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Hypothesis

The Carnitine Palmitoyl-Transferase 2 Cascade Hypothesis for Alzheimer's Disease

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Abstract. Despite decades of intense research, the precise etiology of Alzheimer's disease (AD) remains unclear. In this hypothesis, we present a new perspective on this matter by identifying carnitine palmitoyl transferase-2 (CPT2) as a central target in AD. CPT2 is an enzyme situated within the inner mitochondrial membrane, playing a crucial role in beta-oxidation of fatty acids. It exhibits high sensitivity to hydrogen peroxide. This sensitivity holds relevance for the etiology of AD, as all major risk factors for the disease share a commonality in producing an excess of hydrogen peroxide right at this very mitochondrial membrane. We will explain the high sensitivity of CPT2 to hydrogen peroxide and elucidate how the resulting inhibition of CPT2 can lead to the characteristic phenotype of AD, thus clarifying its central role in the disease's etiology. This insight holds promise for the development of therapies for AD which can be implemented immediately.

Keywords: Alzheimer's disease, AMP-kinase pathway dependent integrated stress response, carnitine palmitoyl transferase 2, CPT2, hydrogen peroxide, hypoxia, longevity, radical oxygen species

THE AMPK-ISR PATHWAY SEEMS DISTURBED IN ALZHEIMERS DISEASE

Despite decades of intense research, the precise etiology of Alzheimer's disease (AD) remains unclear. Although amyloid-β probably plays an important role in causing AD, its precise role is probably complex and has not been precisely elucidated thus far [1].

Recently, we explored and validated the AMP-Kinase-pathway-dependent integrated stress response [2] (AMPK-ISR). The AMPK-ISR is a refined and extended version of the classical AMPK/Sirt1/PGC1 pathway, which has already been implicated in the etiology of AD [3] as part of the widely known mitochondrial cascade hypothesis [4]. The AMPK-ISR encompasses an evolutionarily conserved transcriptional stress response system that regulates the expression of anti-oxidative enzymes, organizes autophagy and mitochondrial biogenesis, repairs cellular damage, and provides energy in response to mild stress. As such, it restores homeostasis and offers protection against environmental stress, aging, and neurodegenerative diseases [2]. Therefore, we suspect that this pathway may play a role in the etiology of AD.

The key regulatory elements of the AMPK-ISR include AMPK (adenosine monophosphate kinase), CPT1 (carnitine palmitoyl transferase 1), CPT2 (car-

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nitine palmitoyl transferase 2), Sirt1 (sirtuin 1), PGC1 (peroxisome proliferator-activated receptor gamma coactivator 1), PPARs (peroxisome proliferator-activated receptors), and UCPs (uncoupling proteins) (Fig. 1A).

In the brains of AD patients, the activity of AMPK [5] and CPT1 are increased [6], whereas the expression of Sirt1 [7], PGC1 [8], PPARs [9], and UCP's [10] are decreased (Fig. 1B). The NAD/NADH ratio in brains cannot be measured reliably in postmortem AD brain tissue, but in animal models for this disease, the NAD/NADH ratio is decreased [11, 12]. The activity of CPT2 in AD is currently unknown, but its inactivation by hydrogen peroxide will be discussed in this paper.

This pattern of inhibition suggests that in AD brains, the AMPK-ISR is blocked between the activation of CPT1 and the increase in the NAD/NADH ratio. As the increase in NAD/NADH ration in response to CPT1 activation has shown to be gov-

