

Functional role of translocator protein and its ligands in ocular diseases (Review)

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Abstract. The 18 kDa translocator protein (TSPO) is an essential outer mitochondrial membrane protein that is responsible for mitochondrial transport, maintenance of mitochondrial homeostasis and normal physiological cell function. The role of TSPO in the pathogenesis of ocular diseases is a growing area of interest. More notably, TSPO exerts positive effects in regulating various pathophysiological processes, such as the inflammatory response, oxidative stress, steroid synthesis and modulation of microglial function, in combination with a variety of specific ligands such as 1-(2-chlorophenyl-N-methylpropyl)-3-isoquinolinecarboxamide, 4'-chlorodiazepam and XBD173. In the present review, the expression of TSPO in ocular tissues and the functional role of TSPO and its ligands in diverse ocular diseases was discussed.

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1. Introduction

Translocator protein (TSPO; 18 kDa) is a highly structurally conserved hydrophobic protein located on the outer mitochondrial membrane (OMM) (1). Discovered in the 1970s, TSPO was originally termed the 'peripheral benzodiazepine receptor' due to its specific binding site for certain benzodiazepines in peripheral tissues (2). In 2006, the name was changed to TSPO to reflect the cholesterol binding and transportation functions of the protein (3). TSPO is also involved in a variety of cellular physiological activities such as mitochondrial quality control, mitochondrial permeability transition pore opening, voltage-dependent anion channel (VDAC) opening, calcium transport, cellular proliferation, programmed apoptosis and reactive oxygen species (ROS) production (4-8). This extensive range of molecular functions has led to the association of TSPO with the pathogenesis of multiple diseases, including central nervous system diseases and cardiovascular anomalies (9-12). Increasing research interest in TSPO as a crucial pathogenic factor and therapeutic target has driven the study of TSPO-specific, high-affinity binding molecules, known as TSPO ligands. TSPO ligands have been shown to have a beneficial effect by agonizing or inhibiting TSPO activity in diseases such as Alzheimer's disease (AD), multiple sclerosis and cardiac arrhythmias (13-15). It was not until the previous decade, however, that the role of TSPO and its ligands in ocular diseases became an area of active research, and a number of promising developments have been identified (16-20). The present article provided a review of the advances in the study of TSPO and its ligands in ocular diseases. First, an overview of TSPO function was presented, and a variety of classical and novel TSPO ligands were described. Next, the expression of TSPO in ocular tissues based on existing studies

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Abbreviations: TSPO, translocator protein; OMM, outer mitochondrial membrane; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; mPTPs, mitochondrial permeability transition pores; ROS, reactive oxygen species; NOX, NADPH oxidase; AD, Alzheimer's disease; PPIX, protoporphyrin IX; PK11195, 1-(2-chlorophenyl-N-methylpropyl)-3-isoquinolinecarboxamide; Ro5-4864, 4'-chlorodiazepam; DBI, diazepam-binding inhibitor; PIGAS, phenylindolyilglyoxylamydes; FGIN-1-27, N,N-di-n-hexyl 2-(4-fluorophenyl) indole-3-acetamide; PET, positron emission tomography; TNF- α , tumor necrosis factor- α ; COX-2, cyclooxygenase-2; LPS, lipopolysaccharide; RGC, retinal ganglion cell; RPE, retinal pigment epithelium; AMD, age-related macular degeneration; CNV, choroidal neovascularization; DR, diabetic retinopathy; DM, diabetes mellitus

Key words: TSPO, ligand, ocular diseases

was summarized. Finally, the role of TSPO and its ligands in different ocular conditions was discussed, including ocular development and aging, age-related macular degeneration (AMD), retinal ischemia, diabetic retinopathy (DR) and glaucoma. The aim of the present review was to highlight the research value of TSPO in ophthalmology and to suggest perspectives on potential therapeutic targets.

2. Structure and distribution of TSPO

TSPO is a highly hydrophobic protein that is expressed in both prokaryotic and eukaryotic cells, from bacteria to humans (21). TSPO is primarily localized to the OMM, adjacent to the VDAC and adenine nucleotide translocase (ANT) (1,22,23). This 169-amino acid protein in humans is encoded by a nuclear gene containing four exons (24) and the mature protein contains five α -helical transmembrane domains (TMs). A cholesterol-recognition amino acid consensus sequence is located in the C-terminus of the TM structure, through which TSPO binds to the lipid membrane (25,26). The 3D structure of TSPO in complex with the TSPO-targeting ligand, (R)-1-(2-chlorophenyl-N-methylpropyl)-3-isoquinolinecarboxamide (PK11195), comprising five transmembrane helices (TM1-TM5) that tightly pack together in the clockwise order TM1-TM2-TM5-TM4-TM3 viewed from the cytosol, has also been revealed (26). TSPO is highly expressed in a variety of tissues, particularly in the gonads, adrenal glands, placenta and brain, due to its high abundance in steroidogenic cells (27). Therefore, the pathogenic effects of TSPO and the potential therapeutic value of TSPO ligands for anxiety disorders, AD and hypogonadism have been thoroughly investigated *in vitro* and *in vivo* (28-30).

3. TSPO is involved in multiple cellular functions

TSPO is involved in a large variety of physiological cellular processes (Fig. 1). First, the subcellular localization of TSPO dictates its critical role in mitochondrial function; it is a regulator of mitochondrial respiration, membrane potential, energy homeostasis and quality control (4,31-33). TSPO also reduces mitophagy, which inhibits essential ubiquitination of proteins, leading to a low efficiency of ubiquitin-dependent mitochondrial protein degradation, and thus, an accumulation of dysfunctional mitochondria (33). Previous studies have reported that TSPO modulates mitochondrial permeability transition pores (mPTPs), such as VDAC and ANT (1,5,9). However, there is conflicting evidence regarding these interactions, which suggests that TSPO is not a key mPTP regulator (8). Accordingly, these interactions remain ambiguous, and we hypothesized that these are possibly indirect or cell type-dependent. Another function of TSPO is to control intracellular signaling pathways between mitochondria and the nucleus, thereby affecting nuclear gene expression (6). Finally, TSPO is associated with multiple general cellular activities, such as cell proliferation, cell differentiation (34) and programmed cell death (35), and also acts as a gatekeeper for the biosynthesis of steroid hormones (8). TSPO is involved in the translocation of cholesterol and porphyrins from the outer to the inner mitochondrial membrane (22), which is a

rate-limiting step in steroid and neurosteroid synthesis (36). TSPO also regulates calcium ion homeostasis in cells and activates calcium-dependent NADPH oxidase (NOX) (37), thereby increasing ROS levels (38,39). An additional study has demonstrated that TSPO is a key regulator of NOX1-dependent neurotoxic ROS production (40). In fact, the function of TSPO is still regarded as debatable, and thus, more future studies are required to elucidate this. For example, one of the most highly researched areas of TSPO is its key role in steroidogenesis. However, Tu *et al* (28,29) successfully established a TSPO systemic knockout mouse model and demonstrated that TSPO is not essential for steroidogenesis. In addition, some studies solely focused on TSPO ligand functions without measuring TSPO levels, thus it remains unclear whether TSPO had a direct impact on the biological events (14,25). Therefore, further experiments are required to explain the aforementioned unclear mechanisms in order to ascertain the role of TSPO.

4. TSPO ligands

TSPO ligands. TSPO ligands are classified as endogenous or synthetic ligands. In previous years, TSPO-ligand interactions have received increasing attention, with multiple studies reporting its positive effects on diseases such as AD, multiple sclerosis, anxiety and vasculopathy (13-15,41). Steroidogenesis and anti-inflammation are the main pathways by which TSPO ligands function (42). However, the independent mechanism, 'mitohormesis', defined as subtoxic stress on mitochondria, may be responsible for the protective effects of TSPO ligands. It is proposed that the ligands suppress ATP synthesis through mitohormesis, leading to activation of the cellular damage-response network. This, in turn, enhances the tolerance of cells to the degeneration processes, including oxidative damage, phototoxicity or DNA damage (43). A summary of classical and novel ligands and their features and effects based on existing studies is described below and in Table I.

Porphyrins. Porphyrins are high-affinity endogenous ligands for TSPO, in the micromolar to nanomolar range (35). Mitochondria are critically involved in porphyrin metabolism and TSPO is closely related to this process (26,44). Porphyrins are involved in heme synthesis, and protoporphyrin IX (PPIX), as a key intermediate in the heme biosynthesis pathway, has been one of the most extensively studied endogenous ligands since its discovery in 1987 (45,46). Previous studies have reported that TSPO-PPIX interactions regulate mitochondrial membrane permeability and thus exert cytoprotective effects (46,47). Porphyrin-induced oxidative stress is thought to be the major mechanism of porphyrin-mediated tissue damage (39,48). Furthermore, Yamamoto *et al* (49) demonstrated that TSPO knockdown leads to the accumulation of PPIX and ROS in glioblastoma cells. Specifically, TSPO possibly functions by binding or sequestering PPIX rather than via TSPO-mediated transmembrane transport (50). Unbound PPIX inhibits heme oxygenase and prevents free heme degradation, thereby maintaining the balance between free and bound heme and preventing cytotoxic effects (51).

