HIGHLIGHTS

In search for novel strategies towards neuroprotection and neuroregeneration: is PPARα a promising therapeutic target?

Peroxisome proliferator activated receptors: In the early 1990s, seminal work on rodent liver demonstrated that the hypolipidemic effect of xenobiotics, referred to as peroxisome proliferators, was mediated by a member of steroid hormone receptor superfamily, thus designated peroxisome proliferator-activated receptors (PPARs) (Issemann and Green, 1990; Dreyer et al., 1992). The research field opened by this discovery led to the identification of three isotypes, namely PPARα (NR1C1), PPARβ/δ (NR1C2), PPARγ (NR1C3), in a wide range of tissues. All these receptors act as ligand-activated transcription factors, binding lipid molecules with different, though overlapping, specificity.

PPARs, as other members of the nuclear receptor superfamily, comprise four domains - one of which binds to specific DNA sequences (PPAR response elements, PPREs) - regulating gene expression as heterodimers with retinoid X receptors (RXRs). PPAR activity is modulated by post-translational modifications, such as phosphorylation, SUMOylation, ubiquitylation, and by several corepressors and coactivators (Feige et al., 2006).

It is now well established that PPARs act as lipid sensors, playing a major role in energy homeostasis, lipid metabolism and ROS production/scavenging, thus being involved in key cell processes, including cell proliferation, death and differentiation. These receptors are regulators of oxidative stress, inflammation and immune response, making them a suitable target for the treatment of chronic inflammatory diseases, diabetes, cancer and neurodegenerative disorders (Feige et al., 2006).

PPARs in the brain: In the nervous tissue, the presence of PPARs has been thoroughly described. *In situ* studies have highlighted differential distribution of the three isotypes in the central nervous system (CNS), during pre- and postnatal development, in the adult, and in the course of aging (reviewed by Fidaleo et al., 2014). The expression of PPARs has also been analyzed *in vitro*, demonstrating the presence in all CNS cell types, namely neurons, astrocytes, oligodendrocytes, microglia, as well as in neural stem cells and in cell lines, including neuroblastoma and glioblastoma (Fidaleo et al., 2014). A systematic quantitative and anatomical expression atlas of nuclear receptors, including PPARs in the adult mouse brain has been accomplished (Gofflot et al., 2007). The possibility to cluster these data into anatomical and regulatory networks opens new perspectives towards the understanding of their functions in the brain. As a matter of fact, the roles played by PPARs in the brain are incompletely understood, especially in relation to the isotype-specific mechanisms of action. On the other hand, the largely overlapping action of the three receptors, recently synthesized by Aleshin and co-workers in the "PPAR triad"concept (Fidaleo et al., 2014) should prompt the researchers to equally take into consideration all the isotypes, when studying physiological or pathological models. Despite this notion, the vast majority of investigations has so far dealt with PPARγ and its agonists, while relatively few studies have addressed the role of PPARα and β/δ.

In this perspective, we will focus on PPARα, with special reference to its potential as a therapeutic target against neurodegeneration. Distribution of the receptor in normal and pathological CNS will be briefly reviewed and related with that of its endogenous ligands, in order to provide some insights into the specific roles played in the nervous tissue. We will then discuss established neuroprotective properties of PPARα ligands, as assessed by *in vivo* and *in vitro* models, and their use as stimulators of neurogenesis and neuroregeneration.

Physiological role of PPARα and its ligands in the brain: The expression of PPARα and its heterodimeric partner RXRα in different brain areas has been rather extensively investigated at mRNA and protein levels while information on the localization of PPARα endogenous ligands is still limited (Fidaleo et al., 2014). Recognised PPARα natural agonists include saturated and unsaturated fatty acids and their metabolites, derived by either catabolic (*e.g.*, intermediates of acyl-CoA β-oxidation pathway and some eicosanoids), or neosynthetic (fatty acid synthase-derived fatty acids) pathways (Chakravarthy et al., 2007; Fidaleo et al., 2014). Interestingly, the endocannabinoid-like molecules oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) have recently been identified as high affinity PPARα ligands, their synthesis being even enhanced by the activated receptor (Fidaleo et al., 2014). Consistent with the functional relationship linking OEA and PEA with PPARα, qualitatively and quantitatively similar patterns of these molecules are found in selected brain regions (*e.g.*, neocortex, hippocampus, thalamus, amygdala, hypothalamus, substantia nigra, cranial motor nuclei), involved in diverse behaviors (*i.e.*, feeding, motor control, reward system, sleep, memory consolidation). For example, *ex vivo* studies point to a role for PPARα signaling pathway in motor control, as well as in emotional and cognitive brain functions, by negatively modulating nicotine-induced excitation of dopamine neurons in mesocorticolimbic and mesostriatal systems (Melis et al., 2013). Also, investigations employing PPARα null mice demonstrated its involvement in spatial learning and memory, through regulation of cyclic AMP response element binding (CREB) and hippocampal plasticity-related genes (Roy et al., 2013).

