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# Is the mitochondrial outermembrane protein VDAC1 therapeutic target for Alzheimer's disease?

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## Abstract

Mitochondrial dysfunction and synaptic damage have been described as early events in Alzheimer's disease (AD) pathogenesis. Recent research using AD postmortem brains, and AD mouse and cell models revealed that amyloid beta (Aβ) and tau hyperphosphorylation are involved in mitochondrial dysfunction and synaptic damage in AD. Further, recent research also revealed that the protein levels of mitochondrial outer membrane protein, voltage-dependent anion channel 1 (VDAC1) are elevated in the affected regions of AD postmortem brains and cortical tissues from APP transgenic mice. In addition, emerging research using AD postmortem brains and AD mouse models revealed that VDAC1 is linked to Aβ and phosphorylated tau, blocks the mitochondrial permeability transition (MPT) pores, disrupts the transport of mitochondrial proteins and metabolites, impairs gating of VDAC, and causes defects in oxidative phosphorylation, leading to mitochondrial dysfunction in AD neurons. The purpose of this article is to review research that has investigated the relationship between VDAC1 and the regulation of MPT pores in AD progression.

## Introduction

Alzheimer's disease (AD) is a late-onset, progressive, age-dependent neurodegenerative disease, characterized by the progressive decline of memory, cognitive functions, and changes in behavior and personality [1–3]. Currently, 5.4 million Americans are living with AD – 5.2 million who are 65 years of age and older, and the remaining 0.2 million under the age of 65. Current estimates are that 16 million persons will have AD by 2050. Of Americans aged 65 years and older, 1 in 8 has AD, and nearly half of people aged 85 years and older have the disease [4]. With lifespan increasing in humans, AD is headed towards becoming the major health concern of elderly persons. In addition to the personal, social, and family hardships that AD creates, the numbers of expected AD patients will translate into extremely high health-care costs. In 2012, the direct costs of caring for those with AD or other dementias in the United States will total an estimated $200 billion [4].



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AD is associated with the loss of synapses, synaptic function, mitochondrial abnormalities, inflammatory responses, and neuronal loss, in addition to 2 major pathological hallmarks: 1) intracellular neurofibrillary tangles and 2) extracellular amyloid beta (Aβ) deposits in the regions of the brain that are responsible for learning and memory. Genetic mutations in APP, PS1, and PS2 genes cause about 1–2% of all AD cases. Several factors contribute to late-onset AD: lifestyle, diet, environmental exposure, genetic variants in the sortilin-related receptor 1 gene clusterin, the complement component receptor 1, CD2AP, CD33, EPHA1, and MS4A4/MS4A6E genes and the ApoE 4/4 genotype [5].

Although AD pathogenesis involves multiple molecular and cellular events, 2 events that occur early in AD development are: 1) synaptic damage and 2) mitochondrial dysfunction [3,6–12]. These 2 events are likely caused by an age-dependent accumulation of Aβ and phosphorylated tau in neurons [9]. Recently, several studies reported mitochondrial abnormalities as additional molecular and cellular events in AD progression. These events include changes in mitochondrial DNA, decreased mitochondrial enzyme activities, abnormal mitochondrial gene expressions, increased mitochondrial fragmentation, and decreased mitochondrial fusion [13]. Recently, we reported Aβ and phosphorylated tau associated with mitochondrial membranes and causing mitochondrial dysfunction. Further, we found that the mitochondrial outer membrane protein, voltage-dependent anion channel 1 (VDAC1) directly interacts with Aβ and phosphorylated tau, and contributes to impairments in mitochondrial pore opening and closing [14. The purpose of this article is to review studies that are evaluating the role of VDAC1 in AD pathogenesis and the relationship between VDAC1 and phosphorylated tau in mitochondrial dysfunction that is known to occur in AD.

## Mitochondrial permeability transition pore

Mitochondria, present in all eukaryotic cells, including neurons, are cytoplasmic organelles that are essential for cell survival and cell death [15]. The half-life of neuronal mitochondria is about one month [16]. A mitochondrion contains 2–10 copies of mtDNA [15]. The number of mtDNA copies and the number of mitochondria per cell are dependent on the energy demand of the cell. Mitochondria are controlled and regulated by mitochondrial and nuclear genomes. A mitochondrial genome is a 16.5-kb, double-stranded circular DNA molecule that is maternally transmitted [17]. mtDNA has 2 strands: a guanine-rich outer strand and cytosine-rich inner strand. mtDNA encodes 13 polypeptides participating in oxidative phosphorylation. mtDNA also encodes the 12S and 16S rRNA genes. The 22 tRNA genes are required for mitochondrial protein synthesis. Nuclear genes encode over 1000 mitochondrial proteins that participate in oxidative phosphorylation (OXPHOS) along with mitochondrial-encoded genes. Nuclear mitochondrial proteins are synthesized in the cytoplasm and are transported into mitochondria.

Mitochondria are the power houses of cells, performing several cellular functions, including intracellular calcium regulation, ATP production, the release of proteins that activate the caspase family of proteases, and the alteration of the reduction-oxidation potential of cells and free-radical scavenging. Structurally, mitochondria are compartmentalized into two biolipid membranes: the inner mitochondrial membrane and the outer mitochondrial membrane [16] (Fig. 1). The inner mitochondrial membrane houses the ETC and provides a highly efficient barrier to the flow of ions. The inner mitochondrial membrane covers the mitochondrial matrix, which contains tricarboxylic acid and beta-oxidation. The outer mitochondrial membrane is basically porous and allows the passage of low molecular-weight substances, between the cytosol and the mitochondrial intermembrane space (Fig. 2). These mitochondrial permeability transition (MPT) pores are formed by the mitochondrial

outer membrane protein VDAC1, the mitochondrial inner membrane protein, the adenine nucleotide translocator (ANT), and the matrix protein cyclophilin D (CypD).

The cross talk and transport of metabolites and proteins between mitochondria and the rest of cell are important to complete OXPHOS and to produce mitochondrial ATP. Increasing evidence suggests that mitochondrial pore opening and pore closure of VDAC are impaired in mitochondria, in brain tissues from patients with neurodegenerative diseases, with inherited mitochondrial diseases, stroke, cancer, and ischemia [18–21].