PK11195. PK11195 was the first non-benzodiazepine TSPO synthetic ligand discovered that acts in the nanomolar

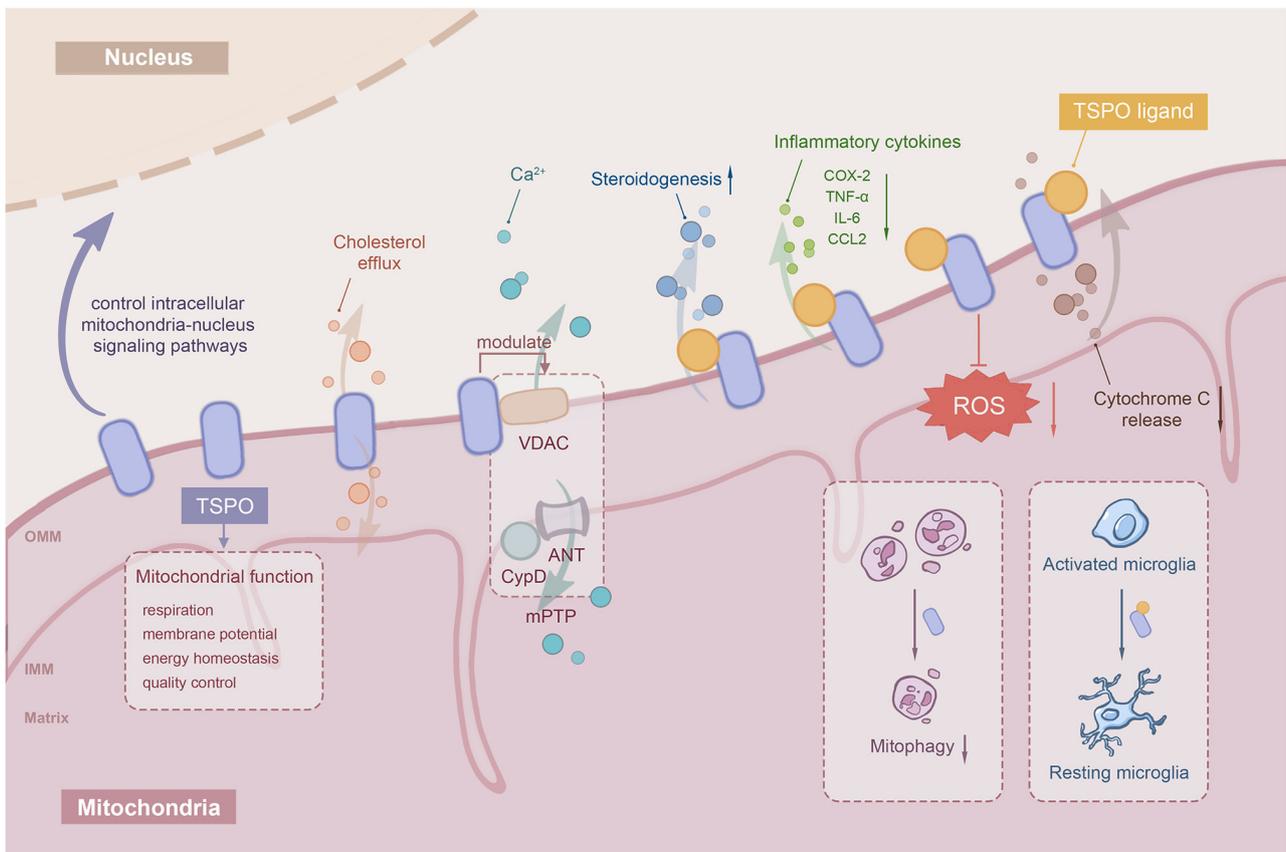


Figure 1. Diagram summarizing the key functions of TSPO and TSPO ligands. TSPO is located in the OMM. TSPO reduces mitophagy and is a regulator of multiple mitochondrial functions including mitochondrial respiration, membrane potential, energy homeostasis and quality control. TSPO controls signaling pathways between mitochondria and the nucleus and thereby affects nuclear gene expression. TSPO is involved in the cholesterol transport from the OMM to the IMM. The close apposition of TSPO and mPTP complexes enables TSPO to regulate the function of mPTP, thereby affecting Ca^{2+} transport across the OMM and maintaining Ca^{2+} homeostasis. TSPO ligands modulate TSPO and promote cell steroidogenesis, downregulate cell inflammatory cytokine expression, decrease ROS production as well as induce the switching of microglia from the active state to favor the resting state. TSPO ligands reduce cytochrome c release from mitochondria to the cytoplasm, thereby inhibiting the mitochondrial apoptosis pathway. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; VDAC, voltage-dependent anion channel; ANT, adenine nucleotide translocase; CypD, cyclophilin D; mPTPs, mitochondrial permeability transition pores; ROS, reactive oxygen species; TSPO, translocator protein; COX-2, cyclooxygenase-2; TNF- α , tumor necrosis factor- α ; IL, interleukin; CCL2, C-C motif chemokine receptor-2.

range (42). PK11195 is considered one of the best-characterized and prototypic ligands of TSPO (50), and is widely used to study the roles and functions of TSPO in various diseases, particularly in *in vivo* studies. [^{11}C]PK11195 has been additionally applied as a radioligand for positron emission tomography (PET). Despite several limitations, such as poor brain permeability, kinetic instability and signal-to-noise ratio issues due to non-specific binding (52-54), [^{11}C]PK11195 remains the standard TSPO-based PET radiotracer for assessing the activation of microglia or macrophages in central nervous system disorders (55,56). PK11195 has also been reported to have anti-neuroinflammation effects in experimental models of AD (10) and pressure-induced retinal ganglion cell (RGC) injury (57). However, further research is needed to elucidate the specific mechanisms underlying these protective effects, including whether they are related to the stimulation effect on steroidogenesis in peripheral tissues and the brain (10,58). PK11195 downregulates the expression of the inflammatory cytokines, tumor necrosis factor- α (TNF- α) and cyclooxygenase-2 (COX-2), in lipopolysaccharide (LPS)-induced human microglia (59,60), and modulation of Ca^{2+} -mediated signaling pathways may be involved in its anti-inflammatory actions (61).

In prostate cancer cells, PK11195 can be used as a sensitizer to the chemotherapy agents mda-7/IL-24 (Ad.mda-7, a novel cytokine gene belonging to the IL-10 gene superfamily) (62), both *in vitro* and *in vivo*. PK11195 promotes cellular autophagy by inactivating the oncoprotein, B-cell lymphoma-2, and by targeting F_1F_0 -ATP synthase (63), an essential component of mPTPs (64). Notably, Gonzalez-Polo *et al* (65) reported that PK11195 promotes starvation-induced cell death through an unexpected pathway independent of TSPO, which directly activates the mitochondrial apoptotic pathway, as demonstrated by cytochrome c release and caspase-3 activation. Although PK11195 is generally considered to be a TSPO antagonist, there is evidence that PK11195 displays a partial agonistic effect on TSPO (66), which is possibly dependent on the cell type and drug concentration.

4'-chlorodiazepam (Ro5-4864). Ro5-4864 is another classical synthetic TSPO ligand that has been used to investigate the characteristics of TSPO in health and disease due to its nanomolar selectivity (67,68). However, the application of Ro5-4864 as a pharmacological agent (67) is limited as its affinity varies with species, from rat (high affinity) to human

Table I. Involved pathways or biological effects of TSPO ligands in *in vitro* and *in vivo* experimental models.

First author/s, year	TSPO ligands	Cell line/animal model	Findings of involved pathways/biological effects	(Refs.)
Yamamoto <i>et al</i> , 2014	PPIX	TSPO-knockdown glioblastoma cells	TSPO deletion leads to the accumulation of PPIX and ROS	(49)
Girard <i>et al</i> , 2012; Ravikumar <i>et al</i> , 2016; Choi <i>et al</i> , 2002	PK11195	LPS-induced human microglia	Downregulates the expression of TNF- α and COX-2, probably relevant to Ca ²⁺ -mediated signaling pathways	(59-61)
Seneviratne <i>et al</i> , 2012		HeLa cells	Regulates cellular autophagy by targeting F ₁ F ₀ -ATP synthase, which is a TSPO-independent pathway	(63)
Ishikawa <i>et al</i> , 2016		Rat <i>ex vivo</i> glaucoma model	Inhibits pressure-induced RGC apoptosis by promoting the TSPO-5 α RD-mediated AlloP synthesis	(57)
Gut <i>et al</i> , 2013	Ro5-4864	Isoprenaline-induced zebrafish larvae	Reverses the glucose level fluctuations	(70)
Bernardi <i>et al</i> , 2022; Baez <i>et al</i> , 2017		Glucose deprivation treated T98G astrocyte cells	Maintains mitochondrial homeostasis by reducing the production of free radicals and inhibits the mitochondrial apoptosis pathway by reducing cytochrome c release and caspase-3 activation	(36,74)
Da Pozzo <i>et al</i> , 2015		AD mouse model	Modulates the production of steroids to exert neuroprotective effects	(71)
Musman <i>et al</i> , 2017		Type-2 diabetes mellitus rat models	Limits cholesterol transport into mitochondria, inhibits oxysterol accumulation and reduces oxidative stress	(139)
Nothdurfter <i>et al</i> , 2012	XBD173	Anxiety disorder population and mouse model	Exerts anxiolytic properties by enhancing neurosteroidogenesis, thereby potentiating GABA-mediated inhibitory postsynaptic currents	(75)
Scholz <i>et al</i> , 2015		Retinal degeneration mouse model	Alleviates neurodegeneration by targeting the microglia/macrophage marker, CD68, and reducing the levels of pro-inflammatory factors, CCL2 and IL-6	(78)
Mages <i>et al</i> , 2019		Retinal ischemia mouse model	Reduces microglia and promotes the transformation toward the anti-inflammatory M2 phenotype, thereby protecting inner retinal neurons	(18)
Lieth <i>et al</i> , 1998; Wagner <i>et al</i> , 2017; Delyfer <i>et al</i> , 2005		Retinal degeneration/ischemia mouse model	Maintains the metabolism of the 'neuron-supportive microenvironment' and protects against neurotoxic injury by regulating glutamine synthetase expression	(123,124,126)
Wolf <i>et al</i> , 2020; Nagineni <i>et al</i> , 2012		Laser-induced CNV mouse model	Inhibits the generation of CNV by inhibiting pro-angiogenic factor and autocrine, IL-1 β , regulating phagocytosis and facilitating the transition of microglia to neuroprotective phenotypes	(40,116)
Biswas <i>et al</i> , 2021; Ibrahim <i>et al</i> , 2020	Etifoxine	High-fat diet/obesity mouse model	Enhances cholesterol metabolism by downregulating the total cholesterol, triglyceride and phospholipid mass both in RPE and in serum	(84,85)
Biswas <i>et al</i> , 2018; Biswas <i>et al</i> , 2017		Choroidal endothelial cells (RF/6A)	Inhibits apoptosis by reducing intracellular lipid accumulation, ox-LDL-induced ROS production and inflammatory cytokine secretion	(17,25)
Wang <i>et al</i> , 2014	DBI/TTN	Retinal inflammation mouse model	DBI-TSPO signaling limits the magnitude of microglial activation and promotes a return to baseline quiescence through macroglia-microglia interactions	(16)

Table I. Continued.

First author/s, year	TSPO ligands	Cell line/animal model	Findings of involved pathways/biological effects	(Refs.)
Santoro <i>et al</i> , 2016	PIGAS	INF γ /LPS-induced rat C6 glioma cells	Downregulates pro-inflammatory enzymes, iNOS and COX-2, expression by modulating neurosteroid synthesis	(91)

TSPO, translocator protein; PPIX, protoporphyrin IX; ROS, reactive oxygen species; PK11195, 1-(2-chlorophenyl-N-methylpropyl)-3-isoquinolinecarboxamide; LPS, lipopolysaccharide; TNF- α , tumor necrosis factor- α ; COX-2, cyclooxygenase-2; RGC, retinal ganglion cell; 5 α RD, 5 α -reductase; AlloP, allopregnanolone; Ro5-4864, 4'-chlorodiazepam; AD, Alzheimer's disease; CCL2, C-C motif chemokine receptor-2; IL-6, interleukin 6; RPE, retinal pigment epithelium; ox-LDL, oxidized low-density lipoprotein; CNV, choroidal neovascularization; DBI, diazepam-binding inhibitor; TTN, triakontatetreuropeptide; PIGAS, phenylindolyglyoxylamides; INF γ , interferon γ ; iNOS, inducible nitric oxide synthase.