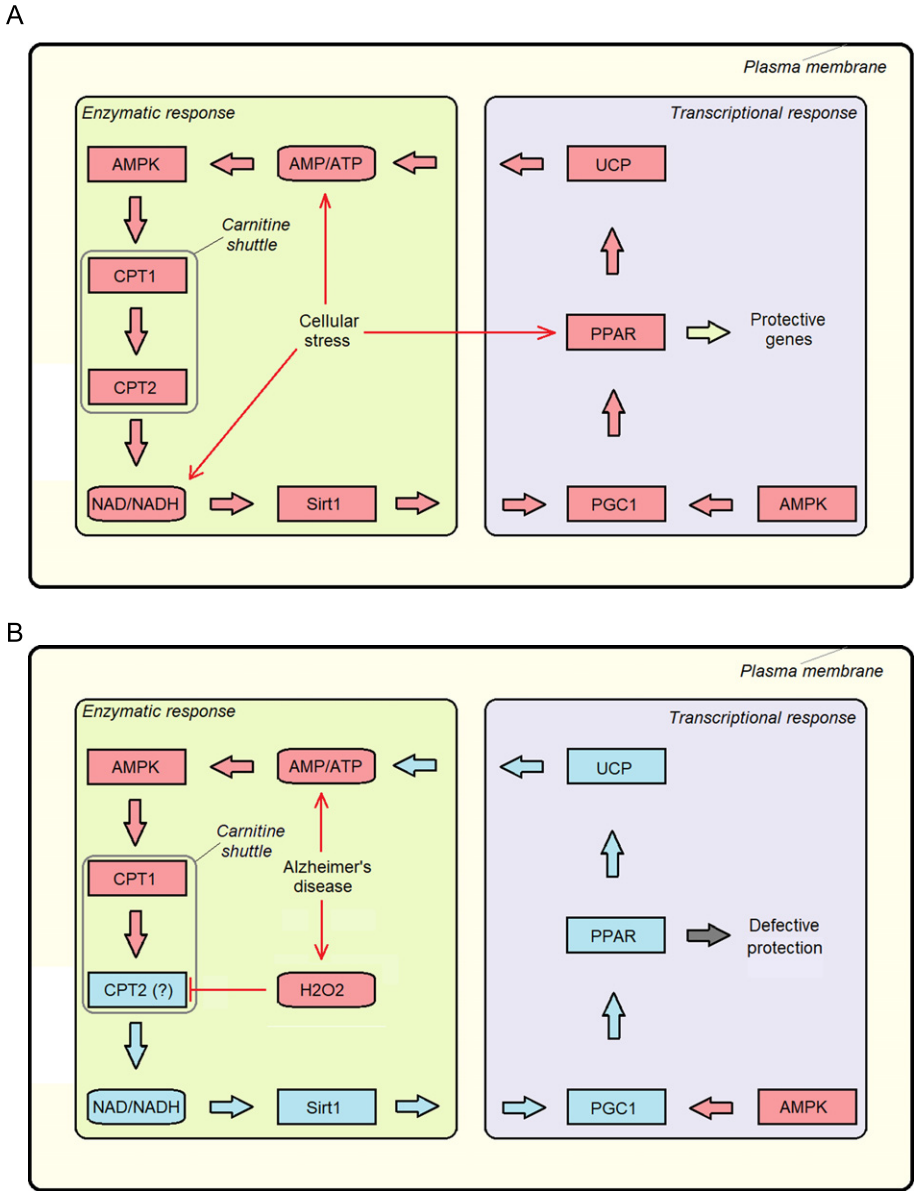

Fig. 1. A) The AMPK pathway dependent integrated stress response (AMPK-ISR), can be activated by various types of mild cellular stress. This results in a transcriptional response, which restores homeostasis. B) In AD, the AMPK-ISR seems blocked after CPT1 activation. Red arrows indicate increased levels or activity; Blue arrows indicate reduced activity. H_2O_2 inhibits CPT2 activity.

erned by the mitochondrial beta-oxidation of fatty acids [13], and this process is indeed inhibited in brains of AD patients [14], the inhibition of beta-oxidation is a likely cause for this block.

The inhibition of the oxidation of fatty acids is caused by CPT2 inhibition

In order to enter mitochondrial beta-oxidation, fatty acids need to be transported from the cytosol into mitochondria by the "carnitine shuttle". This shuttle consists of CPT1, CACT (Carnitine Acyl-Carnitine Translocase) (excluded from Fig. 1 for clarity) and CPT2. CPT1 catalyzes the transfer of fatty acid (Acyl) groups from Acyl-CoA (Acyl Coenzyme A) to carnitine, forming Acyl-carnitines, enabling them to be transferred into the mitochondrion. Transport of Acyl-carnitines to the mitochondrial matrix is catalyzed by CACT. Inside the mitochondrial matrix, on the inner mitochondrial membrane, CPT2 forms back Acyl-CoA from Acyl-carnitines, making it available for mitochondrial beta-oxidation. As both transcriptomic studies in AD patients [15], and proteomics studies in an animal model for AD [16] show that the carnitine shuttle is the most affected pathway of all biochemical pathways studied in AD, a defective carnitine shuttle seems a likely cause for the defective beta-oxidation in AD.

The next question is which element of the carnitine shuttle is defective in AD. For several reasons, we consider CPT2 to be a strong candidate for that. Firstly, a genetic deletion of CPT2 in mice leads to the activation of both AMPK [17] and CPT1 [18], mirroring the observed situation in the brains of AD patients [5, 6]. Secondly, the relevance of CPT2 inhibition for the AD phenotype is further supported by the brain-specific deletion of CPT2, which results in an accumulation of acyl-carnitines in the brain [18]. This tendency is also seen in the cerebrospinal fluid of AD patients [19]. Additionally, the knockout of CPT2 specifically in the brain leads to memory loss in mice [18]. Finally, the genetic deletion of CPT2 in Drosophila leads to an accumulation of lipid droplets in the brain [20], similar to what is observed in the brains of AD patients.

