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Dual PINK Mutant and A*β*42-Dependent Lifespan Shorten and Flight Impairment in Transgenic Drosophila Partially Alleviates by a *Lactococcus lactis* Supplemented Diet

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Abstract

Oxidative stress has been strongly related with Parkinson disease (PD) and Alzheimer disease pathogenesis. We determined the effects of *Lactococcus lactis* (LAL) supplementation on the generated loss-of-function mutants of PINK1 B9, an AR-JP-linked gene and A*β*42 induced phenotypes in a *Drosophila melanogaster* model of PD/AD. Enhanced mutant PINK1 B9 and A*β*42 expression in *D. melanogaster* dopaminergic (DA) neurons can curtail lifespan, flight muscle accompanied by locomotive defects and we have observed longevity methods to assay the effects of LAL on *D. melanogaster* survival. Furthermore, flies expressing mutant PINK1 B9 and A*β*42 in their brain fed LAL had up to the two weeks, or 25%, greater median lifespan than those fed a standard sucrose diet. In addition, LAL improved mutant PINK1 B9 and A*β*42-induced flight impairments in the Drosophila wing. Our microscopy analyses revealed that individuals fed LAL had improved atypical ommatidia as well as an increased thirteen percentage of flight ability than those fed a control diet. We propose that LAL, rich in naturally occurring probiotics and antioxidants, promotes the survival of neurons in brain and wing muscle tissues with increased levels of mutant PINK1 B9 and A*β*42 via a protective cell survival mechanism.

Keywords

***Drosophila melanogaster*, *Lactococcus lactis*, PINK1, Lifespan, Ommatidia, A*β*42**

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1. Introduction

Parkinson disease (PD) is the second most neurodegenerative brain disease in the world and is stand comparison with frequency by Alzheimer disease [1]. Current standards of care have serious limitations and largely address only symptom. The majority of PD cases are sporadic; however, the discovery of genes linked to rate familial forms of disease (encoding α-synuclein, parkin, DJ-1, PINK1, and LRRK2) and studies from experimental animal models has provided crucial insights into molecular mechanisms in disease etiology and identified probable targets for therapeutic intervention. Recent reports implicate mitochondria dysfunction, oxidative damage, abnormal protein accumulation and protein phosphorylation as key molecular mechanisms compromising dopamine neuronal function and survival as the underlying cause of etiology in both sporadic and familial PD [2]. The pathological problems of PD include the loss of dopaminergic (DA) neurons in the substatianigra (SN) and presence of intra-neuronal inclusions known as Lewy bodies (LB) in surviving cells. Affected individuals have both motor and non-motor symptoms ranging from bradykinesia, resting tremor, and muscular rigidity to dementia, depression and olfactory dysfunction. Until now believed to be a totally sporadic disease, linkage studies identified PINK1 as the various gene related to PD. The drosophila *PINK*1 gene encodes a polypeptide of 721a.a with a molecular mass of about 80 kDa. Similar to human PINK1, structural analysis of drosophila PINK1 protein also revealed two characteristic motifs: a mitochondrial targeting motif and a serine/threonine kinase domain. The kinase domain exhibited 60% similarity (42% identity) to that of human PINK1. Consistent with the localization of human PINK1, drosophila PINK1 was also found localized in mitochondria [3]. Recent reports revealed that generated PINK1 loss-of-function mutant flies, $PINK1^{D3}$ and $PINK1^{b9}$, as well as revertants ($PINK1^{rv}$) using RNA-mediated interference technique [4]. Collectively, these results represent that loss of PINK induces indirect flight muscle degeneration and mitochondrial impairment.

Oxidative stress (OS) is consistently related with the etiology of PD or AD; however, its role in disease progression remains unclear. A cell undergoes OS when the net balance between the generated reactive oxygen species (ROS) and the available antioxidant defense mechanisms favours the former. Autopsy analysis of PD or AD patient brains certifies higher levels of OS biomarkers like dysfunctional mitochondria, decreased levels of reduced glutathione, and deficiencies in antioxidant enzymes in the SN or hippocampus of affected individuals [5]. Recent report suggests that pluripotent stem cell-derived DA neurons from a PD patient with a SNCA triplication augment α-synuclein and production of H_2O_2 in Aβ42 flies which is a vital step in the oxidative stress caused by the Aβ, are susceptible to OS [6] [7]. These data suggest that the combination of OS and excess PINK, α-synuclein, Tau and Aβ42 may play a pivotal role in the progression of PD and AD.