(low affinity) (21). Another limitation is that the affinity of [³H] Ro5-4864 can be influenced by temperature, as found in brain membrane binding assays (69). Gut *et al* (70) reported that Ro5-4864 is sufficient to reverse the glucose level fluctuations induced by isoprenaline in zebrafish larvae, suggesting that Ro5-4864 may influence glucose homeostasis in response to altered mitochondrial energy balance. Ro5-4864 also has certain features that are similar to that of PK11195. For example, Ro5-4864 stimulates steroid formation (10), contributes to the neuroprotective effect (but differentiates from PK11195 activity under different experimental conditions) (71) and inhibits pro-inflammatory factors and ROS production in human microglia incubated with LPS (16,59). Furthermore, Ro5-4864 antagonizes the anxiolytic and antidepressant-like effects of other TSPO ligands. For example, the antidepressant properties of PK11195 induced in mice subjected to the forced swimming test was blocked by Ro5-4864 administration (72,73). Ro5-4864 has also been shown to contribute to the maintenance of mitochondrial homeostasis, reducing cytochrome c release and caspase-3 activation, thereby inhibiting the mitochondrial apoptosis pathway (36). Furthermore, Baez *et al* (74) demonstrated that Ro5-4864 maintains mitochondrial function in the T89G astrocyte cell line in the presence of glucose deprivation damage by reducing the production of free radicals.

XBD173. XBD173 (AC-5216; Emapunil) is a novel high-affinity and selective phenylpurine ligand of TSPO, which has been well investigated and widely used in the research and therapy of anxiety disorders (41,75,76). GABA-mediated inhibitory postsynaptic currents were potentiated by XBD173 in both animal experiments and clinical studies (75), and this neurotransmission appeared to be mediated indirectly through the generation of neurosteroids and could be prevented by the 5 α -reductase inhibitor, finasteride (76). According to previous studies, the role of XBD173 in microglia has been well defined and has been shown to alleviate the neurotoxic effect of microglia and inhibit cell proliferation and migration (16,77). The XBD173/TSPO axis appears to target the microglia/macrophage marker, CD68, and inhibit the pro-inflammatory genes, C-C motif chemokine receptor-2 and interleukin (IL)-6, to alleviate apoptosis, thereby alleviating

neurodegenerative dysfunction (78). Mages *et al* (18) further investigated the exact response patterns of the major glial cell types of the retina in a retinal ischemia mouse model. In the study it was reported that XBD173 treatment resulted in lower microglia accumulation, and the microglial activation profile showed a transformation towards the anti-inflammatory M2 phenotype. However, the aforementioned effects of XBD173 on microglia could be reversed to some extent, since the reduced microglial cell number induced by XBD173 could transiently rise again (18). In addition, even with XBD173 intervention, cellular morphological changes (from ramified to amoeboid) representing microglial activation were observed, suggesting that XBD173 only partially inhibits microglial activation (18).

Etifoxine. To the best of our knowledge, Etifoxine (etafenoxine; Stresam[®]) is currently the only commercially available TSPO ligand (79). Etifoxine was developed in the 1960s for anxiety disorders and is administered for this purpose in >42 different countries (80). However previous studies have since reported its therapeutic potential in peripheral nerve injury and demyelination, and chemotherapy-induced pain (14,80,81). As a non-benzodiazepine anxiolytic, etifoxine appears to exercise its functions by binding to the β 2 or β 3 subunits of the GABA_A receptor complex (82). Etifoxine also regulates GABA_A receptors indirectly by stimulating neurosteroid production in both the central and peripheral nervous systems (24,83). Furthermore, Biswas *et al* (84) suggested that etifoxine modulates cholesterol homeostasis, promoting cholesterol efflux and transporting cholesterol to the liver, which is the most important function of this ligand. The authors also found that etifoxine enhanced the expression of cholesterol metabolism and transport enzymes in high-fat diet mice and significantly decreased the total cholesterol, triglycerides and phospholipid mass in the retinal pigment epithelium (RPE). This finding was consistent with that of a study by Ibrahim *et al* (85), which suggested that serum lipid levels were downregulated in etifoxine-treated mice. Furthermore, etifoxine leads to a higher conversion of cholesterol into high-density lipoprotein while preventing intracellular lipid accumulation, and therefore protecting cells against apoptosis by inhibiting oxidized low-density lipoprotein-induced ROS production and inflammatory cytokine secretion (17,25). In addition, etifoxine

stimulates neurosteroid synthesis; however, Costa *et al* (86) suggested that this effect depends more on residence time at the TSPO binding site rather than the binding affinities.

Other reported TSPO ligands. Diazepam-binding inhibitor (DBI) is an endogenous agonistic ligand of TSPO (3). DBI-TSPO interactions have been demonstrated to occur in the production of steroids such as pregnenolone, in both *in vivo* and *in vitro* experiments (87,88). DBI or its cleavage product, triakontatetrapeptide, binds to TSPO during a form of macroglia-microglia interaction, limiting the magnitude of microglial activation and promoting a return of microglia to the resting state, which is a hallmark of the down-regulation of inflammation (16). Novel TSPO synthetic ligands include N,N-di-n-hexyl 2-(4-fluorophenyl) indole-3-acetamide (FGIN-1-27), 7-chloro-N,N,5-trimethyl-4-oxo-3-phenyl-3,5-dihydro-4H-pyridazino(4,5-b)indole-1-acetamide (SSR180575) and phenylindolyglyoxylamides (PIGAS), all of which possess steroidogenic properties (12) and promote cholesterol metabolism and transporter gene expression (25). SSR180575 is anti-apoptotic and protects mitochondria against dysfunction in ischemia-reperfusion injury models (89,90). PIGAS displays anti-inflammatory capacity, as evidenced by decreased inducible nitric oxide synthase and COX-2 expression, possibly by modulating astrocyte function through neurosteroid synthesis (91).

5. Functional roles of TSPO and TSPO ligands in ocular diseases

Overview. Next, the expression of TSPO in ocular tissues, the mechanisms by which TSPO is involved in the progression of various ocular diseases (Fig. 2) and the role of TSPO ligands in therapeutic interventions are discussed, based on existing studies.

Current status of research on TSPO expression in ocular tissues. In recent years, the role of TSPO in ocular diseases has gained attention. However, it must be acknowledged that the number of studies on TSPO expression in ocular cells and tissues has been limited. Biswas *et al* (17,25) reported high TSPO expression in the RPE and choroidal endothelium of mice. This observation was consistent with that of a study by Mages *et al* (18), in which specific cell types were enriched via immunomagnetic separation and then protein expression levels were measured using mass spectrometry analysis. It was noted that TSPO was most highly expressed in RPE cells, vascular cells and Müller cells, while expression was lower in microglia and almost undetectable in retinal neurons (16,18,77). By contrast, Biswas *et al* (17) reported strong TSPO signals in the ganglial cell layer by immunofluorescence analysis in mouse eye sections. Differences in TSPO expression and localization may be due to differences in sample collection and immunoassay approaches. In addition, TSPO expression has been detected in human retina and RPE (77,92,93), with both containing high expression at the mRNA level. When the central and peripheral sections of the retina and RPE were isolated separately, the peripheral RPE exhibited higher TSPO expression than the central RPE (92). It remains unclear whether this infers any functional implications. To the best of

our knowledge, there have been no further reports of TSPO expression in other ocular structures such as the cornea, conjunctiva, lens, meibomian gland and lacrimal gland.

TSPO in ocular development and aging. Wang *et al* (16) investigated whether the endogenous functions of TSPO expression are relevant to retinal developmental processes. TSPO is expressed in microglia with an amoeboid phenotype that invades the neural retina during early development. In adulthood, as microglia undergo a morphological transformation to a ramified cell shape, TSPO expression is barely detectable (94). There are several theories that might help explain this, including that TSPO is possibly involved in the physiological processes of microglial development (95,96), such as complement-mediated synapse elimination and phagocytosis of dead neurons (66). Temporary expression of TSPO may also be associated with the migration process of microglia during retina development (97), and the expression terminated when microglia reach the final site. On the other hand, certain researchers suggest that TSPO is unnecessary for healthy development and aging following the study of several generated systemic TSPO-knockout mouse lines (28,98,99). For example, Klee *et al* (92) considered that ocular development is not strongly associated with TSPO expression as no ocular phenotype was observed in TSPO-knockout mice aged 0-48 weeks old. Furthermore, TSPO mRNA expression did not show significant differences in human retina and RPE tissues among different age groups of donors (from 17-90 years old) (92), suggesting that TSPO expression may not be involved in the regulation of normal development and aging. These differential findings led us to consider whether there are compensatory mechanisms for TSPO deletion in the knockout models such that marked phenotypes are not evident during the development process. Furthermore, as a stress-related protein, the regulatory effects of TSPO in multiple pathways may be more critical in disease states but not play a major role in normal physiological development. At present, the potential mechanisms remain to be validated by additional studies.

