dopaminergic system that is so severely compromised in PD (Braak & Braak, 2000; Dauer & Przedborski, 2003) and it is important to note that zebrafish dopaminergic neurons have been very well mapped and are functionally comparable to the human's (Du et al., 2016; Ma, 2003; Matsui & Sugie, 2017; Xi et al., 2011). A stable transgenic line, Tg (*sncga*:GFP) generated by Tol2-mediated transgenesis (Asakawa & Kawakami, 2009) produced embryos with green fluorescent protein (GFP) signal distribution that recapitulated the spatial expression pattern of *sncga* revealed by WISH (Chen et al., 2009b).

Transient knockdown of a specific gene function during zebrafish development can be achieved by delivering antisense morpholino oligonucleotides (MO) into the fertilised egg. In zebrafish embryo morphants, gene expression begins to recover as the embryos grow and the cellular MO concentration dilutes (Moulton, 2017), allowing for an observation window of gene inhibition for the first 3 to 4 days of embryogenesis (Bedell et al., 2011; Bill et al., 2009; Corey & Abrams, 2001). Having demonstrated that *sncb* and *sncga* were expressed in the dopaminergic neurons, Milanese et al. (2012) targeted the two synucleins for MO knockdown. Inhibition of *sncb* and *sncga* did not result in lethality or systemic defect, and the morphants' CNS appeared normal (Milanese et al., 2012). However, the loss of *sncb* and

sncga did impair motor function, with the morphants exhibiting obvious hypokinesia between 3 and 4 dpf which resolved to normal spontaneous motor activity by 5 dpf for the single knockdown morphants, while double knockdown morphants took longer to recover. Low dopamine levels were detected in all morphants. Collectively the results suggested *sncb* and *sncga* are not required for CNS morphologic development but are necessary for early development or differentiation of dopamine neurons (Milanese et al., 2012). Very importantly, Milanese et al. (2012) demonstrated the hypokinetic phenotype seen in *sncb* and *sncga* morphants could be rescued by human SNCA mRNA. This would support the idea of functional redundancy between the synuclein genes in zebrafish and explain the loss of SNCA from the zebrafish genome (Sager et al., 2010).

As *sncb* has been identified as the clear ortholog for human SNCB, that leaves *sncga* as the presumptive putative functional homolog for SNCA in zebrafish. Notably, the zebrafish *sncga* sequence has been reported to contain N-terminal repeats and hydrophobic regions that are similar to SNCA (Milanese et al., 2012). In one study, Sncga (referred to as γ -1 by the authors) was firstly shown to form fibrils similar to that seen with human SNCA *in vitro*, and formed intracellular γ 1 aggregates *in vivo* when γ -1 was over-expressed (Lulla et al., 2016). The authors subsequently used dithiocarbamate pesticide ziram to induce neurotoxicity and describe synuclein involvement in zebrafish embryos. A previous *in vitro* study had shown ziram to be selectively toxic to dopaminergic neuron cells in primary mesencephalic culture derived from rats, resulting in increased SNCA expression (Chou et al., 2008). In the zebrafish study, γ -1 was shown to be crucial for ziram neurotoxicity as demonstrated by γ -1 MO knockdown embryos being quite protected against ziram-induced damage to telencephalic and diencephalic aminergic neurons, and the use of a molecular tweezer (MT) to disrupt SNCA aggregation being effective against γ -1 as well (Lulla et al., 2016). Taken together, these observations neatly demonstrate the Sncga-like neurotoxic role for zebrafish *sncga*.

The role for SNCB in synucleinopathies is purportedly one of modulation and possible neuroprotection (Fan et al., 2006; Sargent et al., 2018) leading to significant research investment for assessment of therapeutic applications for SNCB (Hashimoto & Spada, 2012; Hashimoto et al., 2001; Park & Lansbury, 2003; Shaltiel-Karyo et al., 2010). In the zebrafish, *sncb* upregulation and *sncb* aggregation were seen following knockdown of the *leucine-rich repeat kinase 2* (*lrrk2*) gene. In the *lrrk2* morphant embryos, *sncb* aggregated in the diencephalon, midbrain, hind-

brain, post optic commissure, and very widely in the hindbrain. Zebrafish *lrrk2* morphants also had significant developmental perturbation and neuron loss, including of dopaminergic neurons (Prabhudesai et al., 2016). *LRRK2* mutations are autosomal dominant and are the most common cause of familial PD (Greggio & Cookson, 2009). The interaction between *LRRK2* and *SNCA* and their links to synucleinopathies have been the subject of much research (Gorostidi et al., 2012; O'Hara et al., 2020; Sen & West, 2009; Taymans & Cookson, 2010), and it should be noted that in the Prabhudesai study, *SNCB* was used as the substitute equivalent to *SNCA* and was detected by Western blot by using rabbit polyclonal antibody for *SNCB* (Prabhudesai et al., 2016). Nonetheless, the zebrafish findings were intriguing as *SNCB* aggregation is not a common report; despite sharing significant sequence similarity with *SNCA*, *SNCB* is reportedly resistant to aggregation (Allison et al., 2014). A current search for *LRRK2* and *SNCB* did not unearth any published articles.

Increased *snbc* expression was also reported in zebrafish embryo morphants of phospholipase A2 group VI (*pla2g6*) (Sánchez et al., 2018), the ortholog of *PLA2G6* which has been associated with neurodegenerative diseases (Gregory et al., 2017; Kurian et al., 2008). Sánchez et al. (2018) were interested in the synucleins as links between *PLA2G6* and *SNCA* have been reported in other animal models; in the absence of *SNCA* in the zebrafish, *snbc* was picked for CNS analysis in *pla2g6* morphants due to it being expressed in the presynaptic terminals. The increase in *snbc* expression and distribution in the brain in *pla2g6*-deficient embryos was thus correlated with *SNCA* elevation in *PLA2G6* patients and *Pla2g6*-KO mice, with the implication that presynaptic synuclein aggregation may be a factor in the pathogenesis of *PLA2G6* neurodegeneration (Sánchez et al., 2018). A more recent study into the neurotoxicity and teratogenic risks of silica nanoparticles in the aquatic environment assayed *snbc* as one of several neurodevelopment, autophagy and parkinsonism-related genes. The group reported no apparent changes to *snbc* mRNA levels in embryos immersed in silica nanoparticle solutions (Li et al., 2021). From their discussion, it appeared that the group was conflating *snbc* and *SNCA*; perhaps *snca* would have been the better gene to assess possible parkinsonism risk in the silica nanoparticle-exposed embryos.

Zebrafish α -synuclein (*SNCA*)?

Interestingly, studies reporting endogenous *SNCA* in zebra-

fish have been published. Khotimah et al. (2015a, 2015b) used rotenone to induce neurotoxicity and synuclein aggregation in adult zebrafish (Khotimah et al., 2015a; Khotimah et al., 2015b). Rotenone is a natural compound isolated from the seeds, stems or roots of several plant species and has been commonly used as an insecticide, pesticide and piscicide. Rotenone directly inhibits mitochondrial complex I of the electron transport chain and selectively damages dopaminergic neurons in the substantia nigra, leading to the progressive mitochondrial dysfunction and increased oxidative stress associated with PD, and its correlation with human PD cases is well documented (Johnson & Bobrovskaya, 2015; Tanner et al., 2011). Importantly, rotenone has long been used to induce parkinsonism in animal models (Innos & Hickey, 2021; Lv et al., 2019; Ünal et al., 2019). In the Khotimah et al. (2015a, 2015b) studies, *SNCA* expression was detected by immunohistochemistry, whereby Western blots showed positive *SNCA* protein signals of approximately 14–16 kDa in untreated controls while rotenone-induced zebrafish showed high expression of aggregated *SNCA* proteins of more than 250 kDa (Khotimah et al., 2015a; Khotimah et al., 2015b). Das et al. (2020) also reported *SNCA* protein detection by Western blot (Das et al., 2020) in a study aimed at assessing the neurodegenerative effects of benzo[a]pyrene (B[a]P), a polycyclic aromatic hydrocarbon often contained in industrial effluents (Chepelev et al., 2015; Das et al., 2020). Adult zebrafish exposed to the neurotoxicant exhibited impaired locomotive function evocative of parkinsonism that is associated with dopaminergic neuron or dopamine loss, alongside significant increase in *SNCA* protein as detected by Western blot (Das et al., 2020). It could be presumed that both these research groups had used commercial antibodies against mammalian *SNCA* that had sufficient homology to target either zebrafish *Snbc* or *Snca* (Vaz et al., 2018; Yurtsever et al., 2020).

Hu et al. (2017) tested the neurotoxicity risks of titanium dioxide nanoparticles in zebrafish embryos and reported increases in *pink1*, *parkin*, *snca* and *uchl1* expression levels (Hu et al., 2017); these are genes implicated with the formation of LBs and parkinsonism. Titanium dioxide nanoparticle neurotoxicity was not fatal but did cause delayed hatching and morphological deformities. The nanoparticles accumulated in larval brains, resulting in reactive oxygen species (ROS) generation and hypothalamus cell death as well as dopaminergic neuron loss, all which greatly incriminated titanium dioxide nanoparticles as risk factors for PD (Hu et al., 2017). The upregulation of *snca* in response to the nanoparticle neurotoxicity would have been

in line with the observation of parkinsonism *in vivo* if not for the fact that zebrafish do not possess the gene. In the same vein, Zhu et al. (2020) reported PD-like symptoms in zebrafish larvae following exposure to Fenvalerate, a type of pyrethroid used as an insecticide, and evaluated *pink1*, *parkin*, *snca* and *uchl1* transcription by qRT-PCR (Zhu et al., 2020). Fenvalerate exposure led to increased mortality, abnormalities in morphology and locomotor behaviour in surviving larvae, dopaminergic neuron loss and significant upregulation in *pink1*, *parkin* and *uchl1*; but no significant difference in *snca* expression in exposed larvae compared to unexposed controls. Intriguingly, the published primers designed for *snca* (Zhu et al., 2020) were in fact targeting NM_001113636.2, which is the reference sequence for *synuclein*, *alpha interacting protein (sncaip)* mRNA, an ortholog of human *SNCAIP* which encodes synphilin-1, a neuronal protein that interacts with SNCA and modulates SNCA aggregation (Engelender et al., 1999; Engelender et al., 2000). Synphilin-1 has also been shown to be one of the protein components in LBs (Wakabayashi et al., 2007) and synphilin-1 mutations have been described in sporadic PD (Marx et al., 2003). Studies are ongoing to understand the possibly cytoprotective role of synphilin-1 in PD pathogenesis (Liu et al., 2016; Shishido et al., 2019; Smith et al., 2010; Tanaka et al., 2004; Hernández-Vargas et al., 2011) and the inadvertent reporting of zebrafish *sncaip/synphilin-1* and its seeming non-response to a neurotoxin (Zhu et al., 2020) offers interesting prospects for further investigations into what role synphilin-1 has in the absence of SNCA.