Further clues toward the understanding of PPARα function in the brain are provided by studies on the expression of its target genes in different brain regions and neuronal/glial cell populations. Overlapping localization of PPARα with catalase (CAT), superoxide dismutase 1 (SOD1) and acyl-CoA oxidase 1 (ACOX1) has been described by our group (Fidaleo et al., 2014), in keeping with the notion that the expression of these genes is regulated by PPARα. These studies

strongly support a CNS role of the receptor in neuroprotection against oxidative damage, by controlling superoxide anion removal (by SOD1), and hydrogen peroxide generation (by SOD1 and ACOX1) and removal (by CAT). Moreover, the involvement of PPARα in brain lipid metabolism is witnessed both by its regulation by fatty acid synthase-produced fatty acids (Chakravarthy et al., 2007), and by its inducing activity towards ACOX1, the rate limiting enzyme of peroxisomal fatty acyl β-oxidation system, and other lipid-regulatory molecules, such as Niemann-Pick disease type 1 C protein, involved in cholesterol trafficking (Chinetti-Gbaguidi et al., 2005). Noteworthy, ACOX1 is not only involved in lipid metabolism, but also leads to the production of acetyl-CoA moieties, necessary for acetylcholine (ACh) synthesis. Thus, in the CNS, PPARα modulates neurotransmission by participating in the synthesis of signaling molecules (H_2O_2, lipids, ACh).

The role of PPARα in neural cells other than neurons should not be underrated. Past work from our group documented the presence of PPARα in astroglia, whose essential role in maintaining proper CNS functioning is well recognized. We found increased expression of PPARα and RXRα in astrocytes differentiating from neural progenitors/stem cells, suggesting a role for PPARα in acquiring the metabolic features characterizing astroglial differentiation (Fidaleo et al., 2014). Strong specific expression of PPARα in ependymal cells has been reported in normal and regenerating CNS, while little is known about the physiological role of the receptor in oligodendroglia and microglia (Fidaleo et al., 2014). Nevertheless, an involvement of PPARα in microglial and astroglial activation in response to neurotoxic stimuli is well documented. Indeed, a concerted anti-inflammatory action of PPAR isotypes has been reported after different physical or chemical insults in both *in vitro* and *in vivo* models (Fidaleo et al., 2014). The specific mechanism whereby ligand-activated PPARα blunts inflammation seems to involve non-genomic actions (tethering and/or squelching), leading to inhibition of other pro-inflammatory transcription factors (Feige et al., 2006).

PPARα as a therapeutic target for neuroprotection and neuroregeneration: The concept that PPARα activity modulates the redox state and neuroinflammation represents the basis for novel therapies against acute and chronic CNS diseases. Indeed, the different neuropathologies, while affecting select neural cell populations and featuring specific pathogenetic mechanisms, all share as common traits oxidative stress and neuroinflammation. Thus, targeting PPARα may result in beneficial effects in a wide array of neuroinflammatory, neurodegenerative and neuropsychiatric conditions (**Figure 1**), and even in normal brain aging.