AMD. AMD is a prevalent cause of severe visual impairment among elderly individuals (100). Clinically, AMD can be categorized into two subtypes: Dry AMD, which accounts for 80-85% of cases and manifests as geographic atrophy, and wet AMD, which accounts for 15-20% of cases and presents as neovascularization (101). The pathogenesis of AMD is multifaceted, with a combination of genetic and environmental factors, while the efficacy of existing therapies is extremely limited since there is no treatment available to prevent the irreversible degeneration of the photoreceptors that leads to loss of central vision (102,103). Dysfunctional cholesterol metabolism is one of the prominent mechanisms underlying the development of AMD (103). The pathological examination of patients with AMD has revealed subretinal deposits of apolipoproteins, cholesterol and cholesteryl ester, indicating a potential association of cholesterol transport disorders in AMD (17,104). Notably, the formation of drusen is associated with poor choroidal endothelial structural and functional integrity, resulting in inadequate nutrient and oxygen delivery to the RPE and photoreceptor cells and impairing their metabolite clearance (105). These processes are particularly associated with the

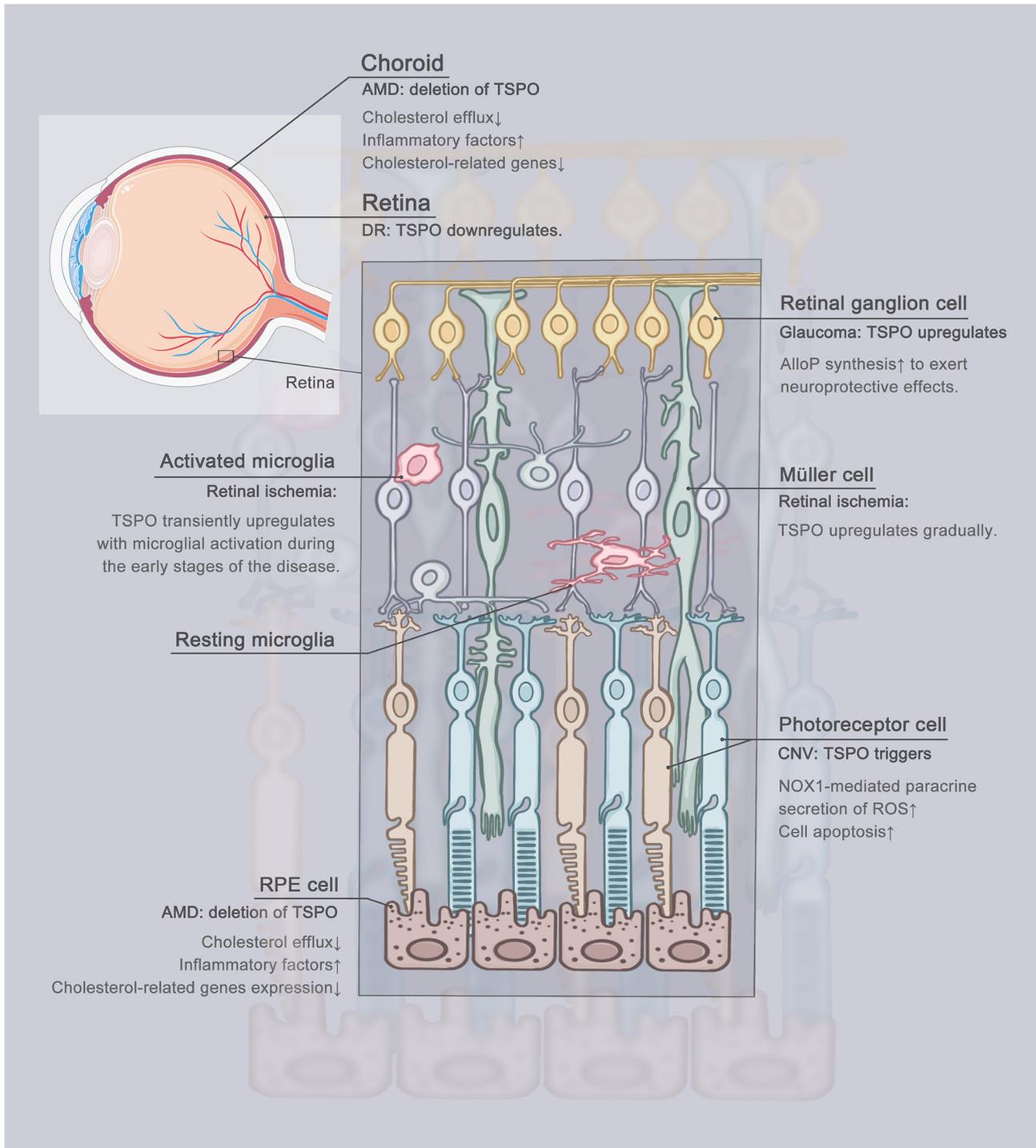


Figure 2. Affected areas or cell types associated with TSPO in various ocular diseases. TSPO, translocator protein; AMD, age-related macular degeneration; DR, diabetic retinopathy; AlloP, allopregnanolone; CNV, choroidal neovascularization; NOX1, NADPH oxidase 1; ROS, reactive oxygen species; RPE, retinal pigment epithelium.

pathogenesis of wet AMD (105,106). Aberrant expression of cholesterol homeostasis genes may lead to cholesterol and lipid dysregulation, thereby worsening AMD progression (107,108). This has been demonstrated in several experimental animal models showing AMD lesion progression (107,109,110). In addition, the release of inflammatory factors, the accumulation of ROS and the phagocytosis of neurons are involved in photoreceptor apoptosis in AMD (111-113). TSPO-knockout mice exhibit AMD-like features, including impaired cholesterol

efflux, increased cholesterol, triglycerides and phospholipids in the RPE and choroid, increased levels of inflammatory factors (IL-1 β and TNF- α) and downregulation of cholesterol-related genes (Nr1h3, Abca1, Abcg1, Cyp27a1 and Cyp46a1) (114). In a laser-induced choroidal neovascularization (CNV) model, TSPO regulated NOX1-mediated paracrine secretion of ROS, which is a crucial pathway for promoting pro-photoreceptor cell apoptosis (40). By contrast, angiogenic factors are generated via a non-NOX-dependent pathway, which can

be reversed by TSPO ligand treatment (115). These findings suggest that TSPO deficiency may contribute to the core pathogenesis of AMD. The TSPO ligand, etifoxine, upregulated cholesterol homeostasis gene expression in both RPE and choroidal vascular endothelial cells, promoting cholesterol efflux in a mouse AMD model (25,84). In addition, in human RPE cells (ARPE-19 cells), the FGIN-1-27, XBD173 and etifoxine ligands also significantly promote cholesterol efflux, whereas in TSPO knockdown RPE cells, this therapeutic effect is reversed (17). The aforementioned results imply that targeting the TSPO-mediated cholesterol efflux process may be effective in improving subretinal lipid accumulation in AMD. ROS production in RPE and choroid endothelial cells is also downregulated following etifoxine treatment (84). For neovascular AMD, XBD173 treatment inhibits the generation of CNV, which is thought to be associated with inhibiting pro-angiogenic factor (Vegf, Ang1 and Ang2) expression, regulating phagocytosis and facilitating the transition of microglia to neuroprotective phenotypes (40). In addition, inhibition of autocrine IL-1 β levels in RPE, which has previously been shown to induce VEGF production, also provides a similar protective effect (116). In general, the mechanisms by which CNVs are formed are complex, and it is likely that TSPO ligands do not inhibit CNVs via a single pathway; thus, specific mechanisms warrant further exploration.

Retinal ischemia. Retinal ischemia is a major cause of visual impairment, often leading to irreversible morphological and functional injuries with depleted ATP stores (117). Extensive research has been devoted to clarifying the mechanisms of ischemia-induced neuronal damage, although debates persist. Retinal ischemic injury involves a cascade of self-reinforcing and destructive events, including neuronal depolarization, calcium imbalance and oxidative stress due to increased energy depletion and enhanced glutamate stimulation (118-121). TSPO was discovered to be upregulated in microglia during the early stages of retinal ischemia injury, which then decreased 4 days later (16). TSPO expression increases over time in Müller cells, which is the primary immune cell type in the retina, indicating an association between TSPO and the active retinal immune response during ischemic injury (16,18). A number of studies have shown that TSPO is closely associated with microglial activation and microglial energy metabolism (98,122). Thus, the transient increase in TSPO expression may be due to a microglia-mediated immune response or an acute response to an imbalanced energy supply in the postischemic phase. Furthermore, mice treated with the TSPO ligand, XBD173, have a significant reduction in neuron death in the postischemic retina. In addition, they have a decreased microglial number accompanied by phenotypic transformation from the M1 type to the M2 type, indicating anti-inflammatory activation (18). This finding concurred with those of Wang *et al* (16) and Karlstetter *et al* (77), who found that XBD173 inhibited microglial neurotoxicity and proliferation. The effect of XBD173 on Müller cells was also notable since it increased the level of DBI in postischemic Müller cells (18). As aforementioned, DBI an endogenous TSPO ligand, can alleviate neuroinflammation and induce microglia to favor the resting state (16). XBD173 regulates glutamine synthetase expression, thereby maintaining the metabolism of a 'neuron-supportive

microenvironment' and protecting against glutamate-overload induced neurotoxic injury (123-126). It should be noted that the aforementioned effect of XBD173 was incomplete, as the transient upregulation of inflammatory markers as well as the downregulation of glutamine synthetase were still detected following XBD173 therapy. Given the high abundance of TSPO in the RPE, its role in retinal ischemia injury should not be overlooked. However, to the best of our knowledge, no relevant studies on the association between TSPO and RPE injury in retinal ischemia have been published to date. In summary, TSPO is involved in the pathogenic reactions of specific cell types to retinal ischemia and XBD173 treatment increased retinal neuron survival, to a certain extent.

DR. DR is the most prevalent complication of diabetes mellitus (DM) (127) and adversely affects the vision of working-aged individuals (128,129). The pathogenesis of DM and DR is complicated and unclear and treatment remains challenging due to recurrent vitreous hemorrhage and progressive loss of function (121). Hyperglycemia is considered to be a leading cause of metabolic disorders in the retinal vasculature via various pathways (130), including the protein kinase C, polyol and hexosamine pathways (131,132). Inflammation and dysfunction of glial cells are also involved in the onset and progression of DR (133,134). Other reported mechanisms of DR include mitochondrial dysfunction and oxidative stress damage, both of which accelerate retinal neuronal apoptosis (135-137). Correspondingly, TSPO has been suggested to play a role in the regulation of glucose metabolism, and the TSPO ligands, Ro5-4864 and PK11195, were both found to reduce the overall glucose levels in zebrafish larvae (70). Moreover, activation of TSPO regulates glucose homeostasis in adipocytes and it has been suggested that the TSPO ligands, PK11195 and FGIN-1-27, increase adipogenesis and glucose absorption by regulating mitochondrial activity and cholesterol transport (138). In addition, Ro5-4864 protects rat models of type-2 DM from metabolic disorders by limiting cholesterol transport into mitochondria, lowering oxysterol accumulation and reducing oxidative stress (139). Furthermore, TSPO relieves diabetic neuropathy by reportedly raising neuroactive steroid levels (11). Thus, TSPO has been identified as a potential target for diabetes management. Despite the increasing number of studies regarding the association between TSPO and DM complications, few investigations on the mechanism of TSPO in DR have been conducted. Zhou *et al* (20) found that TSPO was downregulated in rat models of type-2 DM 12 weeks after commencement of disease modeling. TSPO has previously been proposed to be an appropriate candidate marker of microglial activation (16); it was shown to be transiently upregulated in the early stages of retinal injury along with stress-induced microglial activation, while its expression was lower than that of controls or even absent after microglia returned to a resting state (140,141). Therefore, Zhou *et al* (20) hypothesized that the downregulation of TSPO in the retina indicated the activation of microglia in the retina during the early stages of diabetes. However, this hypothesis requires further study, as microglial activation was not consistently observed in the diabetic models, nor were changes in TSPO expression levels. Guo *et al* (19) reported significantly higher expression levels of TSPO in the peripheral blood of patients

with diabetes than in the controls, and a higher TSPO level in the active proliferative DR subgroup than in the inactive proliferative DR subgroup. Moreover, Guo *et al* (19) found that the TSPO/VDAC complex positively associates with several inflammatory cytokines, such as NOD-like receptor pyrin domain-containing 3, suggesting an important role in DR development and progression.