Meanwhile, Keatinge et al. (2015) utilised the zebrafish model precisely because it lacks SNCA. *Glucocerebrosidase 1 (GBA1)* mutations are the cause of Gaucher's Disease, an autosomal recessive lysosomal disorder (Hruska et al., 2008). Keatinge's group was focused on understanding the mechanisms of *GBA1* mutation in contributing to increased risk of PD in heterozygous *GBA1*^{+/-} carriers, independent of SNCA toxicity (Keatinge et al., 2015). A *gba1*^{-/-} zebrafish line was generated, and juvenile homozygous and heterozygous mutants of the line showed phenotypes associated with Gaucher's Disease but *gba1*^{-/-} zebrafish did not survive past 14 weeks post fertilization (wpf). Without any SNCA influence, dopaminergic neuron degeneration was clearly detected in *gba1*^{-/-} mutants by 12 wpf. Notably both *Sncb* and *Sncga* protein levels were markedly reduced in the *gba1*^{-/-} brains, likely as a consequence of the loss of neuron cells. This study introduced a niche application for the zebrafish in further studies of PD-related pathophysiology unrelated to α -synucleinopathy.

Humanized zebrafish

The high level of genomic conservation between humans and zebrafish allows for the expression of human genes in zebrafish (Barbureau et al., 2021; Cornet et al., 2018, Slijkerman et al., 2018). Of specific relevance to this review, transgenesis of human *SNCA* in zebrafish benefits from the absence of endogenous *SNCA* wherein findings would be reflective of the human *SNCA* rather than complicated by an endogenous protein ortholog, as is seen in transgenic *SNCA* mammalian models (Visanji et al., 2016).

The first published synucleinopathy study using a humanized zebrafish model was by Prabhudesai et al. (2012) who generated a transgenic zebrafish model of *SNCA* toxicity (α -syn-ZF) exogenously expressing *SNCA* fused with DsRed as the reporter gene with HuC neuronal promoter and a T2A peptide inserted between the *SNCA* and DsRed sequence (HuC- α -syn-T2A-DsRed). The T2A peptide would be cleaved post-translationally to release *SNCA* and DsRed in neurons. Embryos injected with the HuC- α -syn-T2A-DsRed construct exhibited DsRed fluorescence in neurons beginning from 12 hpf but also suffered severe morphologic deformities and most were dead by 240 hpf. Severe phenotype and lethality were also seen in embryos injected with HuC- α -syn construct without T2A and DsRed, but not in control embryos expressing HuC-T2A-DsRed, thus implicating *SNCA* as the cause of toxicity. Additionally, *SNCA* aggregates were detected in the neurons of α -syn-ZF larvae. However, the *SNCA* aggregation was prevented when the α -syn-ZF larvae were treated with a novel MT capable of inhibiting the assembly and toxicity of amyloidogenic proteins, thus providing validation for the MT as a potential treatment option for synucleinopathy (Prabhudesai et al., 2012).

O'Donnell et al. (2014) injected their α Syn-2A-GFP transgene construct into single-cell embryos to overexpress human *SNCA* in the Rohon-Beard neurons, which are peripheral mechanosensory neurons located in the dorsal spinal cord of fish and amphibians. Use of a viral 2A system (Luke & Ryan, 2018; Provost et al., 2007) offered a brighter reporter expression than the earlier α -syn-ZF transgenic (Prabhudesai et al., 2012). Morphology and survival of this GFP α -syn transgenic was also improved compared to the α -syn-ZF model, at least when the *CREST3* sensory neuron (Uemura et al., 2005) enhancer rather than *HuC* promoter was used to drive the GFP *SNCA* expression. Here, *SNCA* aggregation and axon degeneration followed by cell death were seen in the neurons of embryos expressing human *SNCA* and GFP, but not in controls expressing GFP

alone (O'Donnell et al., 2014). O'Donnell et al. (2014) were observing the pathological effects of accumulated SNCA on axons (Tagliaferro & Burke, 2016) as well as on the rate of Wallerian degeneration (Conforti et al., 2014; Rotshenker, 2011) via time-lapse confocal microscopy imaging after axonal injury and determined that Wallerian degeneration occurred in the same manner in SNCA-expressing axons and wildtype axons. The axon-protective protein Wallerian degeneration slow (*Wld^s*) (Lingor et al., 2012) was shown to afford some protection against SNCA toxicity but could not prevent neuron cell death. However, the transcriptional coactivator PGC-1 α (Northam & LeMoine, 2019) was able to protect against both neuron demise and axonopathy (O'Donnell et al., 2014), further validating PGC-1 α as a therapeutic target in treating synucleinopathies (Mudò et al., 2012; Ng et al., 2017; Yang et al., 2020; Zheng et al., 2010) and establishing this model for other therapeutic target screens. Matsui & Matsui (2017) also generated a GFP reporter human SNCA transgenic line but noted that the SNCA expression was weak and the fish did not exhibit any SNCA inclusion bodies even after progressing to the advanced age of 18 months. The authors subsequently delivered injections of human SNCA fibrils into the cerebrospinal fluid (CSF) of adult fish. The result of this xenograft was a significant accumulation of human SNCA and SNCA inclusions in the brains of SNCA transgenics, which were not seen in the brains of wildtype zebrafish that underwent the same procedure (Matsui & Matsui, 2017). Notably, the SNCA in the inclusions distributed throughout the brain had undergone post-translational modification phosphorylation at residue Serine-129 (S129). Phosphorylated SNCA S129 has been implicated in the pathological misfolding of SNCA (Anderson et al., 2006; Oueslati et al., 2013) and earmarked as a biomarker for diagnosis of synucleinopathies, including PD (Cariulo et al., 2019). The main achievement of the CSF injections of SNCA fibrils is this study was the demonstration of prion-like propagation of misfolded SNCA, although the authors acknowledged that the brain inclusions seen might have also included endogenous zebrafish synuclein. Prion-like propagation (Tyson et al., 2016) is currently the focus of much intense study to detail the processes and to strategize potential approaches to limit or even stop the loop of misfolded SNCA aggregating and seeding more and more neuron cells (Goedert et al., 2016; Oueslati et al., 2014; Ugalde et al., 2016). The zebrafish can now be added to the stable of animal models for SNCA propagation studies (Bernis et al., 2015).

More recently, Van Laar et al. (2020) constructed a novel

transgenic zebrafish using the mCherry reporter and viral 2A system, Tg (*UAS:hsa.SNCA-2A-nls-mCherry*) that co-expressed human SNCA in the ventral diencephalic dopaminergic neurons that are homologous to the mammalian nigrostriatal system. The human wildtype SNCA was expressed at low levels as to avoid neurodegeneration and dopaminergic neuronal loss or neurobehavioral abnormalities in the larvae (Van Laar et al., 2020). The establishment of this transgenic line generated on the transparent Casper (*roy^{-/-}; nacre^{-/-}*) background (White et al., 2008) allowed for intravital confocal microscopy (Usmani & Mempel, 2021; van Ham et al., 2014) of the whole CNS in the living zebrafish model which would not have been possible in a rodent model. A second transgenic line Tg (*UAS:roGFP2-Orp1*) was developed to express a ratiometric hydrogen peroxide (H_2O_2) biosensor (Nietzel et al., 2019; Kostyuk et al., 2020) on a Casper background which was then crossed with the SNCA transgenics to allow for live *in vivo* measurement of cytoplasmic H_2O_2 flux in dopaminergic neurons. H_2O_2 is an endogenous ROS that has been implicated either as a cause of SNCA aggregation or as a consequence of SNCA accumulation (Lehtonen et al., 2019; Xu et al., 2015). Following acute exposures to either MPTP or rotenone, increased cytoplasmic H_2O_2 flux was observed in the dopaminergic neurons of the SNCA transgenics. Additionally, upon exposure to H_2O_2 , the transgenics' dopaminergic neurons failed to compensate for redox perturbation, reflecting the ROS impact on mitochondrial dysfunction and cellular dysregulation in PD (Dias et al., 2013; Milanese et al., 2018). These transgenics along with an earlier one generated by the same group (Dukes et al., 2016) offer intriguing modalities for *in vivo* imaging of synucleinopathies and PD-related CNS disruptions in a vertebrate model.

Meanwhile, Weston et al. (2021) reported their transgenic line which transiently expressed human SNCA with a C-terminal GFP tag (*SNCA-GFP*) within motor neurons. Their transgenic larvae allowed for tracking of GFP-tagged human wildtype SNCA and A53T point mutation SNCA (Hoenen et al., 2016; Li et al., 2002) in neurons and presynaptic terminals (Weston et al., 2021). The group utilised fluorescence recovery after photobleaching (FRAP) imaging (Ishikawa-Ankerhold et al., 2012) to measure and quantify SNCA mobility in presynaptic neurons, using this method to differentiate between aggregated proteins with limited mobility and non-aggregated SNCA that diffuse freely. This group established the zebrafish larval nervous system as a viable platform to optically study normal and mutant SNCA function as well as SNCA aggregations, and

even detected nitrated SNCA species within the presynaptic terminals (Benner et al., 2008; Chavarría & Souza, 2013) in addition to demonstrating that zebrafish Polo-like kinase (PLK) (Inglis et al., 2009; Mbefo et al., 2010) could phosphorylate human SNCA at S129 in zebrafish neurons. The entire experiment was an elegant use of the zebrafish model to decipher the

mechanisms of human SNCA aggregation and open up avenues for further investigations. Table 2 summarises the zebrafish synuclein models that have been discussed.