## VDAC structure and expression

VDACs, also known as mitochondrial porins, have been found in the outer mitochondrial membrane of paramecium tetraurelia [22] and in the outer mitochondrial membrane of mitochondria in mammals, including humans [23–24]. Studies have found that VDACs are highly conserved [25–28]. Three isoforms of VDAC: VDAC1 and VDAC3 are reported to have 9 exons and VDAC2, 10. The additional exon in VDAC2 is believed to encode part of the 5′-UTR region [29–30] (Fig. 3). VDAC1 has 2 splice variants, and VDAC2 and VDAC3 each have one splice variant.

VDAC1 is believed to be youngest of the 3 isoforms, and VDAC3, the oldest. VDAC1/2 is estimated to have diverged from VDAC3 about 365 60 MY ago, and VDAC1 and VDAC2, about 289±63 MY ago [31–33]. The human VDAC1 has been mapped to chromosome 5, and the human VDAC2, to chromosome 10. The mouse VDAC1 has been mapped to chromosome 11; the mouse VDAC2, to chromosome 14; and the mouse VDAC3, to chromosome 8. Both VDAC1 and VDAC2 cDNA sequences in humans and mice have 90% homology, in contrast to the VDAC3 cDNA sequences, which have 68% homology between humans and mice (NCBI database).

Of the 3 VDAC isoforms, in mammals, VDAC1 is the most widely expressed, followed by VDAC2 and then VDAC3 [34–35]. VDAC1 and VDAC2 are expressed in the heart, liver, and skeletal muscles, and in the brain. VDAC1 is also expressed, but in very low levels, in the testes [29–30]. VDAC3 is expressed in the testes, liver, ovary, adrenal, lung, spleen, and kidney muscles [36].

## VDAC function and physiology

VDAC proteins perform several important functions in the cell, including regulating cell survival, growth, and fertility; maintaining synaptic plasticity through mitochondrial permeability in the transition pore; regulating calcium transport, regulating ATP transport, regulating mitochondrial shape and structural changes; regulating hexokinase interactions with mitochondria; and regulating apoptosis signaling [37–39] (Fig. 4). These functions have been found to be altered in cells from patients with neurodegenerative and mitochondrial diseases, leading to mitochondrial dysfunction. Further, increasing evidence suggests that VDAC interacts with several cytoplasmic proteins, alters channel activity, VDAC closure and reduces VDAC channel conductance [18–21].

VDAC1 and VDAC2 form pores in the mitochondrial outer membrane, specifically in the biolipid layers of the mitochondrial outer membrane, and there is evidence suggesting that recombinant VDAC3 is not able to open the pores [40]. VDACs exhibit voltage dependence, and VDAC1 also closes the mitochondrial pores, when the transmembrane voltage exceeds 20–30 mV [41–42]. In a normal and open state of VDAC, metabolites, including ADP, ATP, inorganic phosphate and other substrates, enter and leave mitochondria after passing through the outer mitochondrial membrane. In a closed state, VDAC does not allow the regular flow of ADP/ATP through the outer membrane (Fig. 2). It is believed that VDAC is constantly

open in metabolic state. However, recent evidence suggests that VDAC closes clearly during apoptosis in diseased neurons. Consequently, with its pores closed, mitochondria may not be able to release ATP into the cytoplasm and/or to uptake ADP, and inorganic phosphate and respiratory substrates from the cytoplasm [43–44]. The anti-apoptotic protein Bcl2-XL has been found to prevent VDAC closure [44], whereas tBid, a pro-apoptotic member, has been found to promotes the pore closure [45]. VDAC appears to be involved in both pro- and anti-apoptosis aspects of mitochondria.

VDAC channel conductance may be impaired in a couple different ways: 1) In neurons from mitochondrial diseases, VDAC may interacts with mutant and cytoskeletal proteins that may have accumulated during disease progression and may have blocked the mitochondrial pores 2. Phosphorylated VDAC may also interact with cytoplasmic proteins, leading to the blockade of mitochondrial pores. In both scenarios, channel conductance would be impaired and may lead to reduced mitochondrial respiration and to mitochondrial dysfunction. In support of VDAC inhibition and subsequent impairment in VDAC channel conductance by cytoskeletal protein, tubulin, Rostovtseva and colleagues [46] reported an abnormal interaction between VDAC and tubulin, resulting in the blockage of mitochondrial pores, disruption in the flux of metabolites between mitochondria and cytoplasm, and the inhibition of mitochondrial respiration [46]. Recently, in a study of brain tissue from postmortem brains of patients with AD, Manczak and Reddy [14] found that VDAC interacted with mutant AD proteins (including Aβ and phosphorylated tau), which in turn blocked mitochondrial pores and interrupted the flow of ATP, ADP, inorganic phosphate substrates, and respiratory substrates between mitochondria and the cytoplasm, ultimately leading to mitochondrial dysfunction [14].

In addition, recent studies revealed that VDAC proteins and their binding partners are modified post-translationally due to VDAC hyperphosphorylation and are involved in the impairment of channel conductance and malfunction of VDAC [21–47–48]. VDAC protein consists of multiple phosphorylation sites, some of which are reported to undergo phosphorylation [21]. VDAC1 is phosphorylated by protein kinase C, leading to a decreased single channel current and open probability [49]. GSK3β protein phosphorylates VDAC1 at Thr51, which in turn disrupts the binding of HK-II to VDAC [50]. Recently, Manczak and Reddy [14] reported phosphorylated tau interacts with VDAC1, leading to pore closure and to reduced mitochondrial function in neurons from APP transgenic mice.

Overall, VDAC performs several functions VDAC channel conductance is regulated by multiple factors. Further research is needed to understand the mechanistic links between VDAC and phosphorylated tau and between VDAC and Aβ.