Glaucoma. Glaucoma is a leading cause of irreversible blindness worldwide and is characterized by progressive optic nerve injury and visual field loss (142,143). Glaucoma is a multifactorial disorder and elevated intraocular pressure is thought to be a major risk factor, leading to morphological deformation of the lamina cribrosa and disturbance of axoplasmic transport in RGC axons (144). Acute or chronic deficiency of these RGC neurotrophic signals ultimately leads to apoptosis (144). Other related pathogenesises of glaucoma include ocular hemodynamic abnormalities, low intracranial pressure, autoimmune dysfunction and mitochondrial dysfunction, while further studies are needed to investigate the detailed mechanisms of RGC injury (145). The neurosteroid, allopregnanolone (AlloP), has been shown to synthesize and exert neuroprotective effects on the retina through the GABA_A receptor pathway in a rat model of acute glaucoma *in vitro* (146,147). As a major mitochondrial cholesterol carrier, TSPO mediates the transport of cholesterol into the mitochondria, which is a key process in the synthesis of pregnenolone (57). Pregnenolone then functions as the starting material for AlloP synthesis (83,148). Under elevated pressure (75 mmHg), TSPO expression is found to be upregulated and TSPO colocalizes with AlloP and 5 α -reductase (5 α RD; a rate-limiting enzyme in the AlloP synthesis pathway) in RGCs, suggesting that RGCs are the principal site of AlloP synthesis (57). Furthermore, the TSPO antagonist, atriol, significantly inhibits the production of AlloP (149), suggesting that TSPO is important in AlloP synthesis. In addition, atriol induces changes in retinal excitotoxicity, including edema-like changes in the inner plexiform layer and bull's eye formation in the inner nuclear layer (150,151). Moreover, the TSPO ligand, PK11195, acts as a selective agonist that promotes TSPO expression and inhibits pressure-induced RGC apoptosis (57). Atriol and 5 α RD inhibitors (24) reverse this effect, suggesting that PK11195 may exert neuroprotective effects by promoting the TSPO-5 α RD-mediated AlloP synthesis pathway, as aforementioned. Hence, TSPO agonists may have therapeutic potential for ocular hypertension injury.

6. Conclusion

Overall, the present review concluded that TSPO is broadly involved in multiple cellular processes as well as mitochondrial functions. TSPO participates in the occurrence and development of various ocular diseases, including AMD, DR, retinal ischemia and glaucoma, with evidence of both protective and aggravating effects in the observed pathology of the diseases. There is increasing evidence that endogenous or synthetic TSPO ligands, which modulate TSPO functions, play beneficial roles in the aforementioned diseases primarily by promoting steroidogenesis, maintaining cholesterol homeostasis, exerting anti-inflammatory effects, decreasing ROS production and regulating microglial activation. These findings

suggest that TSPO may be a key potential therapeutic target. Despite the progress made in understanding the role of TSPO in ocular diseases, the expression of TSPO in ocular surface tissues, the lens, the lacrimal gland and the optic nerve and whether TSPO plays a key role in these diseases remain to be investigated. Moreover, whether TSPO is an essential protein for ocular tissue in healthy individuals is another question that requires more extensive study. At present, the use of TSPO ligands in the ocular environment is limited and there is a lack of comprehensive evaluation of their potential adverse effects. Only by addressing these issues can TSPO and its ligands be applied on a larger scale.

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Authors' contributions

MY and SZ designed the study; MY performed the figure preparation and manuscript draft; and SZ reviewed and edited the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

References

- Shoshan-Barmatz V, Pittala S and Mizrahi D: VDAC1 and the TSPO: Expression, Interactions, and associated functions in health and disease states. *Int J Mol Sci* 20: 3348, 2019.
- Braestrup C and Squires RF: Specific benzodiazepine receptors in rat brain characterized by high-affinity (3H)diazepam binding. *Proc Natl Acad Sci USA* 74: 3805-3809, 1977.
- Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR and Gavish M: Translocator protein (18kDa): New nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* 27: 402-409, 2006.
- Tu LN, Zhao AH, Hussein M, Stocco DM and Selvaraj V: Translocator Protein (TSPO) affects mitochondrial fatty acid oxidation in steroidogenic cells. *Endocrinology* 157: 1110-1121, 2016.

5. Bonsack F and Sukumari-Ramesh S: TSPO: An evolutionarily conserved protein with elusive functions. *Int J Mol Sci* 19: 1694, 2018.
6. Fan J and Papadopoulos V: Mitochondrial TSPO deficiency triggers retrograde signaling in MA-10 mouse tumor leydig cells. *Int J Mol Sci* 22: 252, 2020.
7. Vainshtein A, Veenman L, Shterenberg A, Singh S, Masarwa A, Dutta B, Island B, Tsoglin E, Levin E, Leschiner S, *et al*: Quinazoline-based tricyclic compounds that regulate programmed cell death, induce neuronal differentiation, and are curative in animal models for excitotoxicity and hereditary brain disease. *Cell Death Discov* 1: 15027, 2015.
8. Sileikyte J, Blachly-Dyson E, Sewell R, Carpi A, Menabò R, Di Lisa F, Ricchelli F, Bernardi P and Forte M: Regulation of the mitochondrial permeability transition pore by the outer membrane does not involve the peripheral benzodiazepine receptor (Translocator Protein of 18 kDa (TSPO)). *J Biol Chem* 289: 13769-13781, 2014.
9. Ilkan Z and Akar FG: The mitochondrial translocator protein and the emerging link between oxidative stress and arrhythmias in the diabetic heart. *Front Physiol* 9: 1518, 2018.
10. Arbo BD, Ribeiro MF and Garcia-Segura LM: Development of new treatments for Alzheimer's disease based on the modulation of translocator protein (TSPO). *Ageing Res Rev* 54: 100943, 2019.
11. Mitro N, Cermenati G, Giatti S, Abbiati F, Pesaresi M, Calabrese D, Garcia-Segura LM, Caruso D and Melcangi RC: LXR and TSPO as new therapeutic targets to increase the levels of neuroactive steroids in the central nervous system of diabetic animals. *Neurochem Int* 60: 616-621, 2012.
12. Veenman L and Gavish M: The peripheral-type benzodiazepine receptor and the cardiovascular system. Implications for drug development. *Pharmacol Ther* 110: 503-524, 2006.
13. Barron AM, Garcia-Segura LM, Caruso D, Jayaraman A, Lee JW, Melcangi RC and Pike CJ: Ligand for translocator protein reverses pathology in a mouse model of Alzheimer's disease. *J Neurosci* 33: 8891-8897, 2013.
14. Daugherty DJ, Selvaraj V, Chechneva OV, Liu XB, Pleasure DE and Deng W: A TSPO ligand is protective in a mouse model of multiple sclerosis. *EMBO Mol Med* 5: 891-903, 2013.
15. Wu LP, Gong ZF, Wang H, Zhou ZS, Zhang MM, Liu C, Ren HM, Yang J, Han Y and Zeng CY: TSPO ligands prevent the proliferation of vascular smooth muscle cells and attenuate neointima formation through AMPK activation. *Acta Pharmacol Sin* 41: 34-46, 2020.
16. Wang M, Wang X, Zhao L, Ma W, Rodriguez IR, Fariss RN and Wong WT: Macrogliia-microglia interactions via TSPO signaling regulates microglial activation in the mouse retina. *J Neurosci* 34: 3793-3806, 2014.
17. Biswas L, Zhou X, Dhillon B, Graham A and Shu X: Retinal pigment epithelium cholesterol efflux mediated by the 18 kDa translocator protein, TSPO, a potential target for treating age-related macular degeneration. *Hum Mol Genet* 26: 4327-4339, 2017.
18. Mages K, Grassmann F, Jagle H, Rupprecht R, Weber BHF, Hauck SM and Grosche A: The agonistic TSPO ligand XBD173 attenuates the glial response thereby protecting inner retinal neurons in a murine model of retinal ischemia. *J Neuroinflammation* 16: 43, 2019.
19. Guo Y, Sun Z, Wang L, Jiang R, Shu Q and Xu G: Increased expression of TSPO-VDAC complex is correlated with NLRP3 inflammasome activation in diabetic retinopathy. *Mol Med Rep* 26: 353, 2022.
20. Zhou Y, Ou Y, Ju Z, Zhang X, Zheng L, Li J, Sun Y and Liu X: Visualization of translocator protein (18 kDa) (TSPO) in the retina of diabetic retinopathy rats using fluorine-18-DPA-714. *Ann Nucl Med* 34: 675-681, 2020.
21. Lacapere JJ, Duma L, Finet S, Kassiou M and Papadopoulos V: Insight into the Structural Features of TSPO: Implications for drug development. *Trends Pharmacol Sci* 41: 110-122, 2020.
22. Papadopoulos V, Aghazadeh Y, Fan J, Campioli E, Zirkin B and Midzak A: Translocator protein-mediated pharmacology of cholesterol transport and steroidogenesis. *Mol Cell Endocrinol* 408: 90-98, 2015.
23. Jaremko M, Jaremko L, Jaipuria G, Becker S and Zweckstetter M: Structure of the mammalian TSPO/PBR protein. *Biochem Soc Trans* 43: 566-571, 2015.
24. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, Fan J, Akula N, Groyer G, Adams D and Schumacher M: Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat Rev Drug Discov* 9: 971-988, 2010.
25. Biswas L, Farhan F, Reilly J, Bartholomew C and Shu X: TSPO ligands promote cholesterol efflux and suppress oxidative stress and inflammation in choroidal endothelial cells. *Int J Mol Sci* 19: 3740, 2018.
26. Jaremko L, Jaremko M, Giller K, Becker S and Zweckstetter M: Structure of the mitochondrial translocator protein in complex with a diagnostic ligand. *Science* 343: 1363-1366, 2014.
27. Morohaku K, Pelton SH, Daugherty DJ, Butler WR, Deng W and Selvaraj V: Translocator protein/peripheral benzodiazepine receptor is not required for steroid hormone biosynthesis. *Endocrinology* 155: 89-97, 2014.
28. Tu LN, Morohaku K, Manna PR, Pelton SH, Butler WR, Stocco DM and Selvaraj V: Peripheral benzodiazepine receptor/translocator protein global knock-out mice are viable with no effects on steroid hormone biosynthesis. *J Biol Chem* 289: 27444-27454, 2014.
29. Tu LN, Zhao AH, Stocco DM and Selvaraj V: PK11195 effect on steroidogenesis is not mediated through the translocator protein (TSPO). *Endocrinology* 156: 1033-1039, 2015.
30. Zhao AH, Tu LN, Mukai C, Sirivelu MP, Pillai VV, Morohaku K, Cohen R and Selvaraj V: Mitochondrial translocator protein (TSPO) function is not essential for heme biosynthesis. *J Biol Chem* 291: 1591-1603, 2016.
31. Casellas P, Galiegue S and Basile AS: Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem Int* 40: 475-486, 2002.
32. Corsi L, Geminiani E and Baraldi M: Peripheral benzodiazepine receptor (PBR) new insight in cell proliferation and cell differentiation review. *Curr Clin Pharmacol* 3: 38-45, 2008.
33. Gatliff J, East D, Crosby J, Abeti R, Harvey R, Craigen W, Parker P and Campanella M: TSPO interacts with VDAC1 and triggers a ROS-mediated inhibition of mitochondrial quality control. *Autophagy* 10: 2279-2296, 2014.
34. Varga B, Marko K, Hadinger N, Jelitai M, Demeter K, Tihanyi K, Vas A and Madarász E: Translocator protein (TSPO 18kDa) is expressed by neural stem and neuronal precursor cells. *Neurosci Lett* 462: 257-262, 2009.
35. Veenman L and Gavish M: The role of 18 kDa mitochondrial translocator protein (TSPO) in programmed cell death, and effects of steroids on TSPO expression. *Curr Mol Med* 12: 398-412, 2012.
36. Bernardi P, Carraro M and Lippe G: The mitochondrial permeability transition: Recent progress and open questions. *FEBS J* 289: 7051-7074, 2022.
37. Loth MK, Guariglia SR, Re DB, Perez J, de Paiva VN, Dziedzic JL, Chambers JW, Azzam DJ and Guilarte TR: A novel interaction of translocator protein 18 kDa (TSPO) with NADPH oxidase in microglia. *Mol Neurobiol* 57: 4467-4487, 2020.
38. Gatliff J, East DA, Singh A, Alvarez MS, Frison M, Matic I, Ferraina C, Sampson N, Turkheimer F and Campanella M: A role for TSPO in mitochondrial Ca²⁺ homeostasis and redox stress signaling. *Cell Death Dis* 8: e2896, 2017.
39. Zeno S, Veenman L, Katz Y, Bode J, Gavish M and Zaaroor M: The 18 kDa mitochondrial translocator protein (TSPO) prevents accumulation of protoporphyrin IX. Involvement of reactive oxygen species (ROS). *Curr Mol Med* 12: 494-501, 2012.
40. Wolf A, Herb M, Schramm M and Langmann T: The TSPO-NOX1 axis controls phagocyte-triggered pathological angiogenesis in the eye. *Nat Commun* 11: 2709, 2020.
41. Rupprecht R, Rammes G, Eser D, Baghai TC, Schüle C, Nothdurfter C, Troxler T, Gentsch C, Kalkman HO, Chaperon F, *et al*: Translocator protein (18 kD) as target for anxiolytics without benzodiazepine-like side effects. *Science* 325: 490-493, 2009.
42. Barresi E, Robello M, Costa B, Da Pozzo E, Baglini E, Salerno S, Da Settimo F, Martini C and Taliani S: An update into the medicinal chemistry of translocator protein (TSPO) ligands. *Eur J Med Chem* 209: 112924, 2021.
43. Gut P: Targeting mitochondrial energy metabolism with TSPO ligands. *Biochem Soc Trans* 43: 537-542, 2015.
44. Taketani S, Kohno H, Furukawa T and Tokunaga R: Involvement of peripheral-type benzodiazepine receptors in the intracellular transport of heme and porphyrins. *J Biochem* 117: 875-880, 1995.
45. Snyder SH, Verma A and Trifiletti RR: The peripheral-type benzodiazepine receptor: A protein of mitochondrial outer membranes utilizing porphyrins as endogenous ligands. *FASEB J* 1: 282-288, 1987.
46. Veenman L, Vainshtein A, Yasin N, Azrad M and Gavish M: Tetrapyrroles as endogenous TSPO ligands in eukaryotes and prokaryotes: Comparisons with synthetic ligands. *Int J Mol Sci* 17: 880, 2016.