Alzheimer's disease (AD) is an age-related neurodegenerative disease and the most common cause of dementia. The pathogenesis of AD is yet entirely clear and despite the increasing knowledge regarding the mechanism, no effective disease-modifying therapy is yet available. Recently, many reports have shown to play a pivotal role in the synaptic damage, impairment of homeostasis, inflammation as well as toxicity in relation to AD etiology. Membranes can also be injury by the ROS (reactive oxygen species) that are produced by Aβ aggregates in the presence of metals such as copper, zinc or iron [8]. Subsequent pathophysiological processes include mitochondrial damage [9], phosphor-tau with consequent axonal transport damage and the trigger of cell death [10] [11]. However, until recently it has been impossible to take a global view to ask which biological processes are essential for the induction of the disease and which are downstream consequences of neurotoxicity [12]. Knowing which biological processes are directly involved in initiating AD will allow us to key on those upstream targets that have the greatest therapeutic potential.

Lactococcus lactis (LAL) is an excellent source of dietary antioxidants. The scavenger potential of *Lactococcus lactis* (LAL) in OS and vascular disease has been described and recent studies in drosophila suggest that microbiota may be benefic to individuals suffering from neurodegenerative diseases [13]. Therefore, in this study we describe the protective effects of LAL concentrate on a drosophila Tg model of dual PD/AD. Enhanced lifespan loss and flight impairments caused by directed expression of PINK1b9 and Aβ42 in the DA neurons and brain neurons and developing eye, respectively, is improved by supplementing growth media with LAL.

We have designed a model of AD that is based on the expression of the human Aβ42 in fly neurons by coupling it to an N-terminal secretion signal peptide [14]. The Aβ1-42 but not the Aβ1-40 control accumulates in the brain and results in decreased lifespan and impaired locomotor performance. These phenotypes are more marked in Tg (transgenic) flies expressing the mutant of the Aβ1-42, which causes increased aggregation of Aβ and is responsible for early stage familial AD [15]. Here, we use our *Drosophila* Tg (transgenic) model with combina-

tion with PD and AD can help unravel the role of OS and unveil potential antioxidant therapies in AD and PD that are critical for lifespan loss and flight impairments from dual PINK1b9 and Aβ-mediated neurotoxicity with *Lactococcus lactis* (LAL) supplementation *in vivo*.

2. Materials and Methods

2.1. Fly Stocks and Culture

The *UAS-PINK*1 *b*9 [4] and *UAS-Aβ* [16] flies were generously provided by Professor J.K. Chung (Seoul National University) and Professor G.S. Jo (University of *Kunkuk*), respectively. *GMR-Gal*412 and *UAS-mCD*8-*GFP* flies were obtained from the Bloomington Drosophila Stock Center at Indiana University. The transgenes are each representative of three independent transgenic lines. Flies were maintained at 25˚C in a 12:12 light:dark period on a standard cornmeal-yeast-molasses-agar medium (65 g/L cornmeal, 15 g/L nutritional yeast extract, 5.5 g/L agar in water supplemented with 0.1 g/ml methyl 4-hydroxybensoate in ethanol and 2.5 ml propionic acid per L of medium).

2.2. Longevity Assays

Longevity assays in the secondary screen and flies were reared on either 0.25% sucrose or *Lactococcus lactis* (LAL, Korean Collection for Type Cultures, Korea) supplemented medium then collected under gaseous CO_2 every 24 hours until a minimum of 100 adult females of each genotype were obtained. Briefly, flies were reared on either 0.25% sucrose or 5×10^5/100 µl or 5×10^8/100 µl cell number of LAL with 0.25% sucrose supplemented Tomato Juice medium (skim milk 100 g, tomato juice extract 100 ml, yeast extract 5 g, with distilled water 1000 ml) then collected under gaseous CO_2 every 24 hours until a minimum of 50 adult males of each genotype were obtained. Live flies were counted and their food changed on days 1, 3 and 5 of a 7 day cycle. For assessing the efficacy of *Lactococcus lactis* (LAL, isolated from w^{1118}) was dissolved in PBS and the solution added to fly food to give a final concentration of 5% v/v. Survival curves were plotted using the Kaplan-Meier estimator. The statistical significance was calculated using the log rank test within the SPSS 11.0 statistical package. The null hypothesis in all of the longevity assays was that the presence of the PINK1b9 made no difference to the longevity of the flies expressing the Aβ1-42 transgene.