Therefore, it seems likely that the suppression of beta-oxidation in AD is caused by the inhibition of CPT2. This inhibition blocks the increase of NAD in response to activation of AMPK and CPT1, thus inhibiting the AMPK-ISR in the brains of individuals with AD.

Why is CPT2 inhibited in Alzheimer's disease?

As CPT2 is known to be highly sensitive to oxidation, we wondered whether CPT2 could be inactivated by radical oxygen species (ROS) as formed in AD. Remarkably we realized that not only amyloid-β [21], tau [22], possibly via the tau 26–44 fragment [23], advanced glycation end-products [24], *APOE4*, possibly via its 1–272 fragment [25], hypoxia [26], ethanol [27], carbon monoxide [28], and heavy metals [29], all products of risk factors or risk factors themselves for AD, block complex IV of the electron transport chain. This blockade of the electron transport chain is known to lead to the production ROS at complexes I and III of mitochondria. Given the fact that both complex I and III are situated on the inner mitochondrial membrane, precisely where CPT2 resides, a high local exposure of CPT2 to ROS can be anticipated, regardless of the risk factor that caused it. This is a crucial finding, as CPT2 is very sensitive to ROS because it has an oxidation sensitive histidine residue in its active site [30]. This residue chelates metal ions which catalyze the formation of highly reactive hydroxyl radicals from hydrogen peroxide. Hydrogen peroxide is a relatively stable ROS that is formed by dismutase of superoxide anions which are formed upon complex IV inhibition. The formation of hydroxyl radicals at this residue, located right in its active site, is likely to oxidase, and to inactivate CPT2.

How can CPT2 inhibition cause Alzheimer's disease?

The central role for CPT2 inhibition in causing the AD phenotype is summarized in Fig. 2.

A major effect of CPT2 inhibition is that metabolic energy in neurons will get depleted. As neurons hardly burn any lipids for their energy requirements themselves, this is an indirect effect. Neurons not only burn glucose to produce energy but also lactate, which is produced glycolytically by astrocytes. However, upon the inhibition of CPT2 in astrocytes, their NAD levels decrease. This inhibits their glycolysis and therefore their lactate production, causing an energy deficiency in neurons. This energy deficiency will activate AMPK in these cells, leading to the phosphorylation of tau [5] which contributes to the formation of neurofibrillary tangles which are typical for AD. The hyperphosphorylation of AMPK also reduces the number of neuronal synapses and may therefore contribute to AD related memory loss [31].

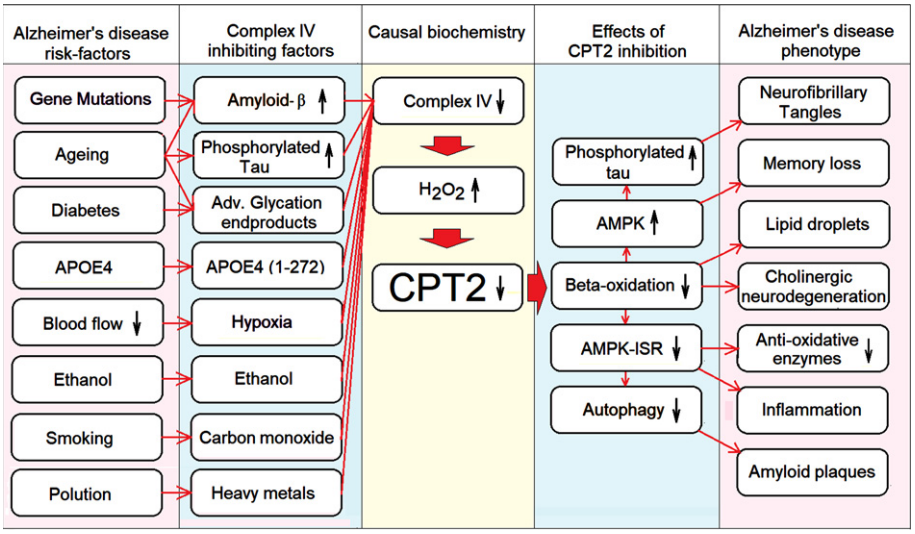

Fig. 2. The CPT2 cascade hypothesis for Alzheimer's disease.

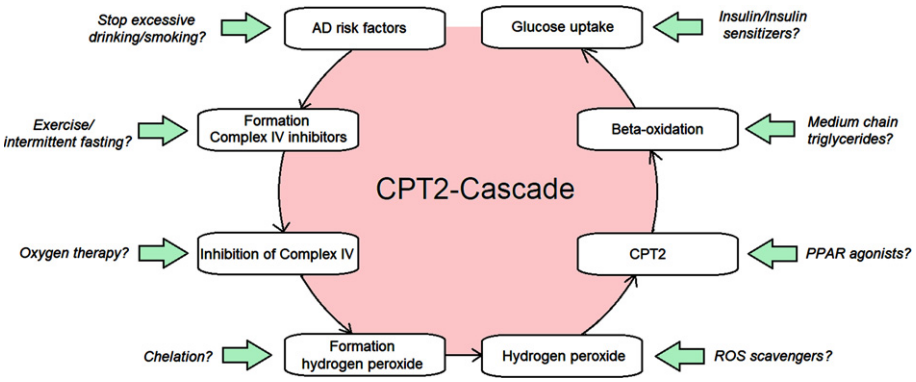

Fig. 3. Potential targets for drug treatment of AD based on the CPT2 cascade hypothesis.