47. Tonon MC, Vaudry H, Chuquet J, Guillebaud F, Fan J, Masmoudi-Kouki O, Vaudry D, Lanfray D, Morin F, Prevot V, *et al*: Endozepines and their receptors: Structure, functions and pathophysiological significance. *Pharmacol Ther* 208: 107386, 2020.
48. Ma Z, An R, Chen M, Wang X and Zhu M: Random versus Block Glycopolymers bearing betulin and porphyrin for enhanced photodynamic therapy. *Biomacromolecules* 23: 5074-5083, 2022.
49. Yamamoto M, Arimura H, Fukushige T, Minami K, Nishizawa Y, Tanimoto A, Kanekura T, Nakagawa M, Akiyama S and Furukawa T: Abcb10 role in heme biosynthesis in vivo: Abcb10 knockout in mice causes anemia with protoporphyrin IX and iron accumulation. *Mol Cell Biol* 34: 1077-1084, 2014.
50. Batoko H, Veljanovski V and Jurkiewicz P: Enigmatic Translocator protein (TSPO) and cellular stress regulation. *Trends Biochem Sci* 40: 497-503, 2015.
51. Korolnek T and Hamza I: Like iron in the blood of the people: The requirement for heme trafficking in iron metabolism. *Front Pharmacol* 5: 126, 2014.
52. Chauveau F, Boutin H, Van Camp N, Dolle F and Tavitian B: Nuclear imaging of neuroinflammation: A comprehensive review of [¹¹C]PK11195 challengers. *Eur J Nucl Med Mol Imaging* 35: 2304-2319, 2008.
53. Dolle F, Luus C, Reynolds A and Kassiou M: Radiolabelled molecules for imaging the translocator protein (18 kDa) using positron emission tomography. *Curr Med Chem* 16: 2899-2923, 2009.
54. Fan Z, Calsolaro V, Atkinson RA, Femminella GD, Waldman A, Buckley C, Trigg W, Brooks DJ, Hinz R and Edison P: Flutriciclamide (18F-GE180) PET: First-in-Human PET study of novel third-generation in vivo marker of human translocator protein. *J Nucl Med* 57: 1753-1759, 2016.
55. Chiabrando D, Marro S, Mercurio S, Giorgi C, Petrillo S, Vinchi F, Fiorito V, Fagoonee S, Camporeale A, Turco E, *et al*: The mitochondrial heme exporter FLVCR1b mediates erythroid differentiation. *J Clin Invest* 122: 4569-4579, 2012.
56. Ni R, Rojdnier J, Voytenko L, Dyrks T, Thiele A, Marutle A and Nordberg A: In vitro characterization of the regional binding distribution of amyloid PET tracer florbetaben and the glia tracers deprenyl and PK11195 in Autopsy Alzheimer's brain tissue. *J Alzheimers Dis* 80: 1723-1737, 2021.
57. Ishikawa M, Yoshitomi T, Covey DF, Zorumski CF and Izumi Y: TSPO activation modulates the effects of high pressure in a rat ex vivo glaucoma model. *Neuropharmacology* 111: 142-159, 2016.
58. Papadopoulos V and Lecanu L: Translocator protein (18 kDa) TSPO: An emerging therapeutic target in neurotrauma. *Exp Neurol* 219: 53-57, 2009.
59. Girard C, Liu S, Adams D, Lacroix C, Sinéus M, Boucher C, Papadopoulos V, Rupprecht R, Schumacher M and Groyer G: Axonal regeneration and neuroinflammation: Roles for the translocator protein 18 kDa. *J Neuroendocrinol* 24: 71-81, 2012.
60. Ravikumar B, Crawford D, Dellovade T, Savinainen A, Graham D, Liere P, Oudinet JP, Webb M and Hering H: Differential efficacy of the TSPO ligands etifoxine and XBD-173 in two rodent models of Multiple Sclerosis. *Neuropharmacology* 108: 229-237, 2016.
61. Choi HB, Khoo C, Ryu JK, van Breemen E, Kim SU and McLarnon JG: Inhibition of lipopolysaccharide-induced cyclooxygenase-2, tumor necrosis factor- α and [Ca²⁺]_i responses in human microglia by the peripheral benzodiazepine receptor ligand PK11195. *J Neurochem* 83: 546-555, 2002.
62. Lebedeva IV, Su ZZ, Sarkar D, Kitada S, Dent P, Waxman S, Reed JC and Fisher PB: Melanoma differentiation associated gene-7, mda-7/interleukin-24, induces apoptosis in prostate cancer cells by promoting mitochondrial dysfunction and inducing reactive oxygen species. *Cancer Res* 63: 8138-8144, 2003.
63. Seneviratne MS, Faccenda D, De Biase V and Campanella M: PK11195 inhibits mitophagy targeting the F1Fo-ATP synthase in Bcl-2 knock-down cells. *Curr Mol Med* 12: 476-482, 2012.
64. Bonora M, Giorgi C and Pinton P: Molecular mechanisms and consequences of mitochondrial permeability transition. *Nat Rev Mol Cell Biol* 23: 266-285, 2022.
65. Gonzalez-Polo RA, Carvalho G, Braun T, Decaudin D, Fabre C, Larochette N, Perfettini JL, Djavaheri-Mergny M, Youlyouz-Marfak I, Codogno P, *et al*: PK11195 potently sensitizes to apoptosis induction independently from the peripheral benzodiazepine receptor. *Oncogene* 24: 7503-7513, 2005.
66. Choi J, Ifuku M, Noda M and Guilarte TR: Translocator protein (18 kDa)/peripheral benzodiazepine receptor specific ligands induce microglia functions consistent with an activated state. *Glia* 59: 219-230, 2011.
67. Awad M and Gavish M: Binding of [3H]Ro 5-4864 and [3H]PK 11195 to cerebral cortex and peripheral tissues of various species: Species differences and heterogeneity in peripheral benzodiazepine binding sites. *J Neurochem* 49: 1407-1414, 1987.
68. Marangos PJ, Patel J, Boulenger JP and Clark-Rosenberg R: Characterization of peripheral-type benzodiazepine binding sites in brain using [3H]Ro 5-4864. *Mol Pharmacol* 22: 26-32, 1982.
69. Kreisl WC, Kim MJ, Coughlin JM, Henter ID, Owen DR and Innis RB: PET imaging of neuroinflammation in neurological disorders. *Lancet Neurol* 19: 940-950, 2020.
70. Gut P, Baeza-Raja B, Andersson O, Hasenkamp L, Hsiao J, Hesselson D, Akassoglou K, Verdin E, Hirschey MD and Stainier DY: Whole-organism screening for gluconeogenesis identifies activators of fasting metabolism. *Nat Chem Biol* 9: 97-104, 2013.
71. Da Pozzo E, Giacomelli C, Barresi E, Costa B, Taliani S, Passetti Fda S and Martini C: Targeting the 18-kDa translocator protein: recent perspectives for neuroprotection. *Biochem Soc Trans* 43: 559-565, 2015.
72. Gavioli EC, Duarte FS, Bressan E, Ferrara P, Farges RC and De Lima TC: Antidepressant-like effect of Ro5-4864, a peripheral-type benzodiazepine receptor ligand, in forced swimming test. *Eur J Pharmacol* 471: 21-26, 2003.
73. Kita A, Kohayakawa H, Kinoshita T, Ochi Y, Nakamichi K, Kurumiya S, Furukawa K and Oka M: Antianxiety and antidepressant-like effects of AC-5216, a novel mitochondrial benzodiazepine receptor ligand. *Br J Pharmacol* 142: 1059-1072, 2004.
74. Baez E, Guio-Vega GP, Echeverria V, Sandoval-Rueda DA and Barreto GE: 4'-chlorodiazepam protects mitochondria in T98G astrocyte cell line from glucose deprivation. *Neurotox Res* 32: 163-171, 2017.
75. Nothdurfter C, Rammes G, Baghai TC, Schüle C, Schumacher M, Papadopoulos V and Rupprecht R: Translocator protein (18 kDa) as a target for novel anxiolytics with a favourable side-effect profile. *J Neuroendocrinol* 24: 82-92, 2012.
76. Kita A, Kinoshita T, Kohayakawa H, Furukawa K and Akaike A: Lack of tolerance to anxiety and withdrawal symptoms in mice repeatedly treated with AC-5216, a selective TSPO ligand. *Prog Neuropsychopharmacol Biol Psychiatry* 33: 1040-1045, 2009.
77. Karlstetter M, Nothdurfter C, Aslanidis A, Moeller K, Horn F, Scholz R, Neumann H, Weber BH, Rupprecht R and Langmann T: Translocator protein (18 kDa) (TSPO) is expressed in reactive retinal microglia and modulates microglial inflammation and phagocytosis. *J Neuroinflammation* 11: 3, 2014.
78. Scholz R, Caramoy A, Bhuckory MB, Rashid K, Chen M, Xu H, Grimm C and Langmann T: Targeting translocator protein (18 kDa) (TSPO) dampens pro-inflammatory microglia reactivity in the retina and protects from degeneration. *J Neuroinflammation* 12: 201, 2015.
79. Schlichter R, Rybalchenko V, Poisbeau P, Verleye M and Gillardin J: Modulation of GABAergic synaptic transmission by the non-benzodiazepine anxiolytic etifoxine. *Neuropharmacology* 39: 1523-1535, 2000.
80. Choi YM and Kim KH: Etifoxine for pain patients with anxiety. *Korean J Pain* 28: 4-10, 2015.
81. Girard C, Liu S, Cadepond F, Adams D, Lacroix C, Verleye M, Gillardin JM, Baulieu EE, Schumacher M and Schweizer-Groyer G: Etifoxine improves peripheral nerve regeneration and functional recovery. *Proc Natl Acad Sci USA* 105: 20505-20510, 2008.
82. Olsen RW and Li GD: GABA(A) receptors as molecular targets of general anesthetics: Identification of binding sites provides clues to allosteric modulation. *Can J Anaesth* 58: 206-215, 2011.
83. Gunn BG, Brown AR, Lambert JJ and Belelli D: Neurosteroids and GABA(A) Receptor Interactions: A focus on stress. *Front Neurosci* 5: 131, 2011.
84. Biswas L, Ibrahim KS, Li X, Zhou X, Zeng Z, Craft J and Shu X: Effect of a TSPO ligand on retinal pigment epithelial cholesterol homeostasis in high-fat fed mice, implication for age-related macular degeneration. *Exp Eye Res* 208: 108625, 2021.
85. Ibrahim KS, Craft JA, Biswas L, Spencer J and Shu X: Etifoxine reverses weight gain and alters the colonic bacterial community in a mouse model of obesity. *Biochem Pharmacol* 180: 114151, 2020.
86. Costa B, Cavallini C, Da Pozzo E, Taliani S, Da Settimo F and Martini C: The anxiolytic etifoxine binds to TSPO Ro5-4864 binding site with long residence time showing a high neurosteroidogenic activity. *ACS Chem Neurosci* 8: 1448-1454, 2017.