## VDAC knockout mouse models

In studies aimed at elucidating the function of VDAC proteins, William Craigen's research group generated VDAC1, VDAC2, and VDAC3 heterozygote (+/−) knockout embryonic stem (ES) cells studied them for mitochondrial respiration and mitochondrial enzymatic activity [51]. The ES cells that were deficient in VDAC1, VDAC2, and VDAC3 were viable but showed a 30% reduction in oxygen consumption and reduced cytochrome oxidase activity. The VDAC3-deficient cells did not show any change in cytochrome oxidase activity relative to wild-type VDAC3 cells. These results indicated that each mouse VDAC isoform is not essential for cell viability [51]. Heterozygote knockout VDAC1, VDAC2, and VDAC3 ES cells were used to produce both hetero- and homozygous knockout mice for VDAC1, VDAC2, and VDAC3, but only VDAC1 and VDAC3 homozygous knockout mice were able to be generated. Further genetic analysis revealed that mutant and wild-type alleles of the VDAC3 locus were transmitted in the expected Mendelian ratios, but

VDAC1$^{-/-}$ mice were bred in less-than-expected numbers, suggesting partial embryonic lethality of VDAC1$^{-/-}$, in particular between embryonic days 10.5 and 11.5. The surviving VDAC1$^{-/-}$ mice were fertile, but were mildly retarded in growth, while the VDAC3$^{-/-}$ male mice were infertile [52]. However, the VDAC heterozygote knockout (VDAC1+/−) and VDAC3+/− mice [52] were fertile and had a normal lifespan.

Using cell culture studies, Cheng et al. [53] studied the role of VDAC2 in the mitochondrial pathway of apoptosis. They found BAK complexed with VDAC2, a VDAC isoform present in low abundance that interacts specifically with the inactive conformer of BAK. Cells deficient in VDAC2 exhibited enhanced BAK oligomerization and were more susceptible to apoptotic death. The overexpression of VDAC2 selectively prevented BAK activation and inhibited the mitochondrial apoptotic pathway. Thus, VDAC2, an isoform restricted to mammals, may regulate the activity of BAK and may provide a connection between mitochondrial physiology and the core apoptotic pathway. Findings from this study may partially explain why VDAC2$^{-/-}$ mice were not able to produce.

To determine the role of VDACs in mitochondrial permeability pore transition, Baines et al. [49] generated simultaneous ablation of VDAC1, VDAC2, and VDAC3 proteins by combining gene deletion and the silencing approach. They found that mitochondria from VDAC 1-, VDAC 3-, and VDAC 1– VDAC 3 null mice exhibited Ca2+- and oxidative stress-induced MPT pores that were indistinguishable from those in wild-type mitochondria. Similarly, Ca2+- and oxidative-stress-induced MPT pores and cell death were unaltered in fibroblasts lacking VDAC1, VDAC 2, VDAC 3, VDAC 1– VDAC 3, and VDAC 1– VDAC 2– VDAC 3. Wild-type and VDAC-deficient mitochondria also exhibited equivalent cytochrome c release, caspase cleavage, and cell death in response to the pro-death Bcl-2 family members Bax and Bid. These results indicate that VDACs are critical for both MPT and Bcl-2 family member-driven cell death.

Overall, these knockout VDAC mouse studies suggest that VDAC2 is important for cell survival, but that VDAC1 and VDAC 2 are also essential for cell death, MPT pores, and mitochondrial functions, but that cells are able to survive with a reduction in VDAC1 and VADC3.

## Mitochondrial dysfunction in Alzheimer's disease

Mitochondrial dysfunction and synaptic damage have been identified as early events in AD pathogenesis, but their underlying mechanisms are not completely understood. Mitochondrial dysfunction has been described in AD postmortem brains [54–60], AβPP transgenic mice [61–67], and cells that express mutant APP and cells treated with Aβ [68–77]. Recently, several groups investigated mitochondrial gene expressions in AD postmortem brains [59–60,78–80] and in AβPP mice [61]. They found mitochondrial-encoded genes abnormally expressed in the brains from the AD patients and the AβPP mice. These abnormal mitochondrial gene expressions may be compensatory responses to mitochondrial dysfunction that Aβ may induced. Several studies found increased free radical production, lipid peroxidation, oxidative DNA and protein damages, and reduced ATP production in brains from AD patients compared to control subjects [54–56,81–83]. In addition, studies of mitochondrial structure in AD postmortem brains and neuronal cells expressing the mutant APP found that Aβ fragments mitochondria and causes structural changes in neurons [57,60,68–71].

## APP, Aβ and their association with mitochondria in Alzheimer's disease

As discussed above, mitochondria dysfunction and oxidative stress have been extensively reported. However, the precisely molecular link between mitochondrial dysfunction and AD

pathogenesis described recently. Using biochemical, molecular, and electron microscopy studies, and AD postmortem brains and brains from AβPP mice, several groups studied the connection between Aβ and mitochondria, and found that A is associated with mitochondria and is responsible for generating increased free radicals and mitochondrial dysfunction [62,65–67,84–87]. Further, a recent study found that Aβ is transported into the inner mitochondrial membrane via the translocase of the outer membrane machinery [85].

Shi Du Yan's group reported on the interaction between Aβ and the mitochondrial matrix proteins ABAD and CypD, which was found to lead to increased ROS production, mitochondrial dysfunction, and cognitive damage in APP mice (J20 line) [62–84]. They also found reduced interaction between Aβ and ABAD, and between Aβ and CypD. They found that reduced ABAD and CypD protects against Aβ toxicity in AD neurons [84,88].

Several studied found APP in mitochondrial membranes, in neurons affected by AD [55,89–91]. These studies also found mitochondrial APP in the N-terminal, inside the mitochondria, and in the C-terminal of the protein that faces the cytosolic side [89,91]. Recently, Devi and colleagues found that full-length APP and the C-terminal truncated APP without the Aβ domain accumulate progressively in mitochondria, in patients with mild, moderate, and severe AD, but not in age-matched subjects without AD [55].

Overall, these studies suggest that APP and Aβ are associated with mitochondrial membranes and are critically involved in mitochondrial dysfunction and neuronal damage in AD progression.

## Phosphorylated tau and its association with mitochondria in Alzheimer's disease

Several groups recently investigated the relationship between tau and mitochondria, and found that N-terminal tau is associated with mitochondrial membranes [92–94]. Amadoro et al. [92] reported that a 20–22 kDa NH2-truncated tau fragment was largely enriched in human mitochondria from synaptosomes of AD brains and that the amount of tau in the terminal fields correlated with pathological synaptic changes and with organelle functional impairment.