Selected flies were then transferred to upright standard plastic shell vials containing the above food medium they were initially exposed to. Each group was maintained at 25˚C and kept in non-crowded conditions (1 to 20 individuals per vial). Flies were scored for viability every 2 days and transferred to fresh medium without anaesthesia according to established protocol [17].

2.3. Behavioural Assays

For the determine of climbing speed, groups of ten 3-day-old females were driven into 18-cm-long vials and incubated for 1 h at room temperature for environmental adaptation. After tapping the flies completely down to the bottom, we marked their climbing time at the 15-cm finish line when more than five flies had arrived. Five trials were performed for each group and repeated with four different groups. The average climbing time (±S.D) was calculated for each genotype. Flight assay was performed as previously described [18] with 3-day-old males ($n > 100$).

2.4. Scanning Electron Microscopy Analysis

Surviving flies were preserved at −80˚C before being mounted on metal studs under a dissecting microscope. Prepared fries were desiccated overnight and gold coated prior to photography at 200 times magnification with a JSM-7610F (JEOL, USA Inc.) scanning electron microscope as per standard methods.

Preparation of drosophila tissue for SEM analysis was determined as following: ten adult flies were pre-fixed for 2 h, at 4˚C, in fresh phosphate-buffered saline (PBS) containing 2.5% glutaraldehyde solution (pH7.4). The specimens were subsequently washed twice, for 20 min each time, in phosphate-buffered sucrose (4%). Tissue dehydration was attained through a graded ethanol series (75%, 80%, 90%, 95% and 100%). Samples were finally subjected to a critical point drying procedure, attached on aluminum stubs, coated with gold in a sputter-coating apparatus (Tousimis, Rockville, Maryland, USA), for 2 min, and visualized under a JSM-7610F Scan-

ning Electron Microscope (JEOL, USA Inc.). For each fly double transgenic 50 eye images, from at least three independent crosses were thoroughly determined. An oval with an area between 3×10^3 - 4.5×10^3 μm^2 was overlaid on the flattest portion of each focused eye with Paintbrush version 2.1.1 for Mac OS X (Soggy Waffles). The external and surface structural organization of each (double transgenic) adult fly eye was examined through a Scanning Electron Microscopy (SEM) approach [19].

3. Results

3.1. Increased Concentration of LAL Protect against PINK11b9 and A*β*42-Induced Initially Mortality

Here we study a reduced lifespan in flies when of PINK1b9 and A*β*42 expression is enhanced in the brain neurons (**Figure 1**). The median survival time of PINK1b9 and A*β*42-expressing flies was reduced by 45% compared to wild type when both groups were fed a control diet. A diet rich in LAL partially rescued the reduced lifespan caused by increased neuronal amounts of PINK1b9 and A*β*42 in D. melanogaster (**Figure 1**). PINK1b9 and A*β*42-expressing flies fed a diet containing thirteen percentage of 5×10^8/100 μl LAL had a 7-day greater median lifespan than those fed a control diet. A concentration of 5×10^5/100 μl LAL dose was slightly observed the survival ratio with PINK1b9 and A*β*42-expressing flies. The median survival values for each group are found in **Table 1**.

3.2. LAL Ameliorates PINK1b9 and A*β*42-Induced Developmental Defects in the Drosophila Eye

A rough external eye phenotype occurs when GMR-Gal4 is used to drive expression of mutant PINK1b9 and A*β*42 in the developing drosophila eye (**Figure 2**). LAL supplementation reduces mean PINK1b9 and A*β*42-induced defects to control levels (**Figure 2**). The mean disruption of mutant PINK1b9 and A*β*42-expressing flies fed a control diet was 42% of the analyzed area. In PINK1b9 and A*β*42-expressing flies, the mean disruption was reduced from 65% for those fed a control diet to 20% and 9% for flies fed 5×10^5/100 μl and 5×10^8/100 μl LAL, respectively. This provides another example of LAL-induced protection in a *Drosophila* tissue that is rich in neurons.