The most direct effect of the inhibition of CPT2 is probably that lipids cannot be burned anymore by beta-oxidation. This may contribute to the formation of lipid droplets in the brains of AD patients [32] and in CPT2 deficient brains of Drosophila [20], although this relationship between CPT2 inhibition and lipid droplet formation is probably more complex than just an accumulation of unburned triglycerides.

Cholinergic neurons, involved in memory retention, will suffer most from an energy deficiency because they need Acetyl-CoA, as formed from lactate, not only for energy but also for the production of acetylcholine.

By inhibiting the AMPK-ISR, CPT2 inhibition will also lead to neuroinflammation and the formation of amyloid plaques. The reason for that is that an intact AMPK-ISR limits inflammatory reaction [33], and orchestrates autophagy, which removes amyloid plaques [34]. Furthermore, the inhibition of the AMPK-ISR will lead to a decreased production of anti-oxidant enzymes which will deteriorate the cellular protection against hydrogen peroxide, and promote the inactivation of CPT2.

The physiological cascade, as set in motion by AD risk factors, and leading to CPT2 inhibition, can explain all major phenotypic manifestations of the disease, and does justice to the complexity of the disease.

EVALUATION OF THE CPT-2 CASCADE HYPOTHESIS FOR ALZHEIMER'S DISEASE

Here, we present the "CPT2 Cascade Hypothesis for Alzheimer's Disease." This hypothesis incorporates all the essential elements of existing theories

regarding AD and aligns remarkably well with the majority, if not all, of the data presented in publications on the etiology of AD. A noteworthy departure from the most prominent hypothesis in AD, the amyloid cascade hypothesis, is that in the CPT2 Cascade Hypothesis, the formation of amyloid plaques and neurofibrillary tangles is regarded as a consequence rather than a cause of AD. This perspective finds support in recent clinical findings that have demonstrated limited clinical benefits from immunotherapy aimed at removing plaques and tangles [35].

When comparing the CPT2 cascade hypothesis with existing hypotheses on AD, it becomes clear that this hypothesis shows most resemblance to the well-established mitochondrial cascade hypothesis [4], as mitochondrial biochemistry, mitochondrial biogenesis, and energy provision to neurons plays an important role in both hypotheses. The CPT2 cascade hypothesis is different from the mitochondrial cascade hypothesis in that it is not limited to non-genetic forms of the disease. In addition, it provides the AMPK pathway dependent integrated stress response as a causal framework of which mitochondria are a regulatory element. Therefore, in a certain sense, mitochondrial damage is not the cause of AD, but the result of a dysfunctional AMPK-ISR which at a higher abstraction level can be seen as the actual cause of AD.

A potential limitation of the CPT2 cascade hypothesis is that it largely results from deductive reasoning (starting from a pathway), whereas previous hypotheses for the disease mainly used inductive reasoning (starting from phenotypic findings). The intrinsic risk of a deductive approach is that only data are collected that are in line with the presumptions made. Deductive reasoning only leads to valuable insights if its premises are right, and care is taken that no data exist that are in conflict with its presumptions. Our premise, that AMPK pathway controls ageing and plays an important role in controlling stress responses and neurodegeneration is widely accepted. Nevertheless, recognizing the importance of the correctness of this presumption, we have extensively validated and refined this pathway as a basis for this paper [2]. Furthermore, we checked all main elements of previous hypothesis to see if these are in line with the CPT2 cascade hypothesis and concluded that scientific data as used to support previous hypotheses, generally also align with our hypothesis.

Independent supportive evidence for the CPT2 cascade hypothesis comes from the fact that specific complex IV inhibition is a known property of mitochondria isolated from brains of AD patients [36], and the fact that in brains of AD patients the expression of CPT2 is negatively correlated with both the severity of AD, and the Mini-Mental State Examination score [37].

The most important insight of the CPT2 cascade hypothesis regarding new treatments for AD is probably that the etiology of AD seems to follow a more or less linear multi-step process (Fig. 3). This implies that synergistic effects can be anticipated if several of these steps would be targeted simultaneously. This may inspire clinicians to use combinations of existing treatments targeting these steps, in the treatment of AD, and such studies could start immediately.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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