Review Article

Promising Genetic Biomarkers of Preclinical Alzheimer’s Disease: The Influence of APOE and TOMM40 on Brain Integrity

Beata Ferencz, Sari Karlsson, and Grégoria Kalpouzos

Aging Research Center, Karolinska Institutet-Stockholm University, Gävlegatan 16, 113 30 Stockholm, Sweden

Correspondence should be addressed to Beata Ferencz, beata.ferencz@ki.se

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Finding biomarkers constitutes a crucial step for early detection of Alzheimer’s disease (AD). Brain imaging techniques have revealed structural alterations in the brain that may be phenotypic in preclinical AD. The most prominent polymorphism that has been associated with AD and related neural changes is the Apolipoprotein E (APOE) ε4. The translocase of outer mitochondrial membrane 40 (TOMM40), which is in linkage disequilibrium with APOE, has received increasing attention as a promising gene in AD. TOMM40 also impacts brain areas vulnerable in AD, by downstream apoptotic processes that forego extracellular amyloid beta aggregation. The present paper aims to extend on the mitochondrial influence in AD pathogenesis and we propose a TOMM40-induced disconnection of the medial temporal lobe. Finally, we discuss the possibility of mitochondrial dysfunction being the earliest pathophysiological event in AD, which indeed is supported by recent findings.

1. Introduction

Alzheimer’s Disease (AD) is one of the leading causes of dementia today and it poses an immense societal challenge as the prevalence is expected to continue to rise [1]. This makes it imperative to identify early preclinical changes in AD with high accuracy, in order for intervention strategies to yield effective outcome and to allow affected individuals to partake in an active treatment plan [2–5]. AD is characterized by early pathological changes in the brain, including senile plaques, neurofibrillary tangles, synapse, and neuronal loss. Neurofibrillary tangle formation may initiate in subcortical nuclei such as the dorsal raphe and locus coeruleus, prior to spreading to transentorhinal regions [6, 7]. Findings also support that pathological changes in AD commence in the medial temporal lobe (MTL) [8–10], primarily in the entorhinal cortex (ERC) and hippocampus (HC) [11–13], which undergo initial gray matter (GM) loss. Recently, attention has also been directed towards the impact of pathological mechanisms on white matter (WM), as up to 50% of AD cases present with global WM deterioration in neuropathological examinations [14, 15]. The temporal succession of GM and WM changes in preclinical AD remains to be determined; so far there is support for both primary and secondary WM changes within the MTL [16, 17].

MCI is regarded as a prodromal state of AD, where individuals present with subjective memory complaints and/or objective memory impairment, but are still intact in daily life and do not meet current AD diagnostic criteria [2, 18, 19]. Amnestic type MCI (aMCI), where memory impairment is considered predominant, has been proposed as a solution for the diagnostic heterogeneity of the overall MCI criteria. The construct of MCI allows for the clinical assessment of prodromal AD, where early interventions could have a beneficial effect [20]. While promising, this therapeutic window is hampered by the fact that not all individuals with MCI convert to AD (6–25%), and almost half return to normal cognitive health within the first year of followup [2, 21]. Moreover, caveats remain regarding MCI and its clinical usefulness, signifying that the most beneficial use of the MCI criteria is by combination with other structural, functional, neuropsychological, genetic, and pathological biomarkers [22]. These
b biomarkers were recently placed into a hypothetic biomarker timeline by Jack and colleagues [23], who proposed that the pathological cascade in AD commences with amyloid and tau pathology, followed by neural injury and dysfunction and finally structural alterations (see Figure 1). Furthermore, they hypothesize that β-amyloid deposition and the following cascade occur earlier in Apolipoprotein (APOE) ε4 carriers. Recent findings have shown brain and cognitive changes up to 10 years prior to the diagnosis of AD, indicating that the combination of biomarkers may provide an alternative timeline [23–25]. A growing body of literature has emphasized the association between genetic and structural brain biomarkers, with imaging quantitative traits within the MTL being a more objective outcome than clinical diagnosis alone. The MTL may act as a mediator between genetic polymorphisms and the clinical expression of AD, indicating the advantage of combined genetic and brain integrity biomarkers [26,27].