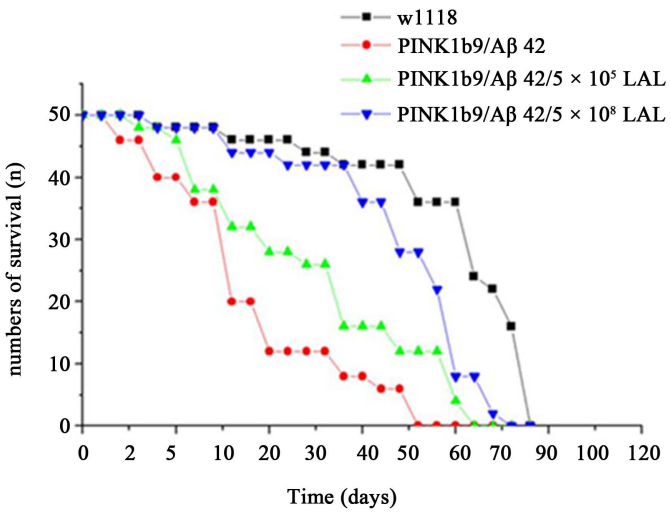

Figure 1. LAL partially rescues Drosophila melanogaster against mutant PINK1b9 and A*β*42-induced initially mortality. Directed expression of mutant PINK1 b9 and A*β*42 (n = 48) in DA neurons shortens lifespan in Drosophila fed a standard diet, as compared to a wild type ($p < 0.05$). Flies fed diets containing 5×10^8 LAL (n = 41) were partially protected against the mutant PINK1 b9 and A*β*42-induced mortality ($p < 0.05$), whereas 5×10^5 LAL (n = 24) had slightly significant effect. Genotypes are w^{1118}; UAS-PINK1/A*β*42/GMR-Gal4 (control) and UAS-PINK1/A*β*42/GMR-Gal4 with LAL supplementation. Errors bars indicate standard error of the mean. P-values were counted by the log-rank (Mantel-Cox) test and multiple comparisons were corrected for using Bonferroni method.

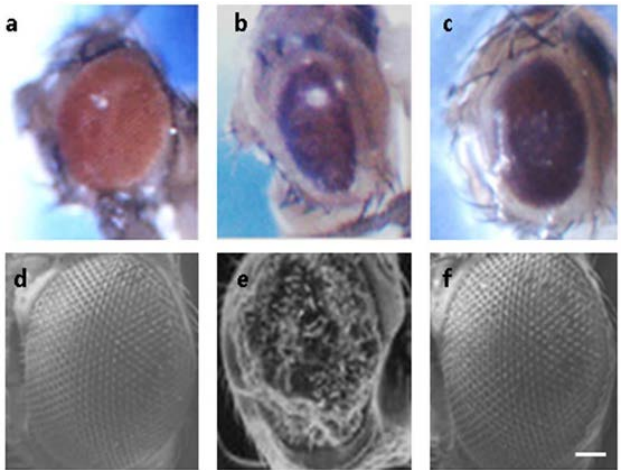

Figure 2. LAL supplementation counteracts mutant PINK1b9 and Aβ42-induced developmental defects of the Drosophila eye. Overexpression of mutant PINK1b9 and Aβ42 during initially eye development observes a rough external eye morphology. Flies supplemented with LAL have suspension levels comparable to control. Stereo-microscope images ((a), (b), (c)) and TEM images ((d), (e), (f)). Left row; w^{1118}, middle row; PINK1 b9/Aβ42 flies, right row, PINK1 b9/Aβ42 flies with LAL supplementation. The eye of a wild-type flies is composed of a regular array ommatidia and Drosophila compound eye morphology organization in mutant PINK1 b9 and Aβ42 double transgenic fly eyes, carrying reduced eye sizes. Scale Bars: 50 μm.

Table 1. Median survival times of transgenic Drosophila melanogaster cultivated on either a control or LAL-supplemented diet.

Genotype (food medium)	Mean survival (days)
w^{1118}	80
UAS-PINK1/Aβ42/GMR-Gal4	52
UAS-PINK1/Aβ42/GMR-Gal4 with LAL (5×10^5)	64**
UAS-PINK1/Aβ42/GMR-Gal4 with LAL (5×10^8)	73**

**$p < 0.05$, flies supplemented with LAL have suspension levels comparable to control. **Represents $p < 0.05$. Genotype are w^{1118}; *UAS-PINK1/Aβ/GMR-Gal4* (control) and *UAS-PINK1/Aβ/GMR-Gal4* with LAL treatment.