87. Kondo D, Saegusa H, Yabe R, Takasaki I, Kurihara T, Zong S and Tanabe T: Peripheral-type benzodiazepine receptor antagonist is effective in relieving neuropathic pain in mice. *J Pharmacol Sci* 110: 55-63, 2009.
88. Tsukagoshi E, Kawaguchi M, Shinomiya T, Yoshikawa M, Kawano T, Okubo M and Sawaki K: Diazepam enhances production of diazepam-binding inhibitor (DBI), a negative saliva secretion regulator, localized in rat salivary gland. *J Pharmacol Sci* 115: 221-229, 2011.
89. Morin D, Musman J, Pons S, Berdeaux A and Ghaleh B: Mitochondrial translocator protein (TSPO): From physiology to cardioprotection. *Biochem Pharmacol* 105: 1-13, 2016.
90. Leducq N, Bono F, Sulpice T, Vin V, Janiak P, Fur GL, O'Connor SE and Herbert JM: Role of peripheral benzodiazepine receptors in mitochondrial, cellular, and cardiac damage induced by oxidative stress and ischemia-reperfusion. *J Pharmacol Exp Ther* 306: 828-837, 2003.
91. Santoro A, Mattace Raso G, Taliani S, Da Pozzo E, Simorini F, Costa B, Martini C, Laneri S, Sacchi A, Cosimelli B, *et al*: TSPO-ligands prevent oxidative damage and inflammatory response in C6 glioma cells by neurosteroid synthesis. *Eur J Pharm Sci* 88: 124-131, 2016.
92. Klee K, Storti F, Barben M, Samardzija M, Langmann T, Dunaief J and Grimm C: Systemic knockout of *Tspo* in mice does not affect retinal morphology, function and susceptibility to degeneration. *Exp Eye Res* 188: 107816, 2019.
93. Storti F, Klee K, Todorova V, Steiner R, Othman A, van der Velde-Visser S, Samardzija M, Meneau I, Barben M, Karademir D, *et al*: Impaired ABCA1/ABCG1-mediated lipid efflux in the mouse retinal pigment epithelium (RPE) leads to retinal degeneration. *Elife* 8: e45100, 2019.
94. Santos AM, Calvente R, Tassi M, Carrasco MC, Martín-Oliva D, Marín-Teva JL, Navascués J and Cuadros MA: Embryonic and postnatal development of microglial cells in the mouse retina. *J Comp Neurol* 506: 224-239, 2008.
95. Lee MA, Sitko AA, Khalid S, Shirasu-Hiza M and Mason CA: Spatiotemporal distribution of glia in and around the developing mouse optic tract. *J Comp Neurol* 527: 508-521, 2019.
96. Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, Nouri N, Micheva KD, Mehalow AK, Huberman AD, Stafford B, *et al*: The classical complement cascade mediates CNS synapse elimination. *Cell* 131: 1164-1178, 2007.
97. Prinz M and Mildner A: Microglia in the CNS: Immigrants from another world. *Glia* 59: 177-187, 2011.
98. Banati RB, Middleton RJ, Chan R, Hatty CR, Kam WW, Quin C, Graeber MB, Parmar A, Zahra D, Callaghan P, *et al*: Positron emission tomography and functional characterization of a complete PBR/TSPO knockout. *Nat Commun* 5: 5452, 2014.
99. Barron AM, Ji B, Kito S, Suhara T and Higuchi M: Steroidogenic abnormalities in translocator protein knockout mice and significance in the aging male. *Biochem J* 475: 75-85, 2018.
100. Grassmann F, Fauser S and Weber BH: The genetics of age-related macular degeneration (AMD)-Novel targets for designing treatment options? *Eur J Pharm Biopharm* 95: 194-202, 2015.
101. Csader S, Korhonen S, Kaarniranta K and Schwab U: The effect of dietary supplementations on delaying the progression of age-related macular degeneration: A systematic review and meta-analysis. *Nutrients* 14: 4273, 2022.
102. Fleckenstein M, Keenan TDL, Guymer RH, Chakravarthy U, Schmitz-Valckenberg S, Klaver CC, Wong WT and Chew EY: Age-related macular degeneration. *Nat Rev Dis Primers* 7: 31, 2021.
103. Pikuleva IA and Curcio CA: Cholesterol in the retina: The best is yet to come. *Prog Retin Eye Res* 41: 64-89, 2014.
104. Curcio CA, Presley JB, Malek G, Medeiros NE, Avery DV and Kruth HS: Esterified and unesterified cholesterol in drusen and basal deposits of eyes with age-related maculopathy. *Exp Eye Res* 81: 731-741, 2005.
105. Bhutto I and Luty G: Understanding age-related macular degeneration (AMD): Relationships between the photoreceptor/retinal pigment epithelium/Bruch's membrane/choriocapillaris complex. *Mol Aspects Med* 33: 295-317, 2012.
106. Biesemeier A, Taubitz T, Julien S, Yoeruek E and Schraermeyer U: Choriocapillaris breakdown precedes retinal degeneration in age-related macular degeneration. *Neurobiol Aging* 35: 2562-2573, 2014.
107. Fritsche LG, Igl W, Bailey JN, Grassmann F, Sengupta S, Bragg-Gresham JL, Burdon KP, Hebbiring SJ, Wen C, Gorski M, *et al*: A large genome-wide association study of age-related macular degeneration highlights contributions of rare and common variants. *Nat Genet* 48: 134-143, 2016.
108. Toomey CB, Johnson LV and Bowes Rickman C: Complement factor H in AMD: Bridging genetic associations and pathobiology. *Prog Retin Eye Res* 62: 38-57, 2018.
109. Malek G, Johnson LV, Mace BE, Saloupis P, Schmechel DE, Rickman DW, Toth CA, Sullivan PM and Bowes Rickman C: Apolipoprotein E allele-dependent pathogenesis: A model for age-related retinal degeneration. *Proc Natl Acad Sci USA* 102: 11900-11905, 2005.
110. Toomey CB, Kelly U, Saban DR and Bowes Rickman C: Regulation of age-related macular degeneration-like pathology by complement factor H. *Proc Natl Acad Sci USA* 112: E3040-E3049, 2015.
111. Langmann T: Microglia activation in retinal degeneration. *J Leukoc Biol* 81: 1345-1351, 2007.
112. Zeng H, Ding M, Chen XX and Lu Q: Microglial NADPH oxidase activation mediates rod cell death in the retinal degeneration in rd mice. *Neuroscience* 275: 54-61, 2014.
113. Zhao L, Zabel MK, Wang X, Ma W, Shah P, Fariss RN, Qian H, Parkhurst CN, Gan WB and Wong WT: Microglial phagocytosis of living photoreceptors contributes to inherited retinal degeneration. *EMBO Mol Med* 7: 1179-1197, 2015.
114. Farhan F, Almarhoun M, Wong A, Findlay AS, Bartholomew C, Williams MTS, Hurd TW and Shu X: Deletion of TSPO causes dysregulation of cholesterol metabolism in mouse retina. *Cells* 10: 3066, 2021.
115. Luckoff A, Scholz R, Sennlaub F, Xu H and Langmann T: Comprehensive analysis of mouse retinal mononuclear phagocytes. *Nat Protoc* 12: 1136-1150, 2017.
116. Nagineni CN, Kommineni VK, William A, Detrick B and Hooks JJ: Regulation of VEGF expression in human retinal cells by cytokines: implications for the role of inflammation in age-related macular degeneration. *J Cell Physiol* 227: 116-126, 2012.
117. Osborne NN, Casson RJ, Wood JP, Chidlow G, Graham M and Melena J: Retinal ischemia: Mechanisms of damage and potential therapeutic strategies. *Prog Retin Eye Res* 23: 91-147, 2004.
118. Osborne NN, Ugarte M, Chao M, Chidlow G, Bae JH, Wood JP and Nash MS: Neuroprotection in relation to retinal ischemia and relevance to glaucoma. *Surv Ophthalmol* 43 (Suppl 1): S102-S128, 1999.
119. Youngblood H, Robinson R, Sharma A and Sharma S: Proteomic biomarkers of retinal inflammation in diabetic retinopathy. *Int J Mol Sci* 20: 4755, 2019.
120. Khayat M, Williams M and Lois N: Ischemic retinal vein occlusion: Characterizing the more severe spectrum of retinal vein occlusion. *Surv Ophthalmol* 63: 816-850, 2018.
121. Heyck M, Bonsack B, Zhang H, Sadanandan N, Cozene B, Kingsbury C, Lee JY and Borlongan CV: The brain and eye: Treating cerebral and retinal ischemia through mitochondrial transfer. *Exp Biol Med (Maywood)* 244: 1485-1492, 2019.
122. Liu GJ, Middleton RJ, Kam WW, Chin DY, Hatty CR, Chan RH and Banati RB: Functional gains in energy and cell metabolism after TSPO gene insertion. *Cell Cycle* 16: 436-447, 2017.
123. Lieth E, Barber AJ, Xu B, Dice C, Ratz MJ, Tanase D and Strother JM: Glial reactivity and impaired glutamate metabolism in short-term experimental diabetic retinopathy. *Penn State Retina Research Group. Diabetes* 47: 815-820, 1998.
124. Wagner L, Pannicke T, Rupprecht V, Frommherz I, Volz C, Illes P, Hirrlinger J, Jäggle H, Egger V, Hayden PG, *et al*: Suppression of SNARE-dependent exocytosis in retinal glial cells and its effect on ischemia-induced neurodegeneration. *Glia* 65: 1059-1071, 2017.
125. Dkhissi O, Chanut E, Wasowicz M, Savoldelli M, Nguyen-Legros J, Minvielle F and Versaux-Botteri C: Retinal TUNEL-positive cells and high glutamate levels in vitreous humor of mutant quail with a glaucoma-like disorder. *Invest Ophthalmol Vis Sci* 40: 990-995, 1999.
126. Delyfer MN, Forster V, Neveux N, Picaud S, Leveillard T and Sahel JA: Evidence for glutamate-mediated excitotoxic mechanisms during photoreceptor degeneration in the rd1 mouse retina. *Mol Vis* 11: 688-696, 2005.
127. Vujosevic S, Aldington SJ, Silva P, Hernández C, Scanlon P, Peto T and Simó R: Screening for diabetic retinopathy: New perspectives and challenges. *Lancet Diabetes Endocrinol* 8: 337-347, 2020.