Several *in vivo* experimental models, mimicking human acute pathologies (brain ischemia, traumatic brain injury, whole brain irradiation, LPS-induced neuroinflammation, viral encephalitis, seizures) or chronic progressive neurodegenerative diseases (Alzheimer's disease, Parkinson's disease, Huntington's disease, frontotemporal lobar degeneration, multiple sclerosis), or even neuropsychiatric disorders (schizophrenia, epilepsy) have been so far investigated, testing natural or synthetic PPARα ligands (reviewed by Fidaleo et al., 2014). These include OEA, PEA, the resveratrol derivative pterostilbene, diverse fibrates (fenofibrate, clofibrate, bezafibrate, gemfibrozyl), as well as other structurally unrelated chemicals (Wy-14643, GW7647 and T33). Irrespective of their chemical structures, all PPARα agonists exert neuroprotective properties, in that they reduce brain damage, neurovascular impairment, inflammation, and oxidative stress, resulting in overall amelioration of behavioral symptoms and neuropathological lesions (**Figure 1**) (Fidaleo et al., 2014).

For example, consistent and recent *in vivo* evidence shows that fenofibrate (i) prevents the short-term motor and cognitive poststroke consequences in mice (Ouk et al., 2014); (ii) protects against hypolocomotion, depressive-like behavior, impairment of learning and memory, and dopaminergic neurodegeneration in MPTP rat models of Parkinson's disease (Barbiero et al., 2014); (iii) reduces cognitive alterations in a neurodevelopmental rat model of schizophrenia, by ameliorating prepulse inhibition disruption (Rolland et al., 2012); (iv) reduces β-amyloid production in an Alzheimer's disease transgenic mouse model (Zhang et al., 2014); (v) reduces or abolishes behavioral and electroencephalographic expressions of nicotine-induced seizures, in a mouse model of epilepsy (Puligheddu et al., 2013).

Similarly to synthetic PPARα agonists, the exogenous administration of naturally occurring molecule PEA (i) ameliorates motor limb function in spinal cord trauma; (ii) reduces infarct size, after transient middle cerebral artery occlusion; (iii) exerts antinociceptive effects associated with changes in thermoceptive threshold; (iv) reverses motor deficits in the MPTP model of Parkinson's disease; (v) protects against β-amyloid-induced learning and memory impairment (all reviewed by Fidaleo et al., 2014). Importantly, the primary involvement of PPARα in mediating the effects of several natural and synthetic substances has been validated by the concomitant treatment of *PPARα* null mice. Nevertheless, secondary participation of other signalling pathways, activated by other PPAR isotypes and/or endocannabinoid receptors cannot be ruled out.

At the cellular level, beneficial effects may rely on the direct or indirect action of the receptor towards different classes of organelles. The biogenesis and/or functionality of mitochondria, peroxisomes and even lysosomes are indeed regulated by PPARα and its cofactors (particularly PGC1α), in cooperation with other nuclear receptors (either partners or not for PPARα) (Fidaleo et al., 2014; Ghosh et al., 2015). Interestingly, PPARα agonists exert pro-survival action not only by protecting neuronal cells against neurotoxic insults, but also directly suppressing cell death, lowering the levels of pro-apoptotic molecules, namely activated caspase 3 and apoptosis inducing factor (Fidaleo et al., 2014). This effect is in agreement with the demonstrated control of cell cycle operated by PPARα in several tissues. Moreover, growing evidence suggests a role for activated PPARα in promoting autophagy, as a survival mechanism under stress conditions, in cooperation with other nuclear receptors (Lee et al., 2014).