Atlante et al. [93] studied the relationship between overexpressed N-terminal tau fragments (1–25 aa and 26–44 aa) and mitochondrial dysfunction. They tested both fragments for ATP synthesis, membrane potential and ANT activity. They found that oxidative phosphorylation was not affected by the N-terminal fragment 1–25 aa tau fragment, but was dramatically impaired by the N-terminal 26–44 aa tau fragment. Both cytochrome c oxidase and the ANT are targets of the N-terminal 26–44 tau fragment, but the ANT is a unique mitochondrial target, responsible for impairment of oxidative phosphorylation by the N-terminal 26–44 tau fragment, which exerts deleterious effects on cellular availability of ATP when the ATP is synthesized in the inner mitochondrial membrane.

Quintanilla et al. [94] reported that the expression of tau that they induced at Asp-421 to mimic caspase cleavage (T4C3) was toxic to immortalized cortical neurons compared with a full-length tau isoform (T4). T4C-expressing cells induced mitochondrial fragmentation and elevated oxidative stress levels, in comparison with T4-expressing cells. Thapsigargin treatment of T4 or T4C3 cells, which causes an increase in intracellular calcium levels, resulted in a significant decrease in mitochondrial potential. It also resulted in the loss of mitochondrial membrane integrity in T4C3 cells when compared with cells expressing T4. Mitochondrial fragmentation and membrane damage were ameliorated in T4C3 cells when

they were pretreated with cyclosporine A or FK506, indicating that the calcium-dependent phosphatase calcineurin in these pathogenic events.

Overall, findings from these studies suggest that the N-terminal fragment of tau may cause mitochondrial dysfunction and defects in oxidative phosphorylation, in AD neurons.

## Elevated VDAC1 levels in AD brains and AD transgenic mice

To our knowledge, very little published evidence is available addressing the involvement of VDAC1 in the progression of disease in AD. To determine the role of VDAC1 in AD progression and pathogenesis, using quantitative real-time RT–PCR with Sybr-Green chemistry, we measured mRNA fold changes for VADC1 in postmortem brain specimens from patients in early and late stages of AD and in postmortem brain specimens from patients without AD [65]. Increased mRNA expression was found in AD brains relative to brains from control subjects. Further, using immunoblotting analysis and quantitative densitometry, VDAC1 protein levels were quantified in frontal cortical tissues from all AD patients and control subjects. Significantly increased VDAC1 was found in AD patients relative to control subjects [14]. These findings suggest that VDAC1 levels progressively increase as AD progresses and may be implicated in AD progression.

To determine whether VDAC1 increases with age in Aβ-overexpressed APP transgenic mice, we also quantified VDAC1 protein levels in cerebral cortex tissues from 6-, 12-, and 24-month APP mice and age-matched non-transgenic wild-type mice. We found significantly increased levels of VDAC1 in the 12- and 24-month-old APP mice, relative to the 6-month-old APP mice, indicating an age-dependent increase of VDAC1 in the cerebral cortex of APP mice. To determine whether mutant APP and/or Aβ elevates VDAC1, we compared VDAC1 immunoblotting and densitometry data with VDAC1 data from the wild-type mice. We found significantly increased levels of VDAC1 in the 6- and 12-month-old APP mice, relative to the 6- and 12-month-old non-transgenic wild-type mice. To determine if aging plays a role in VDAC1 expression, we compared immunoblotting data from the 6-, 12-, and 24-month-old wild-type mice. We found significantly increased levels of VDAC1 in the 12- and 24-month-old non-transgenic wild-type mice, relative to 6-month-old wild-type mice, indicating an age-dependent increase in VDAC1, in the cerebral cortex of the wild-type mice.

Ren and colleagues [95] studied the effect of the Aβ peptide 25–35 on mitochondrial structure and function and on the expression of proteins associated with the mitochondrial permeability transition pore in rat hippocampal neurons. They injected Aβ peptide into hippocampal area CA1. Normal saline was injected as a control to assess the hippocampal structure by transmission electron microscopy. ATPase activity, intracellular Ca2+, and mitochondrial membrane potential were measured. The expression of genes associated with the MPTP, including VDAC1, ANT, and CypD, were evaluated. They found that the Aβ injection damaged the mitochondrial structure of hippocampal neurons, decreased ATPase activity and mitochondrial membrane potential, and increased intracellular Ca2+. The expression levels for VDAC, ANT, and CypD in all groups were significantly higher than those in the normal control group injected after Aβ25–35 peptide. These results indicate that Aβ-25–35 damages mitochondria in rat hippocampal neurons and affects mitochondrial dysfunction, as well as increases the expression of genes associated with MPT pores. Mitochondrial dysfunction may result in increased MPTP gene expression, leading to neurodegenerative effects.

Cuadrado-Tejedor and colleagues [96] studied whether VDAC1 is involved in the release of apoptotic proteins in AD. Through proteomic analysis followed by immunoblotting blotting and immunohistochemical analyses, they found that VDAC1 is overexpressed in the

hippocampus from amyloidogenic AD transgenic mice. VDAC1 was also overexpressed in postmortem brain tissue from AD patients at an advanced stage of disease progression. Interestingly, Aβ soluble oligomers were able to induce the up-regulation of VDAC1 in a human neuroblastoma cell line, further supporting a correlation between Aβ levels and VDAC1 expression. In hippocampal extracts from transgenic mice, a significant increase was observed in the level of VDAC1 that was phosphorylated at an epitope susceptible to phosphorylation by glycogen synthase kinase-3β, whose activity was also increased. The levels of hexokinase I (HXK1), which interacts with VDAC1 and affects its function, were decreased in mitochondrial samples from AD models, indicating that reduced HXKI levels favor a VDAC1 involvement in disease progression of AD [96].

Overall, these studies suggest that VDAC1 increases with the progression of AD. Further, VDAC1 also increases in an age-dependent manner, likely an important factor in better understanding AD progression.