α-Synuclein (SNCA) and the gut-brain-axis

In humans, SNCA is present within the enteric nervous system

Table 2. Summary of zebrafish synuclein models that have been published

Synuclein	Methodology	Life stage	Observations	References
Endogenous synuclein				
Tg (<i>sncga</i> :GFP)	Tg (<i>sncga</i> :GFP)	Embryos	Transgenic GFP expression at 3 dpf in spinal cord, habenula, hindbrain, midbrain, eyes, trigeminal ganglion, vagal ganglion, and posterior lateral line ganglion. Habenula expression is asymmetric, larger GFP domain in left habenula than in right habenula.	Chen et al., 2009b
β-synuclein (<i>sncb</i>) γ-1 synuclein (<i>sncga</i>)	MO knockdown (β MO, γ1 MO, β + γ1 MO)	Embryos, larvae	Slight reduction in <i>slc6a3</i> (<i>dopamine transporter</i> , <i>dat</i>)-positive neurons in double β + γ1 morphants at 2 dpf. Hypokinesia between 3–5 dpf, reduced dopamine levels at 7 dpf. Hypokinesia rescued by human human α-synuclein.	Milanese et al., 2012
β-synuclein	<i>lrrk2</i> MO knockdown	Embryos	β-synuclein aggregation in <i>lrrk2</i> morphant brain at 3 dpf.	Prabhudesai et al., 2016
γ-1 synuclein (<i>sncga</i>)	γ-1 synuclein MO	Embryos	Morphants protected from ziram dithiocarbamate toxicity.	Lulla et al., 2016
γ-1 synuclein (<i>sncga</i>)	γ1-synuclein overexpression in neurons via <i>HuC</i> -ZFy1-T2A-DsRed injection at single-cell stage	Embryos	Malformation, reduced survival, intracytoplasmic aggregation in neurons at 2 dpf.	Lulla et al., 2016
α-synuclein (presumably <i>sncb</i> or <i>sncga</i>)	Rotenone (5 µg/L) immersion for 28 days	Adults	Western Blot detection of α-synuclein aggregates in the brain. Hypokinesia, decreased dopamine levels, increased apoptosis, increased α-synuclein, Caspase-3, Caspase-9 expression and decreased BDNF expression in midbrain.	Khotimah et al., 2015a; Khotimah et al., 2015b
α-synuclein (presumably other synuclein ortholog)	Environmental toxins	Larvae, adults	Increased α-synuclein in embryos exposed to titanium dioxide nanoparticles. PD-like symptoms in zebrafish larvae but no increase in α-synuclein exposed to Fenvalerate type II pyrethroids. Increased α-synuclein in adults exposed to benzo[a]pyrene.	Hu et al., 2017 Zhu et al., 2020 Das et al., 2020
Exogenous α-synuclein transgene				
Human α-syn, SNCA (<i>HuC</i> -α-syn-T2A-dsRed) transgenic	SNCA fused with DsRed reporter gene with <i>HuC</i> neuronal promoter and T2A peptide inserted between SNCA and DsRed sequence. Transgene injected into embryos at one-cell stage.	Embryos	<i>HuC</i> promoter used to drive α-syn DsRed expression in neurons. Embryos have morphological deformities, marked increase in neuron apoptosis as early as 24 hpf, aggregated α-syn in DsRed-positive neurons. Almost all dead by 240 hpf.	Prabhudesai et al., 2012
Human α-synuclein, SNCA (αSyn-2A-GFP) transgenic	<i>CREST3</i> :Gal4:UAS:αSyn-2A-GFP transgene injected into wildtype embryos at one-cell stage	Embryos	<i>CREST3</i> enhancer used to drive expression in peripheral sensory neurons. Most embryos morphologically normal. Human α-syn GFP expression in Rohon-Beard neurons. α-synuclein aggregation by 2 dpf, reduced mitochondrial transport in axons at 2 dpf, axonopathy (axon swelling, beading, fragmentation, degeneration) by 3 dpf.	O'Donnell et al., 2014

Table 2. Continued

Synuclein	Methodology	Life stage	Observations	References
Human α -synuclein xenograft and human <i>α-synuclein</i> (α -syn) transgenic	Human recombinant α -synuclein protein injected into the cerebrospinal fluid (CSF) of 3-month-old AB wildtype and α -syn transgenic (<i>Xenopus</i> neural-specific beta tubulin (NBT) promoter:human <i>α-synuclein</i> IRES GFP) fish	Adults	α -syn transgenic adults with very weak human α -synuclein expression and absence of α -synuclein inclusion bodies at 18-months-old. At 3 months-post-injection, robust accumulation of human α -synuclein and presence of α -synuclein inclusion bodies throughout the brain in 6-month-old α -syn transgenic fish but not in wildtype fish.	Matsui & Matsui, 2017
Human α -synuclein, SNCA (α -Syn) transgenic	Tg (<i>UAS:hsa.SNCA-2A-nls-mCherry</i>) zebrafish expressing human wildtype α -synuclein crossed with Tg (<i>otpb:gal4</i>) Gal4 driver zebrafish line to activate UAS enhancer	Larvae	Co-expression of human α -synuclein (<i>mCherry</i> reporter) in ventral diencephalic dopaminergic neurons at low levels to avoid neurodegeneration or neurobehavioral abnormalities, with fluorescent biosensors for cytoplasmic peroxide flux and glutathione oxidation detection.	Van Laar et al., 2020
Human α -synuclein, SNCA (α -synuclein-GFP; A53T α -synuclein-GFP; S129A α -synuclein-GFP; S129D α -synuclein-GFP) transgenics	Human <i>α-synuclein</i> tagged with C-terminal Green Fluorescent Protein (α -synuclein-GFP) and zebrafish <i>neuroD</i> promoter. Transgene injected into AB/TL wildtype embryos at one-cell stage	Larvae	Human <i>α-synuclein</i> -GFP expression appearing at 2–4 dpf in the soma, axon, and presynaptic terminals of central and peripheral nervous system neurons, including some motor neurons projecting from the spinal cord. Robust expression persisting and neurons appeared healthy until at least 8 dpf. α -synuclein-GFP enriched in presynaptic terminals of neurons at 4 dpf for fluorescence recovery after photobleaching (FRAP) imaging detection of freely diffusing, synaptic vesicle-bound, and aggregated α -synuclein; and immunohistochemistry detection of phosphorylated α -synuclein.	Weston et al., 2021

sncga, γ -synuclein A; GFP, green fluorescent protein; dpf, days post fertilisation; MO, morpholino oligonucleotides; Irrk2, leucine-rich repeat kinase 2; BDNF, brain-derived neurotrophic factor; PD, Parkinson's disease; SNCA, α -synuclein; hpf, hours post fertilisation; CSF, cerebrospinal fluid; S129, Serine-129.

(ENS) but ENS SNCA levels are significantly higher in PD patients, suggestive of altered enteric SNCA. Additionally, LBs have been detected in the gastrointestinal tracts (GITs) of PD patients (Punsoni et al., 2019). In addition to parkinsonism, another common feature of PD is constipation which often predates the motor and posture dysfunctions (Adams-Carr et al., 2016), thus strongly implicating gut involvement. To explain the pathophysiology of idiopathic PD, Braak et al. (2003) had hypothesized that misfolded SNCA aggregates propagated in the gut and then spread to the CNS via postganglionic enteric neurons and the vagus nerve (Braak et al., 2003). Specifically, Braak's hypothesis put forth that the genesis of idiopathic PD is a yet to be known pathogen or perhaps environmental toxin that is inhaled via the nasal cavity, affecting the olfactory bulb, and is also swallowed, leading to the pathogen being present in

the gut and triggering inflammatory reactions that cascade into pathogenic SNCA deposition in the GIT ENS before secondary spreading to the brain (Hawkes et al., 2007). There have been substantial published *in vitro*, *in vivo*, and clinical evidence to give credence to this 'body-first' hypothesis (Rietdijk et al., 2017), but there have also been counterarguments that synucleinopathies are 'brain-first', wherein aggregated SNCA originate in the brain without any peripheral autonomic nervous system involvement (Beach et al., 2021; Borghammer et al., 2021; Lionnet et al., 2018).

Whether or not Braak's hypothesis stands, there is significant focus on the involvement of gut microbiota in α -synucleinopathies such as PD (Haikal et al., 2019; Kaur et al., 2021; Liu et al., 2020; Shen et al., 2021). Sampson et al. (2016) demonstrated that parkinsonism motor symptoms would develop by

transplanting gut bacteria from PD patients into the GIT of SNCA-overexpressing mice (Sampson et al., 2016). Several reviews of animals models utilised for investigating SNCA and the gut, highlighted the challenges involved in deciphering and translating findings to the human condition (Rey et al., 2016; Van Den Berge & Ulusoy, 2022; Vascellari et al., 2021). This review will be summarising zebrafish gut-brain-axis research and the applicability for zebrafish to model transneural SNCA propagation.

Zebrafish gut-brain-axis models

Zebrafish ENS development and gut motility have been well described and gene orthologs identified (Baker et al., 2019; Ganz, 2018; Olsson et al., 2008; Shepherd & Eisen, 2011). Zebrafish gut and enteric innervation are functional as early as 5 dpf and are highly conserved if somewhat simpler compared to the mammalian gut and ENS (Kuil et al., 2021), and the mechanisms of ENS regeneration have been described in zebrafish larvae (Ohno et al., 2021). The studies discussed earlier in this review detailed synuclein gene expressions in zebrafish that were focused quite exclusively on the CNS and spinal cord or limited their characterisations to the embryo and larval stages (Chen et al., 2009b; Sun & Gitler, 2008). mRNA expression levels of zebrafish synuclein transcripts in adult tissues were nonetheless shown to be highest in the brain and low or undetectable in the gut (Chen et al., 2009b). This does not however preclude endogenous synuclein presence in the zebrafish ENS. Determination of the expression level of *snca* in the gut of zebrafish that are treated with rotenone or other parkinsonism-inducing agents could provide for some interesting options in the use of the zebrafish model to chart transneural propagation of synuclein proteins from the gut to the brain or vice-versa.

Zebrafish gut-brain-axis can be studied at larval and adult stages. Zebrafish *sox10* mutant larvae that completely lacked the ENS and as a consequence suffered from bacterial overgrowth, dysbiosis and intestinal hyper-inflammation could be rescued somewhat by transplantation of wildtype ENS precursors into the mutants or by colonizing the *sox10* mutant larval intestine with anti-inflammatory bacteria isolates from a healthy human (Rolig et al., 2017). Borrelli et al. (2016) were the first to report an adult zebrafish gut-brain-axis model. Adult wild type zebrafish between 4–6-months-old were fed probiotics twice daily for 28 days to induce changes in enteric microbiota, neurochemistry and behaviour (Borrelli et al., 2016), thus establishing the feasibility of the model for microbiota and gut-brain crosstalk research. Cuomo et al. (2021) then utilised the model to correlate behavioural

changes with DNA methylation epigenetics and the microbiome following probiotic feeding (Cuomo et al., 2021).

As more data about PD patients' gut microbiota become known (Ma et al., 2019; Pietrucci et al., 2020), the specific bacterial isolates and their impact can be reasonably validated in the zebrafish gut-brain-axis model. Davis et al. (2016) derived germ-free (GF) zebrafish larvae based on earlier protocols for generating gnotobiotic zebrafish larvae (Davis et al., 2016; Pham et al., 2008). The absence of microbiota in GF larvae clearly impacted on locomotor activity and behaviour, which were attenuated by probiotic treatment. The GF larvae were exposed to *Lactobacillus plantarum* directly in the water from 4–6 dpf and although the larvae were too young to really eat, the bacteria could enter into and colonise the gut to establish the microbiome (Davis et al., 2016). Zhang et al. (2021) used GF adults in addition to conventional laboratory fish to study gut microbiota regulation of neurotransmitter secretion (Zhang et al., 2021). Shotgun metagenomic sequencing was performed to identify different microbiota groups based on treatment inductions and elucidate a potential mode of action through the microbiota-gut-brain axis for regulation and neuromodulation (Zhang et al., 2021). Similarly designed experiments could be designed using gnotobiotic humanized SNCA fish to understand the impact of microbiota on synucleinopathy.

Gut dysfunction and by extension, microbiota disruption can also be stress-induced to mimic the pathophysiology of neurodegenerative disorders (Dodiya et al., 2020; Johnson et al., 2018). Cansız et al. (2021) exposed adult zebrafish to rotenone for 30 days to induce inflammation and oxidative stress in the gut-brain axis (Cansız et al., 2021). The rotenone-treated fish suffered locomotor defects indicative of CNS dysfunction. The group did not evaluate for synuclein gene expression but did measure pro-inflammation genes and *brain-derived neurotrophic factor (bdnf)* in the intestines and brain as they demonstrated that caprylic acid could ameliorate neurotoxin-induced inflammation and oxidative stress and augment locomotor activity (Cansız et al., 2021). Administration of caprylic (octanoic) acid by oral gavage to the rotenone-treated zebrafish was done to mimic ketogenic diets that have been reported to have therapeutic potential for PD (Cheng et al., 2009; Shaafi et al., 2016) and the use of the zebrafish model in this manner could be readily applied to oral drug discoveries for synucleinopathies.