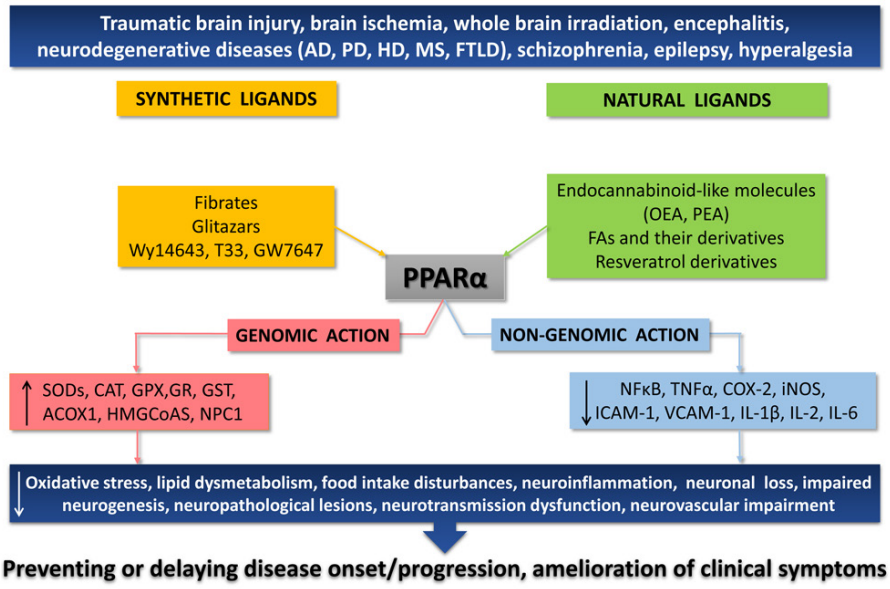

Figure 1 Neuroprotective effects of PPARα agonists.
PPARα: Peroxisome proliferator-activated receptor α; ACOX1: Acyl-CoA oxidase 1; AD: Alzheimer's disease; CAT: catalase; COX-2: cyclooxygenase-2; FAs: fatty acids; FTLD: frontotemporal lobar degeneration; GPX: glutathione peroxidase; GR: glutathione reductase; GST: glutathione S-transferase; HD: Huntington's disease; HMGCoAS: 3-hydroxy-3-methylglutaryl-CoA synthase; ICAM-1: intracellular adhesion molecule-1; IL: interleukin; iNOS: inducible nitric-oxide synthase; MS: multiple sclerosis; NFκB: nuclear factor of kappa light polypeptide gene enhancer in B-cells; NPC1: Niemann-Pick disease type C 1 gene; OEA: oleoylethanolamide; PD: Parkinson's disease; PEA: palmitoylethanolamide; TNFα: tumor necrosis factor α; SODs: superoxide dismutases; VCAM-1: vascular cell adhesion molecule-1.

Importantly, the concept of a role for the receptor in neurogenesis is recently emerging, based on the *in vitro* evidence of its presence in adult neural stem cells, and on *in vivo* studies on models of acute neuropathologies (Fidaleo et al., 2014; Ouk et al., 2014). Indeed, endogenous production of the PPARα ligand OEA and specific PPARα expression in proliferating neural cells was observed after insult. Consistently, fenofibrate was shown to favor brain repair, by preserving and stimulating neurogenesis in damaged areas. Unfortunately, no data are presently available on the involvement of PPARα in neurogenesis during chronic neurodegenerative diseases. Indeed, despite the large body of literature demonstrating disturbances to this process occurring in several pathologies (Mouhieddine et al., 2014) and the effort in developing therapeutic strategies targeting neurogenesis, the beneficial effects of PPARα agonist treatment in neurodegenerative disease models have never been interpreted in view of a pro-neurogenic action of the receptor. Equally lacking are experimental data on the putative role of PPARαin neurogenesis, in either developing, or aging normal brain.

Perspectives and caveat for PPARα agonist-based therapies: In summary, the pleiotropic effects of activated PPARα seem to converge on the molecular pathways shared by different brain pathologies and even normal aging - namely, oxidative stress, inflammation, lipid dysmetabolism, defective neurogenesis, abnormal autophagy and cell death. Therefore, our opinion is that PPARα agonist-based therapy, rather than a *panacea*, should be considered as an add on to other pharmacological approaches, targeting disease-specific pathogenetic mechanisms. Noteworthy, validated treatments against neuroinflammation, such as caloric restriction or hypothermia (Buga et al., 2013), may themselves involve activation of PPARα.

In order to customize therapeutic strategies against specific CNS disorders, the effects of PPARα activation in specific neural cell populations, affected by different diseases, need to be further clarified. Studies addressing this issue should also take into account the expression, concentration and cellular/intracellular localization of the receptor in the targeted brain area. Moreover, knowledge of age-, gender- and pathology-dependent variations in PPARα expression, influencing responsiveness of certain neural cell populations to the treatment, may help select the appropriate patient subgroup and window treatment. Our past and recent studies on PPARα distribution throughout the adult brain (Moreno et al., 2004) and on changes of its concentration and localization in select brain areas (namely, hippocampus and neocortex) related to aging and to Alzheimer's disease pathology (Fanelli et al., 2013; Porcellotti et al., 2015), may constitute a starting point to get further insights into these relevant aspects.