3.3. LAL Suppresses PINK1b9 and Aβ42-Induced Flight Impairments in the Drosophila Wing

To confirm further mitochondria impairment in the muscles, we analyzed quality of mitochondria abundance in the mutants by flight ability. As expected, they showed complete defects in flight ability and these phenotypes were significantly rescued by LAL supplementation. Compared to the w^{1118} flies, PINK1b9 mutants and Aβ42 showed a more than approximately ninety-eight fold reduction in w^{1118} flies, and this was markedly restored by LAL supplementation (**Figure 3**). The BCL-2 protein family determines the commitment of cells to apoptosis, an ancient cell suicide programming genes that is essential for development, tissue homeostasis and immunity. Because substantial results represented that anti-apoptotic Bcl-2 families are involved in the protection of mitochondrial integrity and function [20] [21]. Collectively, these results demonstrate that mutant of PINK1 and Aβ42 expression induces indirect flight muscle degeneration and mitochondria impairment. Overall, these results strongly suggest that mitochondrial dysfunction is the main cause of these aberrant phenotypes of PINK1 mutants and Aβ42 expression.

4. Discussion

Recent reports evidence has suggested that a diet rich in oriental medicine may help delay the Aβ-related degeneration of fly [16]. In other animal study, oriental medicine supplementation improved memory function in older mouse with early memory loss [22]. Moreover, it has been demonstrated that short-term LMK02 supple-

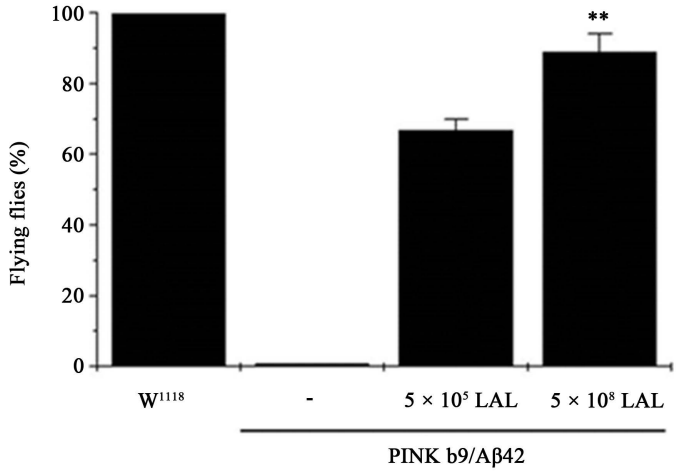

Figure 3. Comparison of flight ability between w^{1118} and PINK1 b9 and Aβ42 flies. Error bars indicate mean ± S.D. N = 25 flies for each group without or with ×LAL supplementation.

mentation increased calbindin-mediated protection against inflammation in aged mice hippocampal neuronal cells [23]. Also, Probiotic Dahi with Lactobacillus acidophilus and *Bifidobacterium bifidum* supplementation alleviates age-oxidative stress and improves expression of biomarkers of aged mice [24]. These reports suggest that the protective effects of oriental medicine or probiotic Lactobacillus sp. are not confirmed to drosophila melanogaster and may extend to mammals.

The free radical/oxidative stress theory of ageing originated in the 1950's and suggests that an organism ages due, in part, to the accumulation of free radical-induced damage to cellular macromolecules. Previous reports have shown that probiotic microbiota supplementation can extend lifespan in a *Caenorhabditis elegans* [25] and drosophila melanogaster [26]. Our results are novel as we have shown that LAL supplementation can extend lifespan and flight ability in a drosophila model of a neurodegenerative disease. The additional antioxidants provided by LAL supplementation may alleviate some of the excess ROS generated during the progression of AD/PD-like cell death resulting in less cellular injury and a longer median survival time in affected flies.

5. Conclusions

Here we advert the first demonstration of the protective effects of LAL in a drosophila model of PINK1b9 and Aβ42-PD/AD. Previous findings have demonstrated that oriental medicine improved both life span and fly eye in a similar drosophila model of AD [16]. Taken together, these results epitomize the value of using drosophila to study PD or AD pathogenesis. Though the literature is relatively new, studies in this versatile organism have helped develop interest in the potential protective effects of dietary antioxidants in medical research. Future studies could aim towards unraveling the interaction of dietary antioxidants, probiotic and the activity of mitochondria biogenesis, such as the ROS and enzymatic antioxidant pathways.