There have been recent reviews that can be referenced on this topic. de Abreu et al. (2019) delineated the core gut microbiome between humans, rodents, and zebrafish, and discussed

zebrafish gut-brain crosstalk and the effects of zebrafish gut microbiota on different systems and on behaviour (de Abreu et al., 2019). Bertotto et al. (2020) reviewed the zebrafish microbiota and detailed larval bacterial gut colonisation methods and experimental assays (Bertotto et al., 2020) as well as the use of zebrafish for studying xenobiotics (Koppel et al., 2017) while Mohanta et al. detailed the zebrafish microbiota-gut-brain-axis homeostasis and mechanisms of brain functioning and modulation of brain neurochemistry (Mohanta et al., 2020). Most recently, Lee et al. (2021) provided a thorough review on using the zebrafish for investigating the association between microbiota-gut-brain-axis and neurological disorders which can include synucleinopathies, and dived into utilising mutant KOs, knockins, and transgenic reporter zebrafish lines to fully exploit this model (Lee et al., 2021).

Conclusion

The zebrafish is a highly amenable research model for investigating synucleinopathies by either utilising the zebrafish's endogenous synucleins that are compensating for the absence of SNCA or by generating humanized transgenics that express the human SNCA. The different forms of synucleinopathies can be recapitulated in this model, which could shed light on the non-PD disorders that have been somewhat overshadowed by the focus on PD. Advanced *in vivo* imaging techniques can now be applied to visualise everything from unique bacterial species in the larval gut to cellular flux in the brain, providing incredible troves of evidence to sieve through for analysis. The establishment of synucleinopathy models would also lead to the feasibility of performing high-throughput screening assays to identify new therapeutics for targeted therapy against synuclein aggregation or to protect neurons from the aggressive onslaught of SNCAs.

Competing interests

No potential conflict of interest relevant to this article was reported.

Funding sources

Not applicable.

Acknowledgements

Not applicable.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

This article does not require IRB/IACUC approval because there are no human and animal participants.

ORCID

Suzita Mohd Noor <https://orcid.org/0000-0001-5344-8165>

Anwar Norazit <https://orcid.org/0000-0003-2724-7591>

References

- Abeliovich A, Schmitz Y, Fariñas I, Choi-Lundberg D, Ho WH, Castillo PE, et al. Mice lacking alpha-synuclein display functional deficits in the nigrostriatal dopamine system. *Neuron*. 2000;25:239-52.
- Adams-Carr KL, Bestwick JP, Shribman S, Lees A, Schrag A, Noyce AJ. Constipation preceding Parkinson's disease: a systematic review and meta-analysis. *J Neurol Neurosurg Psychiatry*. 2016;87:710-6.
- Ahmad M, Attoub S, Singh MN, Martin FL, El-Agnaf OMA. Gamma-synuclein and the progression of cancer. *FASEB J*. 2007;21:3419-30.
- Aleström P, D'Angelo L, Midtlyng PJ, Schorderet DE, Schulte-Merker S, Sohm F, et al. Zebrafish: housing and husbandry recommendations. *Lab Anim*. 2020;54:213-24.
- Allison JR, Rivers RC, Christodoulou JC, Vendruscolo M, Dobson CM. A relationship between the transient structure in the monomeric state and the aggregation propensities of α -synuclein and β -synuclein. *Biochemistry*. 2014;53:7170-83.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol*. 1990;215:403-10.
- Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. *J Biol Chem*. 2006;281:29739-52.
- Appel-Cresswell S, Vilarino-Guell C, Encarnacion M, Sherman H, Yu I, Shah B, et al. Alpha-synuclein p.H50Q, a novel pathogenic mutation for Parkinson's disease. *Mov Disord*. 2013;28:811-3.
- Asakawa K, Kawakami K. The Tol2-mediated Gal4-UAS meth-

- od for gene and enhancer trapping in zebrafish. *Methods*. 2009;49:275-81.
- Bai Q, Burton EA. Zebrafish models of Tauopathy. *Biochim Biophys Acta* 2011;1812:353-63.
- Baker PA, Meyer MD, Tsang A, Uribe RA. Immunohistochemical and ultrastructural analysis of the maturing larval zebrafish enteric nervous system reveals the formation of a neuropil pattern. *Sci Rep*. 2019;9:6941.
- Barbazuk WB, Korf I, Kadavi C, Heyen J, Tate S, Wun E, et al. The syntenic relationship of the zebrafish and human genomes. *Genome Res*. 2000;10:1351-8.
- Barbureau C, Cubedo N, Maurice T, Rossel M. Zebrafish models to study new pathways in tauopathies. *Int J Mol Sci*. 2021;22:4626.
- Barnhill LM, Murata H, Bronstein JM. Studying the pathophysiology of Parkinson's disease using zebrafish. *Biomedicines*. 2020;8:197.
- Beach TG, Adler CH, Sue LI, Shill HA, Driver-Dunckley E, Mehta SH, et al. Vagus nerve and stomach synucleinopathy in Parkinson's disease, incidental Lewy body disease, and normal elderly subjects: evidence against the "body-first" hypothesis. *J Parkinsons Dis*. 2021;11:1833-43.
- Bedell VM, Westcot SE, Ekker SC. Lessons from morpholino-based screening in zebrafish. *Brief Funct Genomics*. 2011;10:181-8.
- Bendor JT, Logan TP, Edwards RH. The function of α -synuclein. *Neuron*. 2013;79:1044-66.
- Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, et al. Nitrated α -synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLOS ONE*. 2008;3:e1376.
- Bernis ME, Babila JT, Breid S, Wüsten KA, Wüllner U, Tamgüney G. Prion-like propagation of human brain-derived alpha-synuclein in transgenic mice expressing human wild-type alpha-synuclein. *Acta Neuropathol Commun*. 2015;3:75.
- Bertotto LB, Catron TR, Tal T. Exploring interactions between xenobiotics, microbiota, and neurotoxicity in zebrafish. *Neurotoxicology*. 2020;76:235-44.
- Beyer K. α -Synuclein structure, posttranslational modification and alternative splicing as aggregation enhancers. *Acta Neuropathol*. 2006;112:237-51.
- Beyer K, Ispierto L, Latorre P, Tolosa E, Ariza A. Alpha- and beta-synuclein expression in Parkinson disease with and without dementia. *J Neurol Sci*. 2011;310:112-7.
- Bill BR, Petzold AM, Clark KJ, Schimmenti LA, Ekker SC. A primer for morpholino use in zebrafish. *Zebrafish*. 2009;6:69-77.
- Blader P, Strähle U. Zebrafish developmental genetics and central nervous system development. *Hum Mol Genet*. 2000;9:945-51.
- Bonini NM, Giasson BI. Snaring the function of α -synuclein. *Cell*. 2005;123:359-61.
- Borghammer P, Horsager J, Andersen K, Van Den Berge N, Raunio A, Murayama S, et al. Neuropathological evidence of body-first vs. brain-first Lewy body disease. *Neurobiol Dis*. 2021;161:105557.
- Borrelli L, Aceto S, Agnisola C, De Paolo S, Dipineto L, Stilling RM, et al. Probiotic modulation of the microbiota-gut-brain axis and behaviour in zebrafish. *Sci Rep*. 2016;6:30046.
- Braak H, Braak E. Pathoanatomy of Parkinson's disease. *J Neurol*. 2000;247:II3-II10.
- Braak H, Rüb U, Gai WP, Del Tredici K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J Neural Transm*. 2003;110:517-36.
- Burré J, Sharma M, Südhof TC. Cell biology and pathophysiology of α -synuclein. *Cold Spring Harb Perspect Med*. 2018;8:a024091.
- Cansız D, Ünal İ, Üstündağ ÜV, Alturfan AA, Altinoz MA, Elmacı İ, et al. Caprylic acid ameliorates rotenone induced inflammation and oxidative stress in the gut-brain axis in Zebrafish. *Mol Biol Rep*. 2021;48:5259-73.
- Cantarero-Prieto D, Leon PL, Blazquez-Fernandez C, Juan PS, Cobo CS. The economic cost of dementia: a systematic review. *Dementia*. 2020;19:2637-57.
- Cariulo C, Martufi P, Verani M, Azzollini L, Bruni G, Weiss A, et al. Phospho-S129 alpha-synuclein is present in human plasma but not in cerebrospinal fluid as determined by an ultrasensitive immunoassay. *Front Neurosci*. 2019;13:889.
- Chavarría C, Souza JM. Oxidation and nitration of α -synuclein and their implications in neurodegenerative diseases. *Arch Biochem Biophys*. 2013;533:25-32.
- Chen M, Martins RN, Lardelli M. Complex splicing and neural expression of duplicated tau genes in zebrafish embryos. *J Alzheimers Dis*. 2009a;18:305-17.
- Chen YC, Cheng CH, Chen GD, Hung CC, Yang CH, Hwang SPL, et al. Recapitulation of zebrafish *snca* expression pattern and labeling the habenular complex in transgenic zebrafish using green fluorescent protein reporter gene. *Dev*