Given that the nervous system is surpassed in PPARα content by other organs (particularly, liver, heart and kidney), systemic undesired effects of any treatment should not be underrated. Concerning fibrates (particularly, fenofibrate), no relevant side effect has been noted, even though monitoring renal function during treatment is recommended (Munigoti and Harinarayan, 2014). More recent studies point to a promising class of synthetic molecules, acting as dual PPARα/γ agonists, namely Glitazars, which are highly effective as hypolipidemic, hypotensive, antiatherogenic, anti-inflammatory and anticoagulant drugs. Among these, Saroglitazar, with predominant PPARα-mediated activity, is considered novel and unique as it was conceptualized to deliver antidyslipidemic and antihyperglycemic effects without any of the adverse effects of other molecules of its family (Munigoti and Harinarayan, 2014).

Conclusions: Clinical trials employing PPARα ligands have so far been limited to the treatment of pathologies unrelated, or only indirectly related, to the brain, while, in our opinion, the time has come to expand these studies to neural disorders. Noteworthy, in humans, PPARα gene polymorphisms are currently being studied, also in relation to their importance as risk factors in specific diseases (namely, Alzheimer's disease). These notions will be indispensible

for future pharmacogenomics paradigms seeking to predict PPARα agonist responders.

An emerging concept suggests that, considering the complex interplay among PPAR isotypes, RXRs, and the endocannabinoid system, combinatorial therapies, aimed at activating multiple receptors, could be especially effective towards neuroprotection and neuroregeneration.

One of the most critical points remains the selection of window treatment, as well as of drug/s dosage, given the heterogeneity of literature data concerning the above parameters. An effort should be made to unify multiple data sources, to indicate optimal conditions to achieve neuroprotection, which is one important point in the future direction. The rapid development of statistical methodology (*i.e.*, cluster analysis, hyerarchical model) provides powerful tools for integrating multi-dimensional data at the analysis stage, in addition to the experimental phase, to purify and compare the treatment effect in a dynamic longitudinal way.

This work was supported by CAL grant to SM from University Roma Tre. The authors are grateful to Francesca Greco for figure editing.

Sandra Moreno*, Maria Paola Cerù

Department of Science-LIME, University Roma Tre, Rome, Italy (Moreno S, Cerù MP)
Department of Life, Health and Environmental Sciences, University of L'Aquila, Coppito (AQ), Italy (Cerù MP)

***Correspondence to:** Sandra Moreno, Ph.D., sandra.moreno@uniroma3.it.*
***Accepted:** 2015-06-07*
***orcid:** 0000-0002-1079-3222 (Sandra Moreno)*
***doi:** 10.4103/1673-5374.165313* ***http**://www.nrronline.org/*
Moreno S, Cerù MP (2015) In search for novel strategies towards neuroprotection and neuroregeneration: is PPARα a promising therapeutic target? Neural Regen Res 10(9):1409-1412.

References

Barbiero JK, Santiago R, Tonin FS, Boschen S, da Silva LM, Werner MF, da Cunha C, Lima MM, Vital MA (2014) PPAR-α agonist fenofibrate protects against the damaging effects of MPTP in a rat model of Parkinson's disease. Prog Neuropsychopharmacol Biol Psychiatry 53:35-44.

Buga AM, Di Napoli M, Popa-Wagner A (2013) Preclinical models of stroke in aged animals with or without comorbidities: role of neuroinflammation. Biogerontology 14:651-662.

Chakravarthy MV, Zhu Y, López M, Yin L, Wozniak DF, Coleman T, Hu Z, Wolfgang M, Vidal-Puig A, Lane MD, Semenkovich CF (2007) Brain fatty acid synthase activates PPARalpha to maintain energy homeostasis. J Clin Invest 117:2539-2552.

Chinetti-Gbaguidi G, Rigamonti E, Helin L, Mutka AL, Lepore M, Fruchart JC, Clavey V, Ikonen E, Lestavel S, Staels B (2005) Peroxisome proliferator-activated receptor alpha controls cellular cholesterol trafficking in macrophages. J Lipid Res 46:2717-2725.

Dreyer C, Krey G, Keller H, Givel F, Helftenbein G, Wahli W (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. Cell 68:879-887.