APOE is one of the primary AD polymorphisms, associated not only with risk and age of onset, but also brain integrity in AD [3,28,29]. Due to its Linkage Disequilibrium (LD) with APOE, Translocase of outer mitochondrial membrane 40 (TOMM40) was previously thought to have minimal influence on the risk of AD [30,31]. Nevertheless, it is now established that TOMM40 influences onset of AD [31–37]. The TOMM40 gene holds promising biomarker properties due to its negative impact on downstream apoptotic processes within the mitochondrial system via possible amyloid beta (Aβ) interplay [30,38,39]. Recently the mitochondrial cascade hypothesis has received increasing support, proposing that mitochondrial dysfunction is the key pathological mechanism in AD, influencing brain structures known to be vulnerable in AD [40,41]. We intend to extend these theories by presenting the mitochondrial disconnection model, an adapted model for mitochondrial involvement in preclinical AD. We also suggest a timeline shift in the biomarker realm, away from the amyloid hypothesis, towards early and primary mitochondrial involvement in the pathophysiology of AD (see Figure 1). The implication of mitochondrial dysfunction in AD is currently supported by genetic and neuropathological research [30,39,41] and has the possibility to shed light on the primary biological insult in the disorder, as well as to provide a new therapeutic window for AD [42,43].

The present paper focuses on recent advances in neuroimaging and genetic biomarkers for preclinical AD. After an overview of structural brain changes in early AD, we discuss the influence of APOE and TOMM40, in an effort to approximate the primary pathological cascade in AD. The mitochondrial disconnection model is an extension of previous findings and is suggested as a workable hypothesis from which the influential role of mitochondria on AD can be assessed.

2. Structural Brain Changes in Early Alzheimer’s Disease

The consensus in the literature is that there is a long preclinical phase of AD, with cognitive as well as structural brain changes commencing years before clinical diagnosis of the disorder [23,24,44]. Indeed, significant brain atrophy can be observed in healthy individuals who will subsequently develop MCI or AD, in comparison to stable controls, within the bilateral medial and lateral temporal lobes, orbitofrontal cortex, posterior cingulate, and precuneus [24]. Interestingly, these preclinical changes correspond to the pattern of GM alterations seen in diagnosed AD [15,45], demonstrating that early AD type pathology is present prior to clinical symptoms [24,46].

Both MCI and AD have characteristic influence on structures in the brain, thereby making them dissociable from nonpathological aging [45,47]. Imaging studies have been able to confirm the Braak staging of neuropathology in AD by showing early structural changes within the MTL (more particularly the ERC and HC), prior to spreading to adjacent cortices [10,13,48–50]. Looking closer at the HC, the lateral CA1 subfield is the most vulnerable in MCI and AD while GM loss in the subiculum is associated with nonpathological age-related changes, denoting region-specific changes within the HC in AD [51]. Moreover, several studies have indicated that the rate of atrophy within the MTL is faster for those who convert from normal aging to MCI as well as from MCI to AD in comparison to those who remain stable [52–54]. This shows that not only atrophy, but also the rate of atrophy in the HC over time could serve as a potential biomarker of preclinical AD. Structural changes in mild-to-moderate AD also occur in areas that are strongly connected to the MTL, such as the retrosplenial cortex, posterior cingulate, precuneus, and lateral posterior parietal regions [55,56]. These findings have been confirmed in individuals with MCI, where medial and lateral temporal as well as parietal atrophy was evident in individuals who converted from MCI to AD, in comparison with those who remained stable [57].