The drosophila compound eye and wing consist of multiple subunits, muscles or ommatidia, composed of several neurons and wing cells. Directed expression of PINK1b9 and Aβ42 during early developmental results is premature degeneration of the retina and muscle with abnormal development of external morphology [4]. Mitochondria are the major intracellular source of reactive oxygen species (ROS). While excessive mitochondrial ROS (mito-ROS) production induces cell injury and death, there is augmenting evidence that non-toxic low levels of mito-ROS could serve as important signaling molecules. Therefore, maintenance of mito-ROS at physiological levels is crucial for cell homeostasis as well as for survival and proliferation. Bcl-2 is to protect the integrity of mitochondrial oxidative phosphorylation and thus limit the mitochondrial dysfunction that is induced by several stimuli of apoptosis. Therefore, our results suggest that a diet containing LAL protects neurons in the eye and wing against PINK1b9 and Aβ42-dependent developmental defects. Both the morphology of atypical ommatidia and wings were improved in flies fed a LAL-supplemental diet. In human, the park in and PINK1 gene encodes an E3 ubiquitin ligase and expression of mitochondrial park in and PINK1 in drosophila eyes and wing muscle suppresses RNAi-induced retinal and wing muscle degeneration in mutant PINK1 flies [4]. Mito-

chondrial PINK1 is a point of major interest in AD/PD pathogenesis and the protective effects of LAL on PINK1b9 and Aβ42-induced injury in the drosophila eye may be due in part to enhanced activity of the mitochondria biogenesis.

Our finding may be referred to a fortified overall antioxidant defense mechanism in PINK1b9 and Aβ42-expressing flies. LAL can increase the expression of enteric probiotic defense molecules in *D. melanogaster*. Additionally, LAL extends lifespan and partially protects PINK1b9 and Aβ42-induced flies under conditions of increased oxidative stress and impairment of flight ability. Similar reports have been represented for several other foods high in dietary antioxidants, including extracts of plant polyphenols [27]-[29], Green Tea Catechin [30] and Black Tea [31]. Although dietary antioxidants likely provide an invaluable secondary support to cells suffering oxidative stress, it appears that their defective effects are dependent on extrinsic/intrinsic antioxidant protective systems.

References

[1] Yoon, J.H., Lee, J.E., Yong, S.W., Moon, S.Y. and Lee, P.H. (2014) The Mild Cognitive Impairment Stage of Dementia with Lewy Bodies and Parkinson Disease: A Comparison of Cognitive Profiles. *Alzheimer Disease and Associated Disorders*, **28**, 151-155. http://dx.doi.org/10.1097/WAD.0000000000000007

[2] Gautier, C.A., Corti, O. and Brice, A. (2014) Mitochondrial Dysfunctions in Parkinson's Disease. *Revista de Neurología*, **170**, 339-343. http://dx.doi.org/10.1016/j.neurol.2013.06.003

[3] Valente, E.M., *et al*. (2004) Hereditary Early-Onset Parkinson's Disease Caused by Mutations in PINK1. *Science*, **304**, 1158-1160. http://dx.doi.org/10.1126/science.1096284

[4] Park, J., Lee, S.B., Lee, S., Kim, Y., Song, S., Kim, S., Bae, E., Kim, J., Shong, M., Kim, J.M. and Chung, J. (2006) Mitochondrial Dysfunction in Drosophila PINK1 Mutants Is Complemented by Parkin. *Nature*, **441**, 1157-1161. http://dx.doi.org/10.1038/nature04788

[5] Schapira, A.H. and Jenner, P. (2011) Etiology and Pathogenesis of Parkinson's Disease. *Movement Disorders*, **26**, 1049-1055. http://dx.doi.org/10.1002/mds.23732

[6] Byers, B., Cord, B., Nguyen, H.N., Schüle, B., Fenno, L., Lee, P.C., Deisseroth, K., Langston, J.W., Pera, R.R. and Palmer, T.D. (2011) SNCA Triplication Parkinson's Patient's iPSC-Derived DA Neurons Accumulate *α-Synuclein* and Are Susceptible to Oxidative Stress. *PLoS ONE*, **6**, e26159. http://dx.doi.org/10.1371/journal.pone.0026159

[7] Huang, X., Atwood, C.S., Hartshorn, M.A., Multhaup, G., Goldstein, L.E., Scarpa, R.C., Cuajungco, M.P., Gray, D.N., Lim, J., Moir, R.D., Tanzi, R.E. and Bush, A.I. (1999) The A Beta Peptide of Alzheimer's Disease Directly Produces Hydrogen Peroxide through Metal Ion Reduction. *Biochemistry*, **38**, 7609-7616. http://dx.doi.org/10.1021/bi990438f