- Dyn. 2009b;238:746-54.
- Cheng B, Yang X, An L, Gao B, Liu X, Liu S. Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity via up-regulating glutathione in a rat model of Parkinson's disease. *Brain Res.* 2009;1286:25-31.
- Chepelev NL, Moffat ID, Bowers WJ, Yauk CL. Neurotoxicity may be an overlooked consequence of benzo[a]pyrene exposure that is relevant to human health risk assessment. *Mutat Res Rev Mutat Res.* 2015;764:64-89.
- Choi TY, Choi TI, Lee YR, Choe SK, Kim CH. Zebrafish as an animal model for biomedical research. *Exp Mol Med.* 2021;53:310-7.
- Choong CJ, Say YH. Neuroprotection of α -synuclein under acute and chronic rotenone and maneb treatment is abolished by its familial Parkinson's disease mutations A30P, A53T and E46K. *Neurotoxicology.* 2011;32:857-63.
- Chou AP, Maidment N, Klintonberg R, Casida JE, Li S, Fitzmaurice AG, et al. Ziram causes dopaminergic cell damage by inhibiting E1 ligase of the proteasome. *J Biol Chem.* 2008;283:34696-703.
- Chu Y, Kordower JH. Age-associated increases of alpha-synuclein in monkeys and humans are associated with nigrostriatal dopamine depletion: is this the target for Parkinson's disease? *Neurobiol Dis.* 2007;25:134-49.
- Conforti L, Gilley J, Coleman MP. Wallerian degeneration: an emerging axon death pathway linking injury and disease. *Nat Rev Neurosci.* 2014;15:394-409.
- Conway KA, Harper JD, Lansbury PT. Accelerated *in vitro* fibril formation by a mutant alpha-synuclein linked to early-onset Parkinson disease. *Nat Med.* 1998;4:1318-20.
- Coon EA, Singer W. Synucleinopathies. *Continuum (Minneapolis Minn).* 2020;26:72-92.
- Corey DR, Abrams JM. Morpholino antisense oligonucleotides: tools for investigating vertebrate development. *Genome Biol.* 2001;2:reviews1015.1.
- Cornet C, Di Donato V, Terriente J. Combining zebrafish and CRISPR/Cas9: toward a more efficient drug discovery pipeline. *Front Pharmacol.* 2018;9:703.
- Cuomo M, Borrelli L, Della Monica R, Coretti L, De Riso G, D'Angelo Lancellotti di Durazzo L, et al. DNA methylation profiles of *Tph1A* and *BDNF* in gut and brain of *L. Rhamnosus*-treated zebrafish. *Biomolecules.* 2021;11:142.
- Das SK, Aparna S, Patri M. Chronic waterborne exposure to benzo[a]pyrene induces locomotor dysfunction and development of neurodegenerative phenotypes in zebrafish. *Neurosci Lett.* 2020;716:134646.
- Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron.* 2003;39:889-909.
- Davis DJ, Bryda EC, Gillespie CH, Ericsson AC. Microbial modulation of behavior and stress responses in zebrafish larvae. *Behav Brain Res.* 2016;311:219-27.
- de Abreu MS, Giacomini ACVV, Sysoev M, Demin KA, Alekseeva PA, Spagnoli ST, et al. Modeling gut-brain interactions in zebrafish. *Brain Res Bull.* 2019;148:55-62.
- Dehal P, Boore JL. Two rounds of whole genome duplication in the ancestral vertebrate. *PLOS Biol.* 2005;3:e314.
- Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's disease. *J Parkinsons Dis.* 2013;3:461-91.
- Dickson DW, Lin W, Liu WK, Yen SH. Multiple system atrophy: a sporadic synucleinopathy. *Brain Pathol.* 1999;9:721-32.
- Ding Y, Lei L, Lai C, Tang Z. Tau protein and zebrafish models for tau-induced neurodegeneration. *J Alzheimers Dis.* 2019;69:339-53.
- Dodiya HB, Forsyth CB, Voigt RM, Engen PA, Patel J, Shaikh M, et al. Chronic stress-induced gut dysfunction exacerbates Parkinson's disease phenotype and pathology in a rotenone-induced mouse model of Parkinson's disease. *Neurobiol Dis.* 2020;135:104352.
- Du Y, Guo Q, Shan M, Wu Y, Huang S, Zhao H, et al. Spatial and temporal distribution of dopaminergic neurons during development in zebrafish. *Front Neuroanat.* 2016;10:115.
- Dukes AA, Bai Q, Van Laar VS, Zhou Y, Ilin V, David CN, et al. Live imaging of mitochondrial dynamics in CNS dopaminergic neurons *in vivo* demonstrates early reversal of mitochondrial transport following MPP⁺ exposure. *Neurobiol Dis.* 2016;95:238-49.
- Elgar G. Plenty more fish in the sea: comparative and functional genomics using teleost models. *Brief Funct Genomic Proteomic.* 2004;3:15-25.
- Engelender S, Kaminsky Z, Guo X, Sharp AH, Amaravi RK, Kleiderlein JJ, et al. Synphilin-1 associates with α -synuclein and promotes the formation of cytosolic inclusions. *Nat Genet.* 1999;22:110-4.
- Engelender S, Wanner T, Kleiderlein JJ, Wakabayashi K, Tsuji S, Takahashi H, et al. Organization of the human synphilin-1 gene, a candidate for Parkinson's disease. *Mamm Genome.* 2000;11:763-6.
- Engeszer RE, Patterson LB, Rao AA, Parichy DM. Zebrafish in the wild: a review of natural history and new notes from the field. *Zebrafish.* 2007;4:21-40.

- Fan Y, Limprasert P, Murray IVJ, Smith AC, Lee VMY, Trojanowski JQ, et al. β -Synuclein modulates α -synuclein neurotoxicity by reducing α -synuclein protein expression. *Hum Mol Genet.* 2006;15:3002-11.
- Flinn L, Breaud S, Lo C, Ingham PW, Bandmann O. Zebrafish as a new animal model for movement disorders. *J Neurochem.* 2008;106:1991-7.
- Fontana BD, Mezzomo NJ, Kalueff AV, Rosemberg DB. The developing utility of zebrafish models of neurological and neuropsychiatric disorders: a critical review. *Exp Neurol.* 2018;299:157-71.
- Ganz J. Gut feelings: studying enteric nervous system development, function, and disease in the zebrafish model system. *Dev Dyn.* 2018;247:268-78.
- George JM. The synucleins. *Genome Biol.* 2001;3:reviews3002.1-3002.6.
- Godoy R, Hua K, Kalyn M, Cusson VM, Anisman H, Ekker M. Dopaminergic neurons regenerate following chemogenetic ablation in the olfactory bulb of adult Zebrafish (*Danio rerio*). *Sci Rep.* 2020;10:12825.
- Goedert M. Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci.* 2001;2:492-501.
- Goedert M, Spillantini MG. Synucleinopathies and tauopathies. In: Brady ST, Siegel GJ, Albers RW, editors. *Basic neurochemistry.* Amsterdam: Elsevier; 2012. p. 829-43.
- Goedert M, Masuda-Suzukake M, Falcon B. Like prions: the propagation of aggregated tau and α -synuclein in neurodegeneration. *Brain.* 2016;140:266-78.
- Gorostidi A, Bergareche A, Ruiz-Martínez J, Martí-Massó JF, Cruz M, Varghese S, et al. α -Synuclein levels in blood plasma from *LRRK2* mutation carriers. *PLOS ONE.* 2012;7:e52312.
- Greggio E, Cookson MR. Leucine-rich repeat kinase 2 mutations and Parkinson's disease: three questions. *ASN Neuro.* 2009;1:AN20090007.
- Gregory A, Kurian MA, Maher ER, Hogarth P, Hayflick SJ. *PLA2G6*-associated neurodegeneration. Seattle, WA: University of Washington; 2017.
- Haikal C, Chen QQ, Li JY. Microbiome changes: an indicator of Parkinson's disease? *Transl Neurodegener.* 2019;8:38.
- Hashimoto M, Rockenstein E, Mante M, Mallory M, Masliah E. β -Synuclein inhibits α -synuclein aggregation: a possible role as an anti-parkinsonian factor. *Neuron.* 2001;32:213-23.
- Hashimoto M, Spada ARL. β -Synuclein in the pathogenesis of Parkinson's disease and related α -synucleinopathies: emerging roles and new directions. *Future Neurol.* 2012;7:155-63.
- Hawkes CH, Del Tredici K, Braak H. Parkinson's disease: a dual-hit hypothesis. *Neuropathol Appl Neurobiol.* 2007;33:599-614.
- Hoenen C, Gustin A, Birk C, Kirchmeyer M, Beaume N, Felten P, et al. Alpha-synuclein proteins promote pro-inflammatory cascades in microglia: stronger effects of the A53T mutant. *PLOS ONE.* 2016;11:e0162717.
- Hoppe SO, Uzunoğlu G, Nussbaum-Krammer C. α -Synuclein strains: does amyloid conformation explain the heterogeneity of synucleinopathies? *Biomolecules.* 2021;11:931.
- Houlden H, Singleton AB. The genetics and neuropathology of Parkinson's disease. *Acta Neuropathol.* 2012;124:325-38.
- Howe K, Clark MD, Torroja CF, Tarrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature.* 2013;496(7446):498-503.
- Howe K. Chapter 31 - The zebrafish genome sequencing project: bioinformatics resources. In: Gerlai RT, editor. *Behavioral and neural genetics of zebrafish.* London: Academic Press; 2020. p. 551-62.
- Hruska KS, LaMarca ME, Scott CR, Sidransky E. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (*GBA*). *Hum Mutat.* 2008;29:567-83.
- Hu Q, Guo F, Zhao F, Fu Z. Effects of titanium dioxide nanoparticles exposure on Parkinsonism in zebrafish larvae and PC12. *Chemosphere.* 2017;173:373-9.
- Ingelsson M. Alpha-synuclein oligomers—neurotoxic molecules in Parkinson's disease and other lewy body disorders. *Front Neurosci.* 2016;10:408.
- Inglis KJ, Chereau D, Brigham EF, Chiou SS, Schöbel S, Frigon NL, et al. Polo-like kinase 2 (PLK2) phosphorylates α -synuclein at serine 129 in central nervous system. *J Biol Chem.* 2009;284:2598-602.
- Innos J, Hickey MA. Using rotenone to model Parkinson's disease in mice: a review of the role of pharmacokinetics. *Chem Res Toxicol.* 2021;34:1223-39.
- Ishikawa-Ankerhold HC, Ankerhold R, Drummen GPC. Advanced fluorescence microscopy techniques—FRAP, FLIP, FLAP, FRET and FLIM. *Molecules.* 2012;17:4047-132.
- Jakes R, Spillantini MG, Goedert M. Identification of two distinct synucleins from human brain. *FEBS Lett.* 1994;345:27-32.
- Jiang W, Li J, Zhang Z, Wang H, Wang Z. Epigenetic upregulation