Although the progression of WM alterations in the brain is still unclear, damage to WM pathways within the MTL can
be detrimental according to the “disconnection hypothesis,” stating that deterioration of WM tracts leads to subsequent disconnection of the brain's circuitry [11, 58]. Not only might there be an overall disconnection, but a specific isolation of the HC that may result from reduced WM integrity within the MTL, mainly in the parahippocampal area, cingulum, fornix, uncinate fasciculus, and perforant pathway [59–61]. Moreover, an alteration in the WM of the precuneus, closely interconnected with the MTL, has also been observed, resulting in the isolation of the hippocampus [59]. Researchers debate the sequential order of GM and WM deterioration in preclinical AD and the Wallerian degeneration hypothesis stipulates that loss of WM integrity is secondary to GM changes. In line with this hypothesis, research has shown GM atrophy to be more efficient in distinguishing between AD patients and healthy controls. More specifically it has been observed that right-sided hippocampal GM loss is a better predictor of diagnostic status of AD than measures of WM integrity [8]. Recently, this hypothesis was supported in a study where primary GM degeneration in the HC was followed by Wallerian degeneration of WM within the interammonic commissure, a pathway connecting the left and right hippocampi [12]. Also similarly, Villain and colleagues found HC atrophy to be followed by loss of WM integrity in the uncinate fasciculus and cingulum bundle, which was corroborated by metabolic alterations in connected cortical areas, demonstrating a significant disruption in connectivity [61]. By contrast, WM deterioration has also been observed in the absence of primary GM changes [60, 62]. For example, in individuals with MCI and AD, loss of WM integrity has been seen in the perforant pathway in absence of GM atrophy [63]. Alteration of the perforant pathway, which connects the ERC and the HC and constitutes a gateway to the limbic system, may contribute to early and likely initial disconnection of the MTL in preclinical AD [60].

WM changes in small pathways of the brain, such as the perforant pathway, are still arduous to discern with the current available neuroimaging techniques. This is particularly important in preclinical AD where the areas implicated are small WM pathways within the MTL. Moreover, the lack of longitudinal studies renders it difficult to determine the temporal order of GM and WM changes in the brain. It has been pointed out that it is disadvantageous to consider the WM changes in the brain in a dichotomized fashion [16, 17]. Instead, a balanced view has been proposed where the temporal order of structural changes in the brain is dependent on the retrogenesis of the specific structure [16, 64]. For instance, in late myelinating pathways connected to the MTL such as the inferior longitudinal fasciculus, primary loss of WM integrity is thought to be of major influence. In early myelinating pathways such as the cerebral peduncles, posterior limb of internal capsule, and forceps major on the other hand, WM degeneration is considered secondary to GM loss. However, it has been proposed that within each brain area, depending on its retrogenetic development, there is a ratio between primary and secondary WM degeneration, possibly explaining why the temporal order of GM and WM changes in early AD has been difficult to ascertain [16].

3. APOE and Preclinical Alzheimer’s Disease

While research in the genetic field has been fraught by small effect sizes and difficulty in replicating findings, APOE has remained a robustly replicated susceptibility gene for AD [42, 65, 66]. Located on chromosome 19, APOE translates into three common allelic variations ε2, ε3, and ε4 [67], the ε4 being strongly associated with risk of developing AD [28, 68, 69]. Furthermore, the ε4 allele has been associated with decreased memory functioning, processing speed, and loss of GM and WM integrity [70–74]. The ε4 allele also modulates risk of progression from MCI to AD. In effect, a recent meta-analysis demonstrated that the presence of one or two ε4 alleles increased the risk of MCI conversion to AD up to four times. However, APOE as a risk factor has low predictivity and sensitivity values as a diagnostic test for AD, leading to the conclusion that APOE genotyping has limited value as a diagnostic tool in clinical practice [75–77].