[8] Bush, A.I. (2003) Themetallobiology of Alzheimer's Disease. *Trends in Neurosciences*, **26**, 207-214. http://dx.doi.org/10.1016/S0166-2236(03)00067-5

[9] Abramov, A.Y., Canevari, L. and Duchen, M.R. (2004) Beta-Amyloid Peptides Induce Mitochondrial Dysfunction and Oxidative Stress in Astrocytes and Death of Neurons through Activation of NADPH Oxidase. *Journal of Neuroscience*, **24**, 565-575. http://dx.doi.org/10.1523/JNEUROSCI.4042-03.2004

[10] Kienlen-Campard, P., Miolet, S., Tasiaux, B. and Octave, J.N. (2002) Intracellular Amyloid-Beta 1-42, but Not Extracellular Soluble Amyloid-Beta Peptides, Induces Neuronal Apoptosis. *Journal of Biological Chemistry*, **277**, 5666-5670. http://dx.doi.org/10.1074/jbc.M200887200

[11] Wei, W., Norton, D.D., Wang, X. and Kusiak, J.W. (2002) Abeta 17-42 in Alzheimer's Disease Activates JNK and Caspase-8 Leading to Neuronal Apoptosis. *Brain*, **125**, 2036-2043. http://dx.doi.org/10.1093/brain/awf205

[12] Cao, W., Song, H.J., Gangi, T., Kelkar, A., Antani, I., Garza, D. and Konsolaki, M. (2008) Identification of Novel Genes That Modify Phenotypes Induced by Alzheimer's β-Amyloid Overexpression in Drosophila. *Genetics*, **178**, 1457-1471. http://dx.doi.org/10.1534/genetics.107.078394

[13] Linder, J.E. and Promislow, D.E. (2009) Cross-Generational Fitness Effects of Infection in *Drosophila melanogaster*. *Fly*, **3**, 143-150. http://dx.doi.org/10.4161/fly.8051

[14] Crowther, D.C., Kinghorn, K.J., Miranda, E., Page, R., Curry, J.A., Duthie, F.A., Gubb, D.C. and Lomas, D.A. (2005) Intraneuronal Aβ, Non-Amyloid Aggregates and Neurodegeneration in a Drosophila Model of Alzheimer's Disease. *Neuroscience*, **132**, 123-135. http://dx.doi.org/10.1016/j.neuroscience.2004.12.025

[15] Nilsberth, C., Westlind-Danielsson, A., Eckman, C.B., Condron, M.M., Axelman, K., Forsell, C., Stenh, C., Luthman, J., Teplow, D.B., Younkin, S.G., Naslund, J. and Lannfelt, L. (2001) The "Arctic" APP Mutation (E693G) Causes Alzheimer's Disease by Enhanced Aβ Protofibril Formation. *Nature Neuroscience*, **4**, 887-893. http://dx.doi.org/10.1038/nn0901-887

[16] Hong, Y.K., Lee, S., Park, S.H., Lee, J.H., Han, S.Y., Kim, S.T., Kim, Y.K., Jeon, S., Koo, B.S. and Cho, K.S. (2012) Inhibition of JNK/dFOXO Pathway and Caspases Rescues Neurological Impairments in Drosophila Alzheimer's Disease Model. *Biochemical and Biophysical Research Communications*, **419**, 49-53. http://dx.doi.org/10.1016/j.bbrc.2012.01.122

[17] Abramoff, M.D., Magalhaes, P.J. and Ram, S.J. (2004) Image Processing with Image. *Biophotonics International*, **11**, 36-42.

[18] Pesah, Y., Pham, T., Burgess, H., Middlebrooks, B., Verstreken, P., Zhou, Y., *et al*. ((2004) Drosophila Parkin Mutants Have Decreased Mass and Cell Size and Increased Sensitivity to Oxygen Radical Stress. *Development*, **131**, 2183-2194. http://dx.doi.org/10.1242/dev.01095

[19] Leulier, F., Ribeiro, P.S., Palmer, E., Tenev, T., Takahashi, K., Robertson, D., *et al*. (2006) Systematic *in Vivo* RNAi Analysis of Putative Components of the Drosophila Cell Death Machinery. *Cell Death and Differentiation*, **13**, 1663-1674. http://dx.doi.org/10.1038/sj.cdd.4401868

[20] Vander Heiden, M.G., Chandel, N.S., Williamson, E.K., Schumacker, P.T. and Thompson, C.B. (1997) Bcl-xL Regulates the Membrane Potential and Volume Homeostasis of Mitochondria. *Cell*, **91**, 627-637. http://dx.doi.org/10.1016/S0092-8674(00)80450-X