- tion of alpha-synuclein in the rats exposed to methamphetamine. *Eur J Pharmacol.* 2014;745:243-8.
- Jo E, McLaurin J, Yip CM, St. George-Hyslop P, Fraser PE. alpha-Synuclein membrane interactions and lipid specificity. *J Biol Chem.* 2000;275:34328-34.
- Johnson ME, Bobrovskaya L. An update on the rotenone models of Parkinson's disease: their ability to reproduce the features of clinical disease and model gene-environment interactions. *Neurotoxicology.* 2015;46:101-16.
- Johnson ME, Stringer A, Bobrovskaya L. Rotenone induces gastrointestinal pathology and microbiota alterations in a rat model of Parkinson's disease. *Neurotoxicology.* 2018;65:174-85.
- Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. *Trends Pharmacol Sci.* 2014;35:63-75.
- Kaur G, Behl T, Bungau S, Kumar A, Uddin MS, Mehta V, et al. Dysregulation of the gut-brain axis, dysbiosis and influence of numerous factors on gut microbiota associated Parkinson's disease. *Curr Neuropharmacol.* 2021;19:233-47.
- Kaur U, Lee JC. Membrane interactions of α -synuclein probed by neutrons and photons. *Acc Chem Res.* 2021;54:302-10.
- Keatinge M, Bui H, Menke A, Chen YC, Sokol AM, Bai Q, et al. Glucocerebrosidase 1 deficient *Danio rerio* mirror key pathological aspects of human Gaucher disease and provide evidence of early microglial activation preceding alpha-synuclein-independent neuronal cell death. *Hum Mol Genet.* 2015;24:6640-52.
- Keller JM, Keller ET. The use of mature zebrafish (*Danio rerio*) as a model for human aging and disease. In: Ram JL, Conn PM, editors. *Conn's handbook of models for human aging.* London: Academic Press; 2018. p. 351-9.
- Khotimah H, Ali M, Sumitro SB, Widodo MA. Decreasing α -synuclein aggregation by methanolic extract of *Centella asiatica* in zebrafish Parkinson's model. *Asian Pac J Trop Biomed.* 2015a;5:948-54.
- Khotimah H, Sumitro SB, Widodo MA. Zebrafish Parkinson's model: rotenone decrease motility, dopamine, and increase α -synuclein aggregation and apoptosis of zebrafish brain. *Int J Pharm Tech Res.* 2015b;8:614-21.
- Kimmel CB. Genetics and early development of zebrafish. *Trends Genet.* 1989;5:283-8.
- Kimmel CB. Patterning the brain of the zebrafish embryo. *Annu Rev Neurosci.* 1993;16:707-32.
- Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn.* 1995;203:253-310.
- Koppel N, Maini Rekdal V, Balskus EP. Chemical transformation of xenobiotics by the human gut microbiota. *Science.* 2017;356:eaag2770.
- Kostyuk AI, Panova AS, Kokova AD, Kotova DA, Maltsev DI, Podgorny OV, et al. *In vivo* imaging with genetically encoded redox biosensors. *Int J Mol Sci.* 2020;21:8164.
- Krüger R, Kuhn W, Müller T, Voitalla D, Graeber M, Kösel S, et al. Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat Genet.* 1998;18:106-8.
- Kuil LE, Chauhan RK, Cheng WW, Hofstra RMW, Alves MM. Zebrafish: a model organism for studying enteric nervous system development and disease. *Front Cell Dev Biol.* 2021;8:629073.
- Kumar A, Anuppalle M, Maddirevula S, Huh TL, Choe J, Rhee M. Peli1b governs the brain patterning via ERK signaling pathways in zebrafish embryos. *Gene.* 2019;694:1-6.
- Kurian MA, Morgan NV, MacPherson L, Foster K, Peake D, Gupta R, et al. Phenotypic spectrum of neurodegeneration associated with mutations in the *PLA2G6* gene (PLAN). *Neurology.* 2008;70:1623-9.
- Lavedan C, Leroy E, Dehejia A, Buchholtz S, Dutra A, Nussbaum RL, et al. Identification, localization and characterization of the human γ -synuclein gene. *Hum Genet.* 1998;103:106-12.
- Lee HJ, Kim C, Lee SJ. Alpha-synuclein stimulation of astrocytes: potential role for neuroinflammation and neuroprotection. *Oxid Med Cell Longev.* 2010;3:283-7.
- Lee JG, Cho HJ, Jeong YM, Lee JS. Genetic approaches using zebrafish to study the microbiota-gut-brain axis in neurological disorders. *Cells.* 2021;10:566.
- Lee VMY, Trojanowski JQ. Progress from Alzheimer's tangles to pathological tau points towards more effective therapies now. *J Alzheimers Dis.* 2006;9(3 Suppl):257-62.
- Lehtonen Š, Sonninen TM, Wojciechowski S, Goldsteins G, Koistinaho J. Dysfunction of cellular proteostasis in Parkinson's disease. *Front Neurosci.* 2019;13:457.
- Lennaerts-Kats H, Ebenau A, Steppe M, van der Steen JT, Meinders MJ, Vissers K, et al. "How long can I carry on?" The need for palliative care in Parkinson's disease: a qualitative study from the perspective of bereaved family caregivers. *J Parkinsons Dis.* 2020;10:1631-42.
- Leong SL, Cappai R, Barnham KJ, Pham CLL. Modulation of alpha-synuclein aggregation by dopamine: a review. *Neu-*

- rochem Res. 2009;34:1838-46.
- Lesage S, Anheim M, Letournel F, Bousset L, Honoré A, Rozas N, et al. G51D α -synuclein mutation causes a novel parkinsonian–pyramidal syndrome. *Ann Neurol*. 2013;73:459-71.
- Li J, Uversky VN, Fink AL. Conformational behavior of human α -synuclein is modulated by familial Parkinson's disease point mutations A30P and A53T. *Neurotoxicology*. 2002;23:553-67.
- Li N, Stewart T, Sheng L, Shi M, Cilento EM, Wu Y, et al. Immunoregulation of microglial polarization: an unrecognized physiological function of α -synuclein. *J Neuroinflammation*. 2020;17:272.
- Li X, Dang J, Li Y, Wang L, Li N, Liu K, et al. Developmental neurotoxicity fingerprint of silica nanoparticles at environmentally relevant level on larval zebrafish using a neurobehavioral-phenomics-based biological warning method. *Sci Total Environ*. 2021;752:141878.
- Lingor P, Koch JC, Tönges L, Bähr M. Axonal degeneration as a therapeutic target in the CNS. *Cell Tissue Res*. 2012;349:289-311.
- Linnertz C, Saucier L, Ge D, Cronin KD, Burke JR, Brown-dyke JN, et al. Genetic regulation of α -synuclein mRNA expression in various human brain tissues. *PLOS ONE*. 2009;4:e7480.
- Lionnet A, Leclair-Visonneau L, Neunlist M, Murayama S, Takao M, Adler CH, et al. Does Parkinson's disease start in the gut? *Acta Neuropathol*. 2018;135:1-12.
- Liu J, Li T, Thomas JM, Pei Z, Jiang H, Engelender S, et al. Synphilin-1 attenuates mutant LRRK2-induced neurodegeneration in Parkinson's disease models. *Hum Mol Genet*. 2016;25:672-80.
- Liu J, Xu F, Nie Z, Shao L. Gut microbiota approach—a new strategy to treat Parkinson's disease. *Front Cell Infect Microbiol*. 2020;10:570658.
- Lu J, Peatman E, Tang H, Lewis J, Liu Z. Profiling of gene duplication patterns of sequenced teleost genomes: evidence for rapid lineage-specific genome expansion mediated by recent tandem duplications. *BMC Genomics*. 2012;13:246.
- Luke GA, Ryan MD. Using the 2A protein coexpression system: multicistronic 2A vectors expressing gene(s) of interest and reporter proteins. In: Damoiseaux R, Hasson S, editors. Reporter gene assays: methods and protocols. New York, NY: Humana Press/Springer; 2018. p. 31-48.
- Lulla A, Barnhill L, Bitan G, Ivanova MI, Nguyen B, O'Donnell K, et al. Neurotoxicity of the Parkinson disease-associated pesticide ziram is synuclein-dependent in zebrafish embryos. *Environ Health Perspect*. 2016;124:1766-75.
- Luo C, Rajput AH, Akhtar S, Rajput A. α -Synuclein and tyrosine hydroxylase expression in acute rotenone toxicity. *Int J Mol Med*. 2007;19:517-21.
- Lv DJ, Li LX, Chen J, Wei SZ, Wang F, Hu H, et al. Sleep deprivation caused a memory defects and emotional changes in a rotenone-based zebrafish model of Parkinson's disease. *Behav Brain Res*. 2019;372:112031.
- Ma PM. Catecholaminergic systems in the zebrafish. IV. Organization and projection pattern of dopaminergic neurons in the diencephalon. *J Comp Neurol*. 2003;460:13-37.
- Ma Q, Xing C, Long W, Wang HY, Liu Q, Wang RF. Impact of microbiota on central nervous system and neurological diseases: the gut-brain axis. *J Neuroinflammation*. 2019;16:53.
- Madden JC, Hewitt M, Przybylak K, Vandebriel RJ, Piersma AH, Cronin MTD. Strategies for the optimisation of *in vivo* experiments in accordance with the 3Rs philosophy. *Regul Toxicol Pharmacol*. 2012;63:140-54.
- Makhija DT, Jagtap AG. Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: comparison with rat model. *J Pharmacol Pharmacother*. 2014;5:39-46.
- Maroteaux L, Campanelli JT, Scheller RH. Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J Neurosci*. 1988;8:2804-15.
- Martins T, Valentim AM, Pereira N, Antunes LM. Anaesthesia and analgesia in laboratory adult zebrafish: a question of refinement. *Lab Anim*. 2016;50:476-88.
- Marx FP, Holzmann C, Strauss KM, Li L, Eberhardt O, Gerhard E, et al. Identification and functional characterization of a novel R621C mutation in the synphilin-1 gene in Parkinson's disease. *Hum Mol Genet*. 2003;12:1223-31.
- Matsui H, Matsui N. Cerebrospinal fluid injection into adult zebrafish for disease research. *J Neural Transm*. 2017;124:1627-33.
- Matsui H, Sugie A. An optimized method for counting dopaminergic neurons in zebrafish. *PLOS ONE*. 2017;12:e0184363.
- Maximino C, de Brito TM, da Silva Batista AW, Herculano AM, Morato S, Gouveia A Jr. Measuring anxiety in zebrafish: a critical review. *Behav Brain Res*. 2010;214:157-71.
- Mbefo MK, Paleologou KE, Boucharaba A, Oueslati A, Schell H, Fournier M, et al. Phosphorylation of synucleins by members of the Polo-like kinase family. *J Biol Chem*. 2010;285:2807-22.
- Mehra S, Sahay S, Maji SK. α -Synuclein misfolding and aggre-