APOE appears to play an essential role for lipid metabolism within the Central Nervous System (CNS) and allelic variations of the gene are thought to modulate neural repair, lipid homeostasis, oxidative stress, and Aβ deposition [43, 68]. As lipids are abundant in the brain and essential for myelination of axons, it comes as no surprise that APOE, being the main cholesterol transport lipoprotein, appears to play an essential role in maintaining brain integrity [78]. Although the mechanism behind the influence of APOE on the brain is not fully elucidated, the protein appears to govern the efficiency of cholesterol delivery to neurons. Particularly, the presence of an ε4 allele reduces the delivery of cholesterol, consequently disturbing lipid homeostasis within the CNS and triggering a cascade leading to the formation of amyloid depositions [79]. The combined amyloid cascade hypothesis [80] and APOE lipid recycling cascade models [81] promote a disturbance in lipid homeostasis as a source for AD pathology [82]. While the amyloid cascade hypothesis has been prominent throughout the last two decades, it was initially based on studies with rare autosomal dominant variants of AD and had pathophysiological shortcomings [83]. Indeed, widespread amyloid deposition is present in AD, but there is no consensus regarding the finite pathophysiological burden of amyloid in the brain and it has been argued that amyloid aggregation is a downstream process in AD not related to clinical manifestation of the disorder [84]. While APOE may influence the amyloid cascade in AD, other neuropathological aspects of the polymorphism have been highlighted, including influence on neuronal repair mechanisms and maintenance of synaptic connections [43]. One way of increasing the predictability of the APOE polymorphisms is by combining genetic and structural brain biomarkers [27, 73].

4. APOE and Structural Integrity

Extensive research has been done on the genetic influence of APOE polymorphisms on brain changes in preclinical AD (Table 1). Support for the influence of APOE on AD-like changes within the brain comes from a Genomewide Association Study (GWAS) on neuroimaging phenotypes in a mixed sample of MCI and AD individuals [85]. The authors
found APOE to be one of the top ten genetic markers to influence overall imaging phenotypes. Espeseth and colleagues [86] demonstrated a modulatory effect of APOE polymorphism on cortical thickness in healthy middle-aged adults. Carriers of an e4 allele showed accelerated cortical thinning in specific regions known to structurally deteriorate in normal aging but also in AD such as prefrontal regions, para-hippocampal cortex, and adjacent occipitotemporal areas (fusiform and lingual gyri), but not the HC. Others have found more region-specific influence of APOE on MTL areas [29, 87, 88]. This effect appears to be left lateralized with the e4 influence on HC volume [89]. The majority of findings converge towards a significant impact of APOE polymorphism on GM integrity within the MTL, mainly the HC.

Further, evidence for the influence of APOE on the MTL comes from longitudinal studies on MCI and conversion to AD (see Table 1). These have found that there is a genetic influence of APOE not only on hippocampal GM loss, but also on the rate of atrophy of the HC [88, 94]. Support for the specific influence of APOE e4 in MCI has been shown, as aMCI individuals have been found more likely to have smaller hippocampi and be carriers of at least one e4 allele than nonamnestic MCI individuals [94]. Also, MCI APOE e4 carriers express AD-type structural alterations such as atrophy in MTL regions (ERC and HC). Those with MCI and an e4 allele who convert to AD also show atrophy in frontal and parietal cortices [48, 95]. Progressive MCI e4 carriers show global AD-type structural changes years before clinical diagnosis of AD [95]. However, APOE e4 does not predict conversion from MCI to AD, while ERC volume reduction at baseline does [48]. Hence, it appears that while APOE may influence structural integrity in areas that are vulnerable in the preclinical stages in AD, APOE polymorphism has limited predictive value on the conversion to AD. The latter finding may, however, be biased by limited sample sizes. Thus, future studies combining structural, genetic and cognitive biomarkers in larger samples may show enhanced predictability.

Given its hypothesized role as the brain’s main lipid transporter [79], APOE impacts WM integrity in preclinical AD [99]. Several studies have confirmed both widespread and localized WM changes throughout the brain in relation to APOE polymorphism in healthy samples (see Table 1). Persson and colleagues [97], for instance, demonstrated an impact of APOE e4 on the WM integrity of the posterior corpus callosum and HC in healthy younger and older individuals, possibly reflecting preclinical signs of AD. Their findings are supported by recently published data showing that the presence of an e4 allele exacerbates age-related WM changes [73]. Moreover, it seems that late myelinating regions are more susceptible to age-related loss of integrity in e4 carriers, leading to progressive disconnection of the brain in APOE e4 carriers [96].