[21] Vander Heiden, M.G. and Thompson, C.B. (1999) Bcl-2 Proteins: Regulators of Apoptosis or of Mitochondrial Homeostasis? *Nature Cell Biology*, **1**, E209-E216. http://dx.doi.org/10.1038/70237

[22] Seo, J.S., Jung, E.Y., Kim, J.H., Lyu, Y.S., Han, P.L. and Kang, H.W. (2010) A Modified Preparation (LMK03) of the Oriental Medicine Jangwonhwan Reduces A$\beta_{1\text{-}42}$ Level in the Brain of Tg-APPswe/PS1dE9 Mouse Model of Alzheimer Disease. *Journal of Ethnopharmacology*, **130**, 578-585. http://dx.doi.org/10.1016/j.jep.2010.05.055

[23] Seo, J.S., Yun, J.H., Baek, I.S., Leem, Y.H., Kang, H.W., Cho, H.K., Lyu, Y.S., Son, H.J. and Han, P.L. (2010) Oriental Medicine Jangwonhwan Reduces A$\beta_{1\text{-}42}$ Level and Beta-Amyloid Deposition in the Brain of Tg-APPswe/PS1dE9 Mouse Model of Alzheimer Disease. *Journal of Ethnopharmacology*, **128**, 206-212. http://dx.doi.org/10.1016/j.jep.2010.01.014

[24] Kaushal, D. and Kansal, V.K. (2012) Probiotic Dahi Containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum* Alleviates Age-Inflicted Oxidative Stress and Improves Expression of Biomarkers of Ageing in Mice. *Molecular Biology Reports*, **39**, 1791-1799. http://dx.doi.org/10.1007/s11033-011-0920-1

[25] Grompone, G., Martorell, P., Llopis, S., González, N., Genovés, S., Mulet, A.P., Fernández-Calero, T., Tiscornia, I., Bollati-Fogolín, M., Chambaud, I., Foligné, B., Montserrat, A. and Ramón, D. (2012) Anti-Inflammatory *Lactobacillus rhamnosus* CNCM I-3690 Strain Protects against Oxidative Stress and Increases Lifespan in *Caenorhabditis elegans*. *PLoS ONE*, **12**, e52493. http://dx.doi.org/10.1371/journal.pone.0052493

[26] Lee, K.A. and Lee, W.J. (2014) Drosophila as a Model for Intestinal Dysbiosis and Chronic Inflammatory Diseases. *Developmental & Comparative Immunology*, **42**, 102-110. http://dx.doi.org/10.1016/j.dci.2013.05.005

[27] Schriner, S.E., Katoozi, N.S., Pham, K.Q., Gazarian, M., Zarban, A. and Jafari, M. (2012) Extension of *Drosophila lifespan* by *Rosa damascena* Associated with an Increased Sensitivity to Heat. *Biogerontology*, **13**, 105-117. http://dx.doi.org/10.1007/s10522-011-9357-0

[28] Peng, C., Chan, H.Y., Huang, Y., Yu, H. and Chen, Z.Y. (2011) Apple Polyphenols Extend the Mean Lifespan of *Drosophila melanogaster*. *Journal of Agricultural and Food Chemistry*, **59**, 2097-2106. http://dx.doi.org/10.1021/jf1046267

[29] Long, J., Gao, H., Sun, L., Liu, J. and Zhao-Wilson, X. (2009) Grape Extract Protects Mitochondria from Oxidative Damage and Improves Locomotor Dysfunction and Extends Lifespan in a Drosophila Parkinson's Disease Model. *Rejuvenation Research*, **12**, 321-331. http://dx.doi.org/10.1089/rej.2009.0877

[30] Li, Y.M., Chan, H.Y., Huang, Y. and Chen, Z.Y. (2007) Green Tea Catechins Upregulate Superoxide Dismutase and Catalase in Fruit Flies. *Molecular Nutrition & Food Research*, **51**, 546-554. http://dx.doi.org/10.1002/mnfr.200600238

[31] Peng, C., Chan, H.Y., Li, Y.M., Huang, Y. and Chen, Z.Y. (2009) Black Tea Theaflavins Extend the Lifespan of Fruit Flies. *Experimental Gerontology*, **44**, 773-783. http://dx.doi.org/10.1016/j.exger.2009.09.004