Fanelli F, Sepe S, D'Amelio M, Bernardi C, Cristiano L, Cimini A, Cecconi F, Cerù MP, Moreno S (2013) Age-dependent roles of peroxisomes in the hippocampus of a transgenic mouse model of Alzheimer's disease. Mol Neurodegener 8:8.

Feige JN, Gelman L, Michalik L, Desvergne B, Wahli W (2006) From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions. Prog Lipid Res 45:120-159.

Fidaleo M, Fanelli F, Ceru MP, Moreno S (2014) Neuroprotective properties of peroxisome proliferator-activated receptor alpha (PPARα) and its lipid ligands. Curr Med Chem 21:2803-2821

Ghosh A, Jana M, Modi K, Gonzalez FJ, Sims KB, Berry-Kravis E, Pahan K (2015) Activation of peroxisome proliferator-activated receptor α induces lysosomal biogenesis in brain cells: implications for lysosomal storage disorders. J Biol Chem 290:10309-10324.

Gofflot F, Chartoire N, Vasseur L, Heikkinen S, Dembele D, Le Merrer J, Auwerx J (2007) Systematic gene expression mapping clusters nuclear receptors according to their function in the brain. Cell 131:405-418.

Issemann I, Green S (1990) Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 347:645-650.

Lee JM, Wagner M, Xiao R, Kim KH, Feng D, Lazar MA, Moore DD (2014) Nutrient-sensing nuclear receptors coordinate autophagy. Nature 516:112-115.

Melis M, Scheggi S, Carta G, Madeddu C, Lecca S, Luchicchi A, Cadeddu F, Frau R, Fattore L, Fadda P, Ennas MG, Castelli MP, Fratta W, Schilstrom B, Banni S, De Montis MG, Pistis M (2013) PPARα regulates cholinergic-driven activity of midbrain dopamine neurons via a novel mechanism involving α7 nicotinic acetylcholine receptors. J Neurosci 33:6203-6211.

Moreno S, Farioli-Vecchioli S, Cerù MP (2004) Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. Neuroscience 123:131-145.

Mouhieddine TH, Kobeissy FH, Itani M, Nokkari A, Wang KK (2014) Stem cells in neuroinjury and neurodegenerative disorders: challenges and future neurotherapeutic prospects. Neural Regen Res 9:901-906.

Munigoti SP, Harinarayan CV (2014) Role of Glitazars in atherogenic dyslipidemia and diabetes: Two birds with one stone? Indian J Endocrinol Metab 18:283-287.

Ouk T, Gautier S, Pétrault M, Montaigne D, Maréchal X, Masse I, Devedjian JC, Deplanque D, Bastide M, Nevière R, Duriez P, Staels B, Pasquier F, Leys D, Bordet RJ (2014) Effects of the PPAR-α agonist fenofibrate on acute and short-term consequences of brain ischemia. Cereb Blood Flow Metab 34:542-551.

Porcellotti S, Fanelli F, Fracassi A, Sepe S, Cecconi F, Bernardi C, Cimini A, Cerù MP, Moreno S (2015) Oxidative stress during the progression of β-amyloid pathology in the neocortex of the Tg2576 mouse model of Alzheimer's disease. Oxid Med Cell Longev 2015:967203.

Puligheddu M, Pillolla G, Melis M, Lecca S, Marrosu F, De Montis MG, Scheggi S, Carta G, Murru E, Aroni S, Muntoni AL, Pistis M (2013) PPAR-alpha agonists as novel antiepileptic drugs: preclinical findings. PLoS One 8:e64541.

Rolland B, Marche K, Cottencin O, Bordet R (2012) The PPARα Agonist Fenofibrate Reduces Prepulse Inhibition Disruption in a Neurodevelopmental Model of Schizophrenia. Schizophr Res Treatment 2012:839853.

Roy A, Jana M, Corbett GT, Ramaswamy S, Kordower JH, Gonzalez FJ, Pahan K (2013) Regulation of cyclic AMP response element binding and hippocampal plasticity-related genes by peroxisome proliferator-activated receptor α. Cell Rep 4:724-737.

Zhang H, Gao Y, Qiao PF, Zhao FL, Yan Y (2014) Fenofibrate reduces amyloidogenic processing of APP in APP/PS1 transgenic mice via PPAR-α/PI3-K pathway. Int J Dev Neurosci 38:223-231.