In conclusion, influence of APOE on GM structural integrity has been consistently demonstrated in areas associated with preclinical AD. By contrast, little is known about the genetic influence of APOE on WM changes in AD and whether these changes are occurring sequentially or in a balanced retrogenetic fashion.

5. TOMM40 and Preclinical Alzheimer’s Disease

Missing heritability is increasingly debated in the literature, as current genetic findings are not able to explain the full extent of the genetic contribution to complex diseases such as AD [65, 100, 101]. While larger sample sizes in GWAS are suggested as a remedy for missing heritability, others suggest that the answer resides in genetic polymorphisms that are in LD with current known ones [65, 102–104]. TOMM40 is becoming increasingly acknowledged as a prominent AD gene [31–37]. In LD with APOE, TOMM40 could hold part of the missing heritability that we are searching for in our efforts to map the genetic influences in AD. Moreover, taking TOMM40 into consideration may contribute to a better understanding of the early and primary pathophysiological cascade that takes place in the preclinical phases of the disorder. This hypothesis is supported by the fact that TOMM40 asserts its influence on mitochondrial survival, a process increasingly highlighted in the pathogenesis of AD [31, 105, 106]. Mitochondrial dysfunction has been associated with several pathological processes in AD, including brain hypometabolism, synaptic pathology, accumulation of Amyloid Precursor Proteins (APP), and Aβ influx to the cell [38, 39, 41]. Mitochondria have recently been implicated in more complex signaling cascades, oxidative stress, and apoptotic processes, indicating that mitochondria are not merely a powerhouse of the cell, rather they appear to govern cell death [107]. The notion of mitochondrial dysfunction in aging and neurodegeneration is not new. In fact, malfunctioning mitochondrial systems have been observed in premature aging [105, 108] as well as neurodegenerative disorders such as AD [40, 109, 110], Parkinson’s and Huntington’s disease [105] and appear to have an early and causal influence on pathological processes in the brain. Damage in mitochondria may exert a specific influence on the pathophysiology of AD through interplay with Aβ and its precursor, the APP [111].

Mitochondria play an essential role in providing energy to cells and are abundant in the neurons and synapses of the CNS. Containing an outer and inner membrane, the organelle is essential for the production of adenosine triphosphate (ATP), which is the energy source of all cells [112]. The outer mitochondrial membrane contains the translocase of outer mitochondrial membrane pore subunit (Tom40). The Tom40 channel forming subunit is one of the primary pores via which proteins can readily enter the mitochondria. The pore is governed by the TOMM40 gene and is essential for mitochondrial survival as the majority of proteins that enter the mitochondria pass through here [107, 113]. In AD specifically, it has been hypothesized that mitochondria exert neurotoxic influence by allowing the influx of Aβ to the cell via the Tom40 import pore. Passage of Aβ through the Tom40 import pore increases Reactive Oxygen Species (ROS) within the organelle. This increase is detrimental for mitochondrial survival and energy production (ATP), ultimately resulting in apoptotic processes of the cell [38, 111, 114]. Further ROS precipitating events include the accumulation of APP in mitochondrial import pores. This
Table 1: Genetic influence of APOE and TOMM40 on cerebral structural integrity.