128. Wang W and Lo ACY: Diabetic retinopathy: Pathophysiology and treatments. *Int J Mol Sci* 19: 1816, 2018.
129. Fung TH, Patel B, Wilmot EG and Amoaku WM: Diabetic retinopathy for the non-ophthalmologist. *Clin Med (Lond)* 22: 112-116, 2022.
130. Brownlee M: The pathobiology of diabetic complications: A unifying mechanism. *Diabetes* 54: 1615-1625, 2005.
131. Naruse K, Nakamura J, Hamada Y, Nakayama M, Chaya S, Komori T, Kato K, Kasuya Y, Miwa K and Hotta N: Aldose reductase inhibition prevents glucose-induced apoptosis in cultured bovine retinal microvascular pericytes. *Exp Eye Res* 71: 309-315, 2000.
132. Romeo G, Liu WH, Asnagli V, Kern TS and Lorenzi M: Activation of nuclear factor-kappaB induced by diabetes and high glucose regulates a proapoptotic program in retinal pericytes. *Diabetes* 51: 2241-2248, 2002.
133. Abcouwer SF: Muller cell-microglia cross talk drives neuroinflammation in diabetic retinopathy. *Diabetes* 66: 261-263, 2017.
134. Sorrentino FS, Allkabes M, Salsini G, Bonifazzi C and Perri P: The importance of glial cells in the homeostasis of the retinal microenvironment and their pivotal role in the course of diabetic retinopathy. *Life Sci* 162: 54-59, 2016.
135. Podesta F, Romeo G, Liu WH, Krajewski S, Reed JC, Gerhardinger C and Lorenzi M: Bax is increased in the retina of diabetic subjects and is associated with pericyte apoptosis in vivo and in vitro. *Am J Pathol* 156: 1025-1032, 2000.
136. Tien T, Zhang J, Muto T, Kim D, Sarthy VP and Roy S: High glucose induces mitochondrial dysfunction in retinal muller cells: Implications for diabetic retinopathy. *Invest Ophthalmol Vis Sci* 58: 2915-2921, 2017.
137. Sasaki M, Ozawa Y, Kurihara T, Kubota S, Yuki K, Noda K, Kobayashi S, Ishida S and Tsubota K: Neurodegenerative influence of oxidative stress in the retina of a murine model of diabetes. *Diabetologia* 53: 971-979, 2010.
138. Li J and Papadopoulos V: Translocator protein (18 kDa) as a pharmacological target in adipocytes to regulate glucose homeostasis. *Biochem Pharmacol* 97: 99-110, 2015.
139. Musman J, Paradis S, Panel M, Pons S, Barau C, Caccia C, Leoni V, Ghaleh B and Morin D: A TSPO ligand prevents mitochondrial sterol accumulation and dysfunction during myocardial ischemia-reperfusion in hypercholesterolemic rats. *Biochem Pharmacol* 142: 87-95, 2017.
140. Ciudin A, Simo-Servat O, Hernandez C, Arcos G, Diego S, Sanabria A, Sotolongo O, Hernández I, Boada M and Simó R: Retinal Microperimetry: A new tool for identifying patients with type 2 diabetes at risk for developing Alzheimer disease. *Diabetes* 66: 3098-3104, 2017.
141. Kinuthia UM, Wolf A and Langmann T: Microglia and inflammatory responses in diabetic retinopathy. *Front Immunol* 11: 564077, 2020.
142. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, *et al*: Global causes of blindness and distance vision impairment 1990-2020: A systematic review and meta-analysis. *Lancet Glob Health* 5: e1221-e1234, 2017.
143. Tham YC, Li X, Wong TY, Quigley HA, Aung T and Cheng CY: Global prevalence of glaucoma and projections of glaucoma burden through 2040: A systematic review and meta-analysis. *Ophthalmology* 121: 2081-2090, 2014.
144. Zukerman R, Harris A, Oddone F, Siesky B, Verticchio Vercellin A and Ciulla TA: Glaucoma heritability: Molecular mechanisms of disease. *Genes (Basel)* 12: 1135, 2021.
145. Quigley HA: Glaucoma. *Lancet* 377: 1367-1377, 2011.
146. Ishikawa M, Yoshitomi T, Zorumski CF and Izumi Y: Neurosteroids are endogenous neuroprotectants in an ex vivo glaucoma model. *Invest Ophthalmol Vis Sci* 55: 8531-8541, 2014.
147. Belelli D and Lambert JJ: Neurosteroids: endogenous regulators of the GABA(A) receptor. *Nat Rev Neurosci* 6: 565-575, 2005.
148. Weir CJ, Ling AT, Belelli D, Wildsmith JA, Peters JA and Lambert JJ: The interaction of anaesthetic steroids with recombinant glycine and GABAA receptors. *Br J Anaesth* 92: 704-711, 2004.
149. Midzak A, Akula N, Lecanu L and Papadopoulos V: Novel androstenediol interacts with the mitochondrial translocator protein and controls steroidogenesis. *J Biol Chem* 286: 9875-9887, 2011.
150. Izumi Y, Benz AM, Kurby CO, Labruyere J, Zorumski CF, Price MT and Olney JW: An ex vivo rat retinal preparation for excitotoxicity studies. *J Neurosci Methods* 60: 219-225, 1995.
151. Izumi Y, Kirby CO, Benz AM, Olney JW and Zorumski CF: Muller cell swelling, glutamate uptake, and excitotoxic neurodegeneration in the isolated rat retina. *Glia* 25: 379-389, 1999.



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