- gation: implications in Parkinson's disease pathogenesis. *Biochim Biophys Acta Proteins Proteomics*. 2019;1867:890-908.
- Melki R. Role of different alpha-synuclein strains in synucleinopathies, similarities with other neurodegenerative diseases. *J Parkinsons Dis*. 2015;5:217-27.
- Milanese C, Sager JJ, Bai Q, Farrell TC, Cannon JR, Greenamyre JT, et al. Hypokinesia and reduced dopamine levels in zebrafish lacking β - and γ 1-synucleins. *J Biol Chem*. 2012;287:2971-83.
- Milanese C, Cerri S, Ulusoy A, Gornati SV, Plat A, Gabriels S, et al. Activation of the DNA damage response *in vivo* in synucleinopathy models of Parkinson's disease. *Cell Death Dis*. 2018;9:818.
- Mohanta L, Das BC, Patri M. Microbial communities modulating brain functioning and behaviors in zebrafish: a mechanistic approach. *Microb Pathog*. 2020;145:104251.
- Moulton JD. Using morpholinos to control gene expression. *Curr Protoc Nucleic Acid Chem*. 2017;68:4.30.1-4.30.29.
- Moussavi Nik SH, Newman M, Ganesan S, Chen M, Martins R, Verdile G, et al. Hypoxia alters expression of zebrafish microtubule-associated protein tau (*mapta*, *maptb*) gene transcripts. *BMC Res Notes*. 2014;7:767.
- Mudò G, Mäkelä J, Di Liberto V, Tselykh TV, Olivieri M, Piepponen P, et al. Transgenic expression and activation of PGC-1 α protect dopaminergic neurons in the MPTP mouse model of Parkinson's disease. *Cell Mol Life Sci*. 2012;69:1153-65.
- Musgrove REJ, King AE, Dickson TC. Neuroprotective upregulation of endogenous alpha-synuclein precedes ubiquitination in cultured dopaminergic neurons. *Neurotox Res*. 2011;19:592-602.
- Musgrove RE, Horne J, Wilson R, King AE, Edwards LM, Dickson TC. The metabolomics of alpha-synuclein (SNCA) gene deletion and mutation in mouse brain. *Metabolomics*. 2014;10:114-22.
- Najib NHM, Nies YH, Abd Halim SAS, Yahaya MF, Das S, Lim WL, et al. Modeling Parkinson's disease in zebrafish. *CNS Neurol Disord Drug Targets*. 2020;19:386-99.
- Ng CH, Basil AH, Hang L, Tan R, Goh KL, O'Neill S, et al. Genetic or pharmacological activation of the *Drosophila* PGC-1 α ortholog spargel rescues the disease phenotypes of genetic models of Parkinson's disease. *Neurobiol Aging*. 2017;55:33-7.
- Nietzel T, Elsässer M, Ruberti C, Steinbeck J, Ugalde JM, Fuchs P, et al. The fluorescent protein sensor roGFP2-Orp1 monitors *in vivo* H₂O₂ and thiol redox integration and elucidates intracellular H₂O₂ dynamics during elicitor-induced oxidative burst in *Arabidopsis*. *New Phytol*. 2019;221:1649-64.
- NLM. SNCA - synuclein alpha. [Internet]. 2017 [cited 2021 Jun 21]. <https://www.ncbi.nlm.nih.gov/kis/ortholog/6622/?scope=7776>
- Northam C, LeMoine CMR. Metabolic regulation by the PGC-1 α and PGC-1 β coactivators in larval zebrafish (*Danio rerio*). *Comp Biochem Physiol A Mol Integr Physiol*. 2019;234:60-7.
- Nuytemans K, Theuns J, Cruts M, Van Broeckhoven C. Genetic etiology of Parkinson disease associated with mutations in the *SNCA*, *PARK2*, *PINK1*, *PARK7*, and *LRRK2* genes: a mutation update. *Hum Mutat*. 2010;31:763-80.
- O'Donnell KC, Lulla A, Stahl MC, Wheat ND, Bronstein JM, Sagasti A. Axon degeneration and PGC-1 α -mediated protection in a zebrafish model of α -synuclein toxicity. *Dis Model Mech*. 2014;7:571-82.
- O'Hara DM, Pawar G, Kalia SK, Kalia LV. LRRK2 and α -synuclein: distinct or synergistic players in Parkinson's disease? *Front Neurosci*. 2020;14:577.
- Ohnesorge N, Heidl C, Lewejohann L. Current methods to investigate nociception and pain in zebrafish. *Front Neurosci*. 2021;15:632634.
- Ohno M, Nikaido M, Horiuchi N, Kawakami K, Hatta K. The enteric nervous system in zebrafish larvae can regenerate via migration into the ablated area and proliferation of neural crest-derived cells. *Development*. 2021;148:dev195339.
- Olsson C, Holmberg A, Holmgren S. Development of enteric and vagal innervation of the zebrafish (*Danio rerio*) gut. *J Comp Neurol*. 2008;508:756-70.
- Ostrovova N, Petrucelli L, Farrer M, Mehta N, Choi P, Hardy J, et al. α -Synuclein shares physical and functional homology with 14-3-3 proteins. *J Neurosci*. 1999;19:5782-91.
- Oueslati A, Schneider BL, Aebischer P, Lashuel HA. Polo-like kinase 2 regulates selective autophagic α -synuclein clearance and suppresses its toxicity *in vivo*. *Proc Natl Acad Sci USA*. 2013;110:E3945-54.
- Oueslati A, Ximerakis M, Vekrellis K. Protein transmission, seeding and degradation: key steps for α -synuclein prion-like propagation. *Exp Neurobiol*. 2014;23:324-36.
- Parichy DM, Elizondo MR, Mills MG, Gordon TN, Engeszer RE. Normal table of postembryonic zebrafish development: staging by externally visible anatomy of the living fish. *Dev Dyn*. 2009;238:2975-3015.
- Park JY, Lansbury PT Jr. β -Synuclein inhibits formation of α -syn-

- nuclein protofibrils: a possible therapeutic strategy against Parkinson's disease. *Biochemistry*. 2003;42:3696-700.
- Parkinson J. An essay on the shaking palsy. 1817. *J Neuropsychiatry Clin Neurosci*. 2002;14:223-36.
- Perez RG, Waymire JC, Lin E, Liu JJ, Guo F, Zigmond MJ. A role for alpha-synuclein in the regulation of dopamine biosynthesis. *J Neurosci*. 2002;22:3090-9.
- Periquet M, Fulga T, Myllykangas L, Schlossmacher MG, Feany MB. Aggregated α -synuclein mediates dopaminergic neurotoxicity *in vivo*. *J Neurosci*. 2007;27:3338-46.
- Peters OM, Shelkovernikova T, Highley JR, Cooper-Knock J, Hortobágyi T, Troakes C, et al. Gamma-synuclein pathology in amyotrophic lateral sclerosis. *Ann Clin Transl Neurol*. 2015;2:29-37.
- Pham LN, Kanther M, Semova I, Rawls JE. Methods for generating and colonizing gnotobiotic zebrafish. *Nat Protoc*. 2008;3:1862-75.
- Pietrucci D, Teofani A, Unida V, Cerroni R, Biocca S, Stefani A, et al. Can gut microbiota be a good predictor for Parkinson's disease? A machine learning approach. *Brain Sci*. 2020;10:242.
- Piovesan A, Antonaros F, Vitale L, Strippoli P, Pelleri MC, Caracausi M. Human protein-coding genes and gene feature statistics in 2019. *BMC Res Notes*. 2019;12:315.
- Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science*. 1997;276:2045-7.
- Prabhudesai S, Sinha S, Attar A, Kotagiri A, Fitzmaurice AG, Lakshmanan R, et al. A novel "molecular tweezer" inhibitor of α -synuclein neurotoxicity *in vitro* and *in vivo*. *Neurotherapeutics*. 2012;9:464-76.
- Prabhudesai S, Bensabeur FZ, Abdullah R, Basak I, Baez S, Alves G, et al. LRRK2 knockdown in zebrafish causes developmental defects, neuronal loss, and synuclein aggregation. *J Neurosci Res*. 2016;94:717-35.
- Proukakis C, Dudzik CG, Brier T, MacKay DS, Cooper JM, Millhauser GL, et al. A novel α -synuclein missense mutation in Parkinson disease. *Neurology*. 2013;80:1062-4.
- Provost E, Rhee J, Leach SD. Viral 2A peptides allow expression of multiple proteins from a single ORF in transgenic zebrafish embryos. *Genesis*. 2007;45:625-9.
- Punsoni M, Friedman JH, Resnick M, Donahue JE, Yang DF, Stopa EG. Enteric pathologic manifestations of alpha-synucleinopathies. *Appl Immunohistochem Mol Morphol*. 2019;27:543-8.
- Quilty MC, King AE, Gai WP, Pountney DL, West AK, Vickers JC, et al. Alpha-synuclein is upregulated in neurons in response to chronic oxidative stress and is associated with neuroprotection. *Exp Neurol*. 2006;199:249-56.
- Ravi V, Venkatesh B. The divergent genomes of teleosts. *Annu Rev Anim Biosci*. 2018;6:47-68.
- Recchia A, Debetto P, Negro A, Guidolin D, Skaper SD, Giusti P. Alpha-synuclein and Parkinson's disease. *FASEB J*. 2004;18:617-26.
- Rey NL, George S, Brundin P. Review: spreading the word: precise animal models and validated methods are vital when evaluating prion-like behaviour of alpha-synuclein. *Neuropathol Appl Neurobiol*. 2016;42:51-76.
- Rietdijk CD, Perez-Pardo P, Garssen J, van Wezel RJA, Kraneveld AD. Exploring Braak's Hypothesis of Parkinson's disease. *Front Neurol*. 2017;8:37.
- Rodriguez L, Marano MM, Tandon A. Import and export of misfolded α -synuclein. *Front Neurosci*. 2018;12:344.
- Rolig AS, Mittge EK, Ganz J, Troll JV, Melancon E, Wiles TJ, et al. The enteric nervous system promotes intestinal health by constraining microbiota composition. *PLOS Biol*. 2017;15:e2000689.
- Rotshenker S. Wallerian degeneration: the innate-immune response to traumatic nerve injury. *J Neuroinflammation*. 2011;8:109.
- Sager JJ, Bai Q, Burton EA. Transgenic zebrafish models of neurodegenerative diseases. *Brain Struct Funct*. 2010;214:285-302.
- Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, et al. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell*. 2016;167:1469-80.e12.
- Sánchez E, Azcona LJ, Paisán-Ruiz C. *Pla2g6* deficiency in zebrafish leads to dopaminergic cell death, axonal degeneration, increased beta-synuclein expression, and defects in brain functions and pathways. *Mol Neurobiol*. 2018;55:6734-54.
- Sargent D, Bétemps D, Drouyer M, Verchere J, Gaillard D, Arsac JN, et al. Investigating the neuroprotective effect of AAV-mediated β -synuclein overexpression in a transgenic model of synucleinopathy. *Sci Rep*. 2018;8:17563.
- Savica R, Boeve BF, Mielke MM. When do α -synucleinopathies start? An epidemiological timeline: a review. *JAMA Neurol*. 2018;75:503-9.