## Elevated VDAC1 and its interaction with Aβ in neurons from AD tissues

As discussed above, in AD neurons, we found large amounts of Aβ associated with the outer mitochondrial membrane [65]. Further to determine, if Aβ is associated with outer mitochondrial membrane, immunoprecipitation analysis was conducted, using cortical protein lysates from brains of AD patients at different stages of disease progression, and from brains of APP and APP/PS1 transgenic mice and of age-matched wild-type mice. These histologic studies involved the use of Aβ (6E10 monoclonal A11 oligomeric Aβ) and VDAC1 antibodies [14]. A 4 kDa Aβ and a 100 kDa full-length APP were found in VDAC1 immunoprecipitation elutes from definite and severe AD patients and from 20-month-old APP and APP/PS1 mice. Similar to monomeric Aβ, oligomeric Aβ was also found in immunoprecipitation elutes from definite and severe AD patients and from APP and APP/PS1 mice. Findings from these studies, clearly suggest that both monomeric and oligomeric Aβ interact with VDAC1 and blocks the mitochondrial pores, interrupting the transport of metabolites and proteins between mitochondria and the rest of the cell.

Thinnes and colleagues [97] proposed that GxxxG motif of N-terminal part of VDAC1 might interact with GxxxG motif of C terminal part of Aβ peptide. The GxxxG motifs are established as aggregation/membrane perturbation motifs. Aβ, a C-terminal cleaved product from APP by beta-secretase BACE1 and gamma-secretase, has been insinuated to induce AD via apoptosis by opening type-1 porin/VDAC in cell membranes of hypometabolic neuronal cells. Considering the ubiquitous expression modus of APP, beta- and gamma-secretases and type-1 VDAC/eukaryotic porin a basic model of apoptosis might be given.

Ramirez and colleagues [98] studied VDAs involvement in AD pathogenesis using affected regions, including frontal cortex and hippocampus, from AD patients and controls subjects. They found pl-VDAC and mERα present in the caveolae tissues from the human cortex and hippocampus, in a complex with scaffolding caveolin-1 which likely provides mERα stability at the plasma membrane. In AD brains, VDAC was accumulated in the caveolae and in dystrophic neurites of senile plaques, whereas mERα was expressed in astrocytes surrounding the plaques. Together with previous data on murine neurons demonstrating the participation of pl-VDAC in Aβ-induced neurotoxicity, Ramirez et al. data suggest that the mitochondrial channel may be involved in membrane dysfunction similar to that observed in AD neuropathology.

Overall, findings from these studies suggest that VDAC1 normally interacts with Aβ, and this abnormal interaction increases with AD progression. Further, there is evidence to support pl-VDAC being linked with the caveolae of the cortex and hippocampus, and that VDAC1 is heavily localized in dystrophic neurites of senile plaques.

## Elevated VDAC1 and its interaction with phosphorylated tau in Alzheimer's disease

A physiological link between abnormal and phosphorylated tau, and mitochondrial dysfunction has been proposed in AD [99], suggesting that VADC1 may be associated with mitochondrial dysfunction found in AD. Researchers reported N-terminal tau associated with mitochondria in brain tissues from AD patients and AD mouse models. To determine whether phosphorylated tau interacts with VDAC1, we conducted immunoprecipitation analysis, using cortical protein lysates from the brains of AD patients and of control subjects, and APP/PS1, 3xTg.AD in brain tissues from transgenic mice and age-matched wild-type mice. We found a 32-kDa band of VDAC1 in the phosphorylated tau immunoprecipitation elutes in the brains from AD patients and from APP/PS1, 3xTg.AD transgenic mice. These results were cross-checked using a VDAC1 antibody for immunoprecipitation and a phosphorylated tau antibody for immunoblotting. We found a 60 kDa band of phosphorylated tau protein in the VDAC1-immunoprecipitation elutes, in the brain tissue from AD patients and from APP/PS1, 3xTg.AD transgenic mice. These findings suggest that phosphorylated tau interacts with VDAC1. These observations were further confirmed by double-labeling immunofluorescence analyses of VDAC1 and phosphorylated tau in brain tissues from AD patients and 3XTg.AD mice.

Overall, the results from our study suggest that VDAC1 interacts with phosphorylated tau in AD neurons and that these interactions increase with disease progression. The VDAC1-phosphortylated tau complexes blocks mitochondrial pores, interrupt the flux of metabolites between mitochondrial membranes and cytoplasm, impairs the gating of VDAC channel, leading to mitochondrial dysfunction and neuronal damage in AD neurons.

## Conclusions and future directions

A large body evidence suggests that mitochondrial dysfunction and oxidative stress are involved in AD progression and pathogenesis. Further, recent research on AD postmortem brains, brain tissues from AD mouse models, and cells that express Aβ and phosphorylated tau revealed that Aβ and phosphorylated tau are associated with mitochondria, resulting in mitochondrial dysfunction and synaptic damage in AD-affected neurons. More specifically, several recent studies found Aβ interacting with the mitochondrial matrix proteins CypD and ABAD, and that this interaction leads to increased ROS production and mitochondrial dysfunction. In addition, recent research from the Reddy lab, that used postmortem brain tissues from AD patients and tissues from AD mouse models, revealed that in the mitochondrial outer membrane protein, VDAC1 is interacts with both Aβ and phosphorylated tau, resulting in the blockage of MPT pores and a disruption in the transport of proteins and metabolites between mitochondria and the rest of cell. Results from this study also showed that VDAC1 likely causes defects in oxidative phosphorylation and mitochondrial dysfunction in AD neurons. It is well-established that MPT pores are formed by VDAC1, ANT and CypD. Proper maintenance of pore opening and pore closure in mitochondria is critical for the transport of metabolites – including ADP, inorganic phosphorous, ATP, and proteins – from the cytoplasm to the mitochondria and vice versa. However, as described earlier, in the mitochondrial matrix, CypD is elevated, it interacts with Aβ in AD neurons, and may cause mitochondrial structural and functional abnormalities. Further, hippocampal neurons treated with Aβ exhibited elevated levels of the pore forming proteins VDAC1, CypD and ANT, and they revealed altered mitochondrial membrane potential and altered intracellular Ca2+ levels.

Elevated levels of VDAC1 were found in postmortem brain tissues from AD brains and AD transgenic mice, and evidence indicating an interaction between VDAC1 and Aβ and

between VDAC1 and phosphorylated tau were found in both brain tissues [14]. Complexes of VDAC1-Aβ and VDAC1-phosphorylated tau may be involved in the blockage of mitochondrial pores, leading to the interruption of mitochondrial protein and metabolite transport, and to the impairment of VDAC channel conductance. In turn, these changes may lead to mitochondrial dysfunction in AD neurons. Based on these observations, reduced levels of VDAC1, Aβ, and phosphorylated tau may reduce the interactions between VDAC1 and Aβ, and between VDAC1 and phosphorylated tau in AD neurons–, resulting in the maintenance of normal mitochondrial pore opening and pore closure, ultimately leading to mitochondria supplying ATP to nerve terminals (as is the case in healthy neurons). If these hypotheses hold, then reduced interaction between VDAC1 and Aβ and phosphorylated tau may prove useful in boosting synaptic and cognitive functions in AD.

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## Research Highlights

1. Mitochondrial dysfunction is an early event in Alzheimer's disease.
2. VDAC1 protein levels are elevated in AD brains and cortical tissues from APP transgenic mice.
3. VDAC1 is interacted with Aβ and phospho tau, disrupts the transport of metabolites.
4. VDAC1+Aβ and Aβ+phospho tau complexes cause defects in OXPHOS in AD neurons.

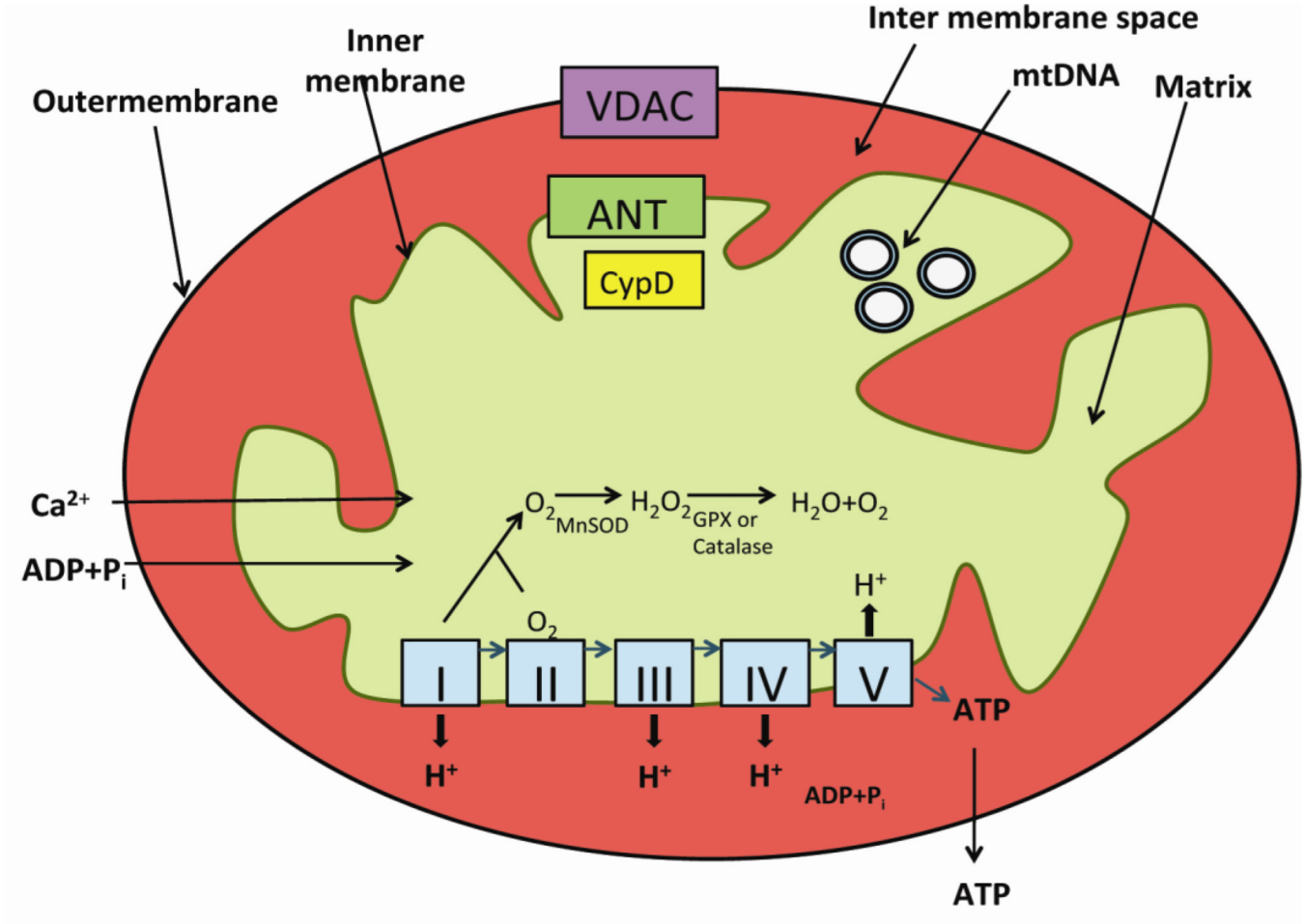


**Figure 1.**

The structure of mitochondria. Mitochondria are compartmentalized into two biolipid membranes: the inner mitochondrial membrane and the outer mitochondrial membrane. The inner mitochondrial membrane houses the mitochondrial respiratory chain or electron transport chain (ETC) and provides a highly efficient barrier to ionic flow. In the ETC - complexes I, III leak electrons to oxygen, producing primarily superoxide radicals, and superoxide radicals are dismutated by manganese superoxide dismuase and produce $H_2O_2$. Further, ETC involves the reduction of $H_2O_2$ to $H_2O$ and $O_2$ by catalase or glutathione peroxidase accepting electrons donated by NADH and $FADH_2$ and then yielding energy for the generation of ATP from adenosine diphosphate and inorganic phosphate.