- Sen S, West AB. The therapeutic potential of LRRK2 and α -synuclein in Parkinson's disease. *Antioxid Redox Signal*. 2009;11:2167-87.
- Senior SL, Ninkina N, Deacon R, Bannerman D, Buchman VL, Cragg SJ, et al. Increased striatal dopamine release and hyperdopaminergic-like behaviour in mice lacking both alpha-synuclein and gamma-synuclein. *Eur J Neurosci*. 2008;27:947-57.
- Shaafi S, Najmi S, Aliasgharpour H, Mahmoudi J, Sadigh-Etemad S, Farhoudi M, et al. The efficacy of the ketogenic diet on motor functions in Parkinson's disease: a rat model. *Iran J Neurol*. 2016;15:63-9.
- Shaltiel-Karyo R, Frenkel-Pinter M, Egoz-Matia N, Frydman-Marom A, Shalev DE, Segal D, et al. Inhibiting α -synuclein oligomerization by stable cell-penetrating β -synuclein fragments recovers phenotype of Parkinson's disease model flies. *PLOS ONE*. 2010;5:e13863.
- Shen T, Yue Y, He T, Huang C, Qu B, Lv W, et al. The association between the gut microbiota and Parkinson's disease, a meta-analysis. *Front Aging Neurosci*. 2021;13:636545.
- Shepherd I, Eisen J. Development of the zebrafish enteric nervous system. *Methods Cell Biol*. 2011;101:143-60.
- Shi L, Huang C, Luo Q, Xia Y, Liu H, Li L, et al. Pilot study: molecular risk factors for diagnosing sporadic Parkinson's disease based on gene expression in blood in MPTP-induced rhesus monkeys. *Oncotarget*. 2017;8:105606-14.
- Shishido T, Nagano Y, Araki M, Kurashige T, Obayashi H, Nakamura T, et al. Synphilin-1 has neuroprotective effects on MPP⁺-induced Parkinson's disease model cells by inhibiting ROS production and apoptosis. *Neurosci Lett*. 2019;690:145-50.
- Siddiqui IJ, Pervaiz N, Abbasi AA. The Parkinson disease gene SNCA: evolutionary and structural insights with pathological implication. *Sci Rep*. 2016;6:24475.
- Slijkerman R, Goloborodko A, Broekman S, de Vrieze E, Heterschijt L, Peters T, et al. Poor splice-site recognition in a humanized zebrafish knockin model for the recurrent deep-intronic c.7595-2144A>G mutation in USH2A. *Zebrafish*. 2018;15:597-609.
- Smith WW, Liu Z, Liang Y, Masuda N, Swing DA, Jenkins NA, et al. Synphilin-1 attenuates neuronal degeneration in the A53T alpha-synuclein transgenic mouse model. *Hum Mol Genet*. 2010;19:2087-98.
- Specht CG, Schoepfer R. Deletion of the alpha-synuclein locus in a subpopulation of C57BL/6J inbred mice. *BMC Neurosci*. 2001;2:11.
- Spillantini MG, Divane A, Goedert M. Assignment of human alpha-synuclein (SNCA) and beta-synuclein (SNCB) genes to chromosomes 4q21 and 5q35. *Genomics*. 1995;27:379-81.
- Spillantini MG, Schmidt ML, Lee VMY, Trojanowski JQ, Jakes R, Goedert M. α -synuclein in Lewy bodies. *Nature*. 1997;388:839-40.
- Stefanis L. α -Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med*. 2012;2:a009399.
- Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: from tank to bedside. *Trends Neurosci*. 2014;37:264-78.
- Strähle U, Scholz S, Geisler R, Greiner P, Hollert H, Rastegar S, et al. Zebrafish embryos as an alternative to animal experiments—a commentary on the definition of the onset of protected life stages in animal welfare regulations. *Reprod Toxicol*. 2012;33:128-32.
- Sun Z, Gitler AD. Discovery and characterization of three novel synuclein genes in zebrafish. *Dev Dyn*. 2008;237:2490-5.
- Tagliaferro P, Burke RE. Retrograde axonal degeneration in Parkinson disease. *J Parkinsons Dis*. 2016;6:1-15.
- Tanaka M, Kim YM, Lee G, Junn E, Iwatsubo T, Mouradian MM. Aggregates formed by α -synuclein and synphilin-1 are cytoprotective. *J Biol Chem*. 2004;279:4625-31.
- Tannenbaum J, Bennett BT. Russell and Burch's 3Rs then and now: the need for clarity in definition and purpose. *J Am Assoc Lab Anim Sci*. 2015;54:120-32.
- Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korrell M, et al. Rotenone, paraquat, and Parkinson's disease. *Environ Health Perspect*. 2011;119:866-72.
- Taymans JM, Cookson MR. Mechanisms in dominant parkinsonism: the toxic triangle of LRRK2, α -synuclein, and tau. *BioEssays*. 2010;32:227-35.
- Thawkar BS, Kaur G. Zebrafish as a promising tool for modeling neurotoxin-induced Alzheimer's disease. *Neurotox Res*. 2021;39:949-65.
- Toni M, Cioni C. Fish synucleins: an update. *Mar Drugs*. 2015;13:6665-86.
- Touchman JW, Dehejia A, Chiba-Falek O, Cabin DE, Schwartz JR, Orrison BM, et al. Human and mouse alpha-synuclein genes: comparative genomic sequence analysis and identification of a novel gene regulatory element. *Genome Res*. 2001;11:78-86.
- Tyson T, Steiner JA, Brundin P. Sorting out release, uptake and

- processing of alpha-synuclein during prion-like spread of pathology. *J Neurochem.* 2016;139:275-89.
- Uemura O, Okada Y, Ando H, Guedj M, Higashijima S, Shimazaki T, et al. Comparative functional genomics revealed conservation and diversification of three enhancers of the *isl1* gene for motor and sensory neuron-specific expression. *Dev Biol.* 2005;278:587-606.
- Ugalde CL, Finkelstein DI, Lawson VA, Hill AF. Pathogenic mechanisms of prion protein, amyloid- β and α -synuclein misfolding: the prion concept and neurotoxicity of protein oligomers. *J Neurochem.* 2016;139:162-80.
- Ünal İ, Üstündağ ÜV, Ateş PS, Eğilmezer G, Alturfan AA, Yiğitbaşı T, et al. Rotenone impairs oxidant/antioxidant balance both in brain and intestines in zebrafish. *Int J Neurosci.* 2019;129:363-8.
- Usmani SM, Mempel TR. Chapter 10 - Intravital microscopy. In: Ross BD, Gambhir SS, editors. *Molecular imaging: principles and practice Volume 2.* London: Academic Press; 2021. p. 167-92.
- Vaccaro R, Toni M, Casini A, Vivacqua G, Yu S, D'este L, et al. Localization of α -synuclein in teleost central nervous system: immunohistochemical and Western blot evidence by 3D5 monoclonal antibody in the common carp, *Cyprinus carpio*. *J Comp Neurol.* 2015;523:1095-124.
- Van Den Berge N, Ulusoy A. Animal models of brain-first and body-first Parkinson's disease. *Neurobiol Dis.* 2022;163:105599.
- van Ham TJ, Brady CA, Kalicharan RD, Oosterhof N, Kuipers J, Veenstra-Algra A, et al. Intravital correlated microscopy reveals differential macrophage and microglial dynamics during resolution of neuroinflammation. *Dis Models Mech.* 2014;7:857-69.
- Van Laar VS, Chen J, Zharikov AD, Bai Q, Di Maio R, Dukes AA, et al. α -Synuclein amplifies cytoplasmic peroxide flux and oxidative stress provoked by mitochondrial inhibitors in CNS dopaminergic neurons *in vivo*. *Redox Biol.* 2020;37:101695.
- Varga M. The doctor of delayed publications: the remarkable life of George Streisinger (1927–1984). *Zebrafish.* 2018;15:314-9.
- Vargas KJ, Colosi PL, Girardi E, Park JM, Chandra SS. α -Synuclein facilitates clathrin assembly in synaptic vesicle endocytosis [Internal]. *bioRxiv.* 2021 [cited 2022 Jan 10]. <https://doi.org/10.1101/2020.04.29.069344>
- Hernández-Vargas RH, Fonseca-Ornelas L, López-González I, Riesgo-Escovar J, Zurita M, Reynaud E. Synphilin suppresses α -synuclein neurotoxicity in a Parkinson's disease *Drosophila* model. *Genesis.* 2011;49:392-402.
- Vascellari S, Melis M, Palmas V, Pisanu S, Serra A, Perra D, et al. Clinical phenotypes of Parkinson's disease associate with distinct gut microbiota and metabolome enterotypes. *Biomolecules.* 2021;11:144.
- Vaz RL, Outeiro TF, Ferreira JJ. Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: a systematic review. *Front Neurol.* 2018;9:347.
- Visanji NP, Brotchie JM, Kalia LV, Koprach JB, Tandon A, Watts JC, et al. α -Synuclein-based animal models of Parkinson's disease: challenges and opportunities in a new era. *Trends Neurosci.* 2016;39:750-62.
- Volff JN. Genome evolution and biodiversity in teleost fish. *Heredit.* 2005;94:280-94.
- Wakabayashi K, Tanji K, Mori F, Takahashi H. The Lewy body in Parkinson's disease: molecules implicated in the formation and degradation of α -synuclein aggregates. *Neuropathology.* 2007;27:494-506.
- Wang Y, Liu W, Yang J, Wang F, Sima Y, Zhong Z, et al. Parkinson's disease-like motor and non-motor symptoms in rotenone-treated zebrafish. *Neurotoxicology.* 2017;58:103-9.
- Wang YC, Feng GY, Xia QJ, Hu Y, Xu Y, Xiong L, et al. Knockdown of α -synuclein in cerebral cortex improves neural behavior associated with apoptotic inhibition and neurotrophin expression in spinal cord transected rats. *Apoptosis.* 2016;21:404-20.
- Weston LJ, Cook ZT, Stackhouse TL, Sal MK, Schultz BI, Tobias ZJC, et al. *In vivo* aggregation of presynaptic alpha-synuclein is not influenced by its phosphorylation at serine-129. *Neurobiol Dis.* 2021;152:105291.
- White RM, Sessa A, Burke C, Bowman T, LeBlanc J, Ceol C, et al. Transparent adult zebrafish as a tool for *in vivo* transplantation analysis. *Cell Stem Cell.* 2008;2:183-9.
- Wyatt C, Bartoszek EM, Yaksi E. Methods for studying the zebrafish brain: past, present and future. *Eur J Neurosci.* 2015;42:1746-63.
- Xi Y, Yu M, Godoy R, Hatch G, Poitras L, Ekker M. Transgenic zebrafish expressing green fluorescent protein in dopaminergic neurons of the ventral diencephalon. *Dev Dyn.* 2011;240:2539-47.
- Xu Y, Li K, Qin W, Zhu B, Zhou Z, Shi J, et al. Unraveling the role of hydrogen peroxide in α -synuclein aggregation us-

- ing an ultrasensitive nanoplasmonic probe. *Anal Chem.* 2015;87:1968-73.
- Yang X, Xu S, Qian Y, He X, Chen S, Xiao Q. Hypermethylation of the gene coding for PGC-1 α in peripheral blood leukocytes of patients with Parkinson's disease. *Front Neurosci.* 2020;14:97.
- Yurtsever İ, Üstündağ ÜV, Ünal İ, Ateş PS, Emekli-Alturfan E. Rifampicin decreases neuroinflammation to maintain mitochondrial function and calcium homeostasis in rotenone-treated zebrafish. *Drug Chem Toxicol.* 2020:1-8.
- Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, Ampuero I, et al. The new mutation, E46K, of alpha-synuclein causes Parkinson and Lewy body dementia. *Ann Neurol.* 2004;55:164-73.
- Zhang Z, Peng Q, Huo D, Jiang S, Ma C, Chang H, et al. Melatonin regulates the neurotransmitter secretion disorder induced by caffeine through the microbiota-gut-brain axis in zebrafish (*Danio rerio*). *Front Cell Dev Biol.* 2021;9:678190.
- Zheng B, Liao Z, Locascio JJ, Lesniak KA, Roderick SS, Watt ML, et al. PGC-1 α , a potential therapeutic target for early intervention in Parkinson's disease. *Sci Transl Med.* 2010;2:52ra73.
- Zhu J, Xia R, Liu Z, Shen J, Gong X, Hu Y, et al. Fenvalerate triggers Parkinson-like symptom during zebrafish development through initiation of autophagy and p38 MAPK/mTOR signaling pathway. *Chemosphere.* 2020;243:125336.
- Zou J, Fan YJ, Meng YQ, Xu H, Fan J. An exploratory analysis of γ -synuclein expression in endometrioid endometrial cancer. *BMJ Open.* 2012;2:e000611.