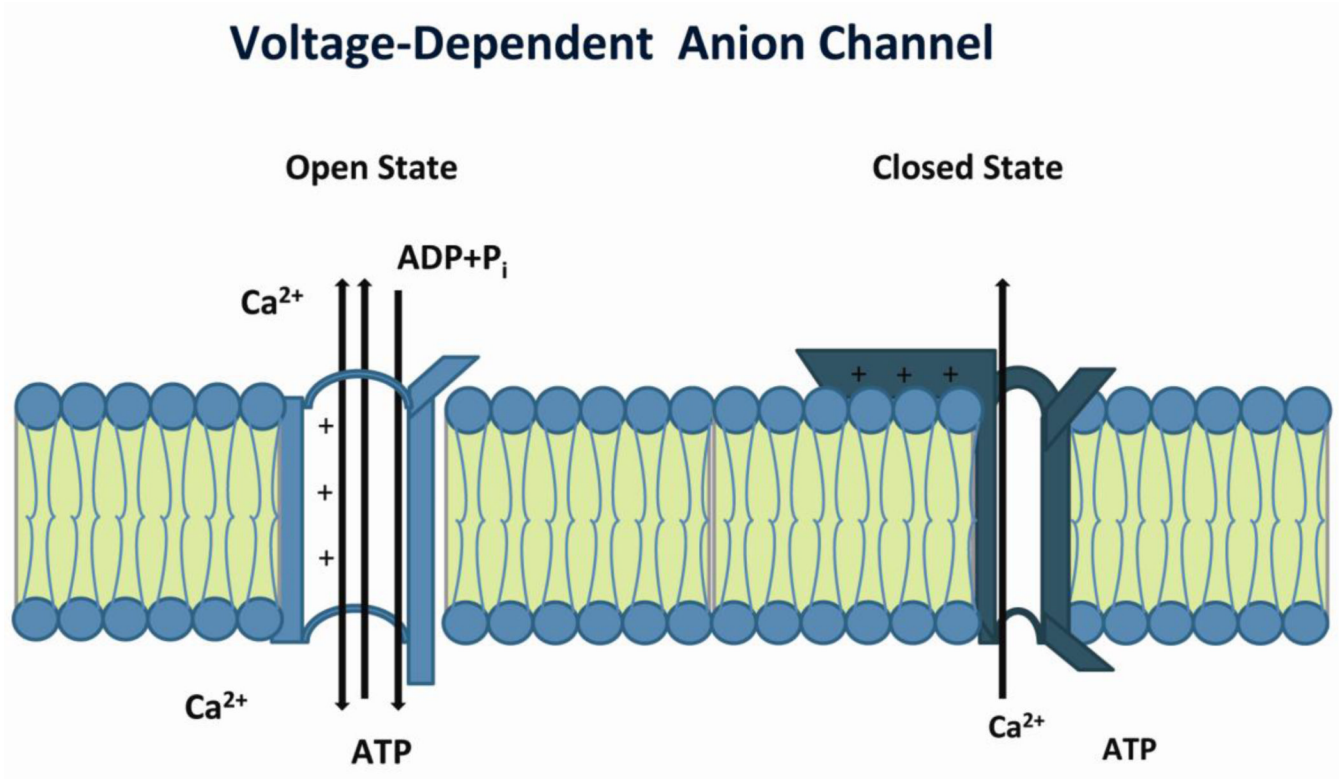


**Figure 2.**
Schematic representation of mitochondrial permeability transition pore opening and closure. In an open state of mitochondrial permeability transition pore, metabolites, including ADP/ATP, inorganic phosphate, pyruvate and other substrates and ions, enter and leave mitochondria after passing through the outer mitochondrial membrane. In a closed state, VDAC does not allow the regular flow of ADP/ATP through the outer membrane.

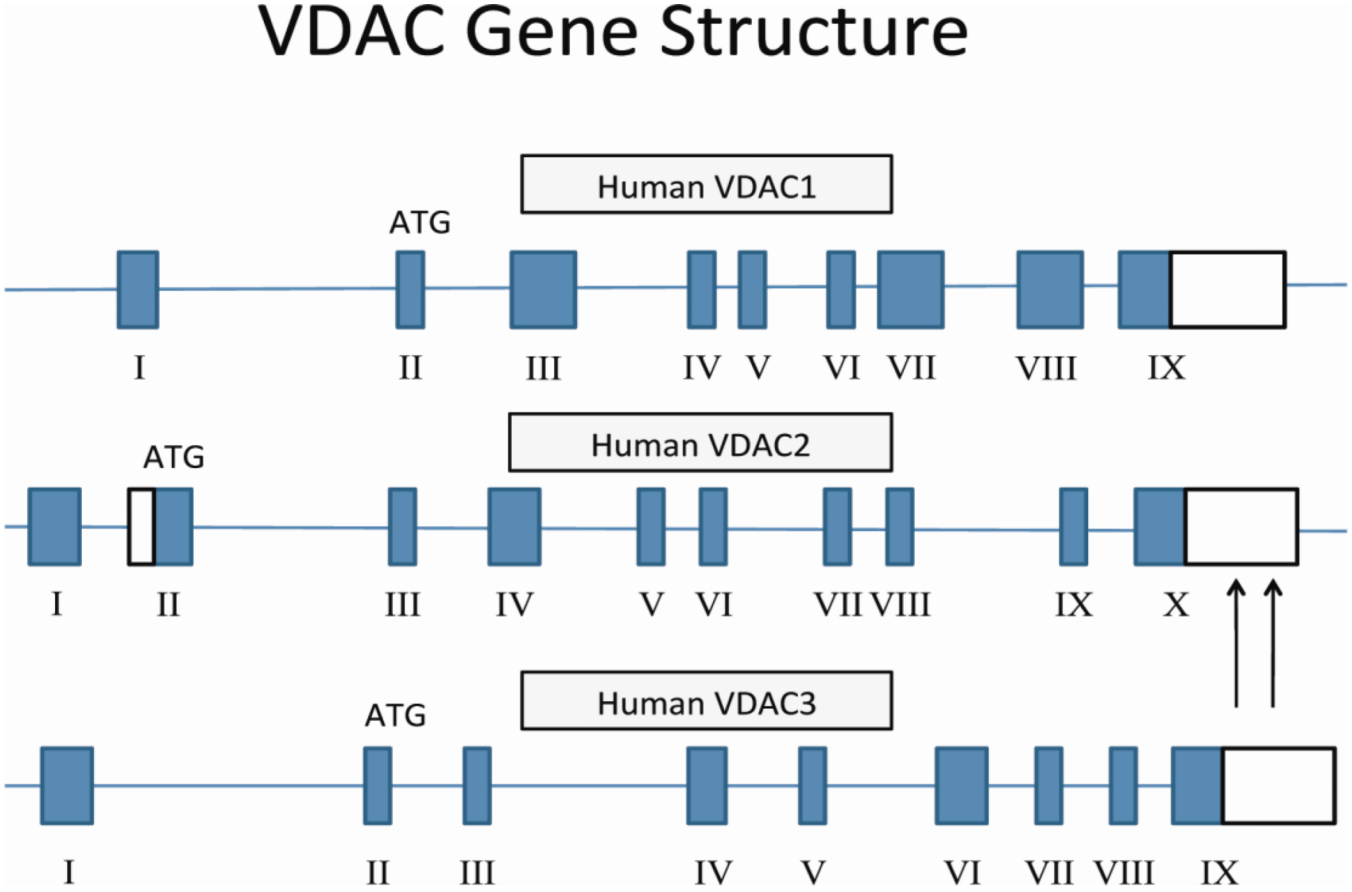


**Figure 3.**
The structure of human VDAC gene. The human VDAC gene has 3 isoforms; isoform 1 or VDAC1 has 9 exons with a start codon, ATG in exon 2 and a long polyadenylation site. Isoform 2 and VDAC2 has 10 exons with multiple polyadenylation sites, and isoform 3 has 3 has 9 exons. VDAC1 has 2 splice variants, and VDAC2 and VDAC3 each have one splice variant.

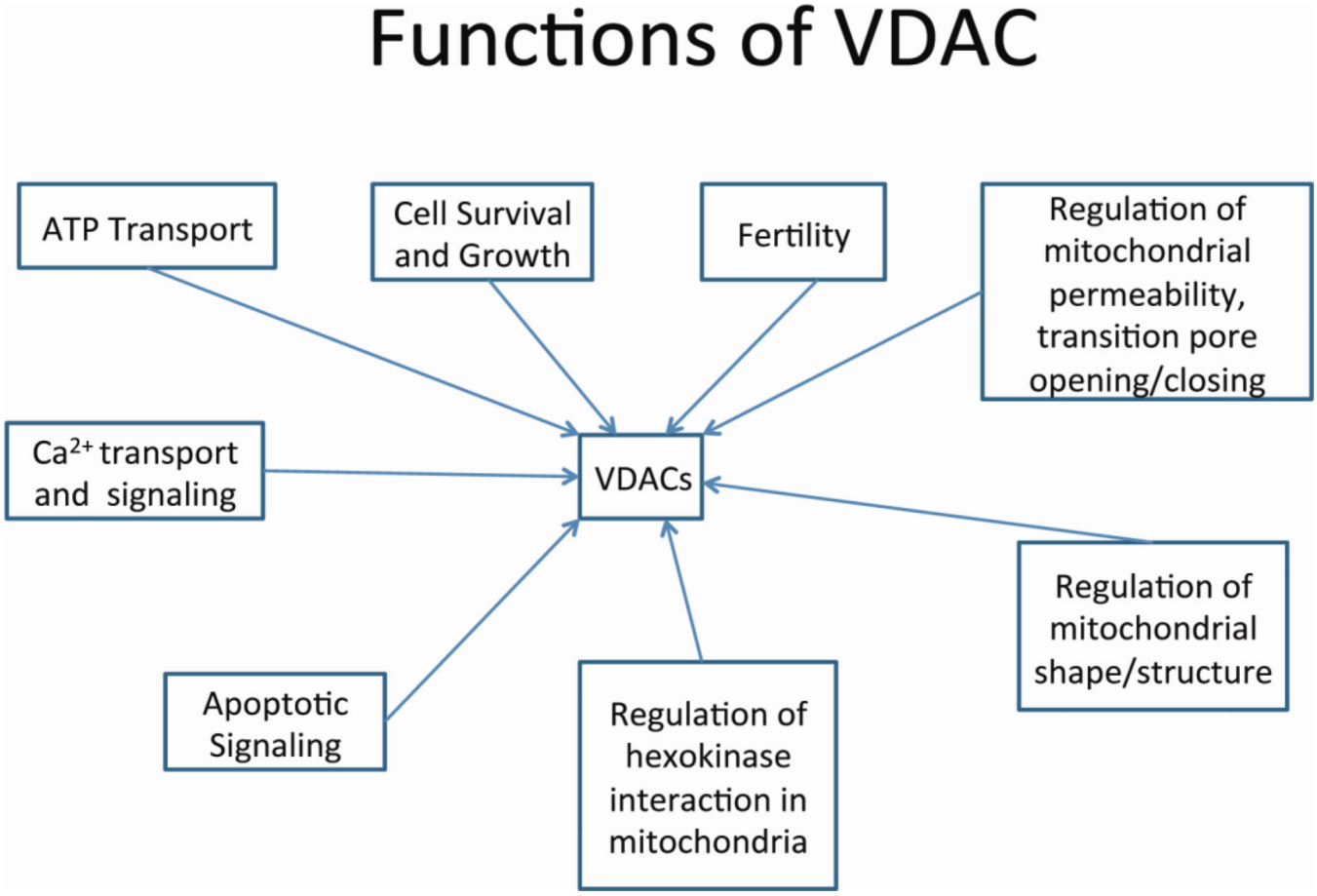


**Figure 4.**
VDACs perform multiple functions – 1) regulating cell survival and cell growth, 2) fertility; 3) maintaining synaptic plasticity through MPT pore, 4) regulating calcium transport, 5) regulating ATP transport, 6) regulating mitochondrial shape and structural changes, 7) regulating hexokinase interactions with mitochondria and 8) regulating apoptosis signaling.