<table>
<thead>
<tr>
<th>Author</th>
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<th>Method</th>
<th>Structural integrity</th>
<th>Conclusion</th>
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<td><strong>APOE</strong></td>
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<tr>
<td>Pievani et al.</td>
<td>Across APOE (e4) n = 28</td>
<td>Volumetry region based</td>
<td>Smaller HC in APOE e4+</td>
<td>ε4+ carriers have greater atrophy in the HC.</td>
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<td>2011 [29]</td>
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<tr>
<td>Bendlin et al.</td>
<td>Across APOE (e4) &amp; family history of AD n = 136</td>
<td>DTI whole brain</td>
<td>Parental history of AD</td>
<td>While no main effect of APOE was observed on DTI measures, parental history of AD was associated with reduced WM integrity in brain areas deteriorated in AD, which in turn interacted with APOE.</td>
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<tr>
<td>2010 [90]</td>
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<td>Reduced FA in cingulum, tapetum, uncinate fasciculus, HC, and adjacent WM</td>
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<tr>
<td>Pievani et al.</td>
<td>Across APOE (e4) n = 29</td>
<td>Volumetry whole brain</td>
<td>APOE ε4+</td>
<td>Significant atrophy in Bil temporal lobes, occipital lobes, retrosplenial, and posterior cingulate Highest GM reduction &gt;20%: entorhinal cortex, anterior temporal pole, superior and middle temporal gyrus, ventral, and dorsal occipital cortex APOE ε4+ versus ε4− Global GM reduction comparable (RH: 14 versus 15%; LH: 16 versus 17%) ε4+ more atrophy in medial and lateral temporal lobes, and right occipital pole</td>
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<td>2009 [91]</td>
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<td>APOE ε4+ Partially overlapping with additive, extending from posterior MTL to inferior lateral temporal cortex Additive model GM reduction in Bil MTL (HC, amygdala, parahippocampal gyrus), fusiform cortex, and orbitofrontal cortex Dose-dependent decrease in medial and anterior temporal lobe volume per allelic (e4) load. Variable regional association indicating that APOE works differently on mechanisms of disease expression.</td>
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<td>Filippini et al.</td>
<td>Across APOE e4 n = 100</td>
<td>Volumetry whole brain</td>
<td>No significant differences between e4+ and e4− on MTL atrophy, WM HI</td>
<td>APOE does not modulate white and gray matter in AD. While APOE influences risk of AD it appears not to modulate pathological processes after diagnosis.</td>
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<td>2009 [92]</td>
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<td>Barber et al.</td>
<td>AD across APOE e4 n = 25</td>
<td>Visual scoring</td>
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<td>1999 [93]</td>
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<td>MTL atrophy WM HI</td>
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<td><strong>TOMM40</strong></td>
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<td>Potkin et al.</td>
<td>AD (n = 229) Healthy Controls (n = 194)</td>
<td>Volumetry region-based GWAS on HC QT</td>
<td>Case-control analysis identified APOE and a new risk gene TOMM40 at 10−6 (10−11 at a haplotype level between APOE &amp; TOMM40 rs11556505) 25 SNPs were associated with QT HC, including APOE</td>
<td>APOE has an effect on brain atrophy independent from overrepresentation in AD. A novel risk gene, TOMM40, was found to be associated with AD.</td>
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<td>2009 [27]</td>
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<td><strong>Mild Cognitive Impairment</strong></td>
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<td><strong>APOE</strong></td>
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<td>Spampinato et al.</td>
<td>Stable versus Progressive MCI (n = 55) across APOE (e4)</td>
<td>Volumetry whole brain Longitudinal</td>
<td>Progressive APOEe4+ 1 year prior to diagnosis: GM atrophy in right temporal lobe, HC, insula 1 year FU: GM atrophy Bil HC, parietal, insula, caudate Stable APOEe4+ 1 year FU GM atrophy Bil insula, temporal lobe APOEe4+ converters show early GM loss 1 year prior to diagnosis, and atrophy progresses in e4+ converters to AD. However, some MTL atrophy is present in APOE e4+ nonconverters, reflecting nonlinear effects of APOE e4.</td>
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<td>2011 [88]</td>
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### Table 1: Continued.

<table>
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<tr>
<th>Author</th>
<th>Population</th>
<th>Method</th>
<th>Structural integrity</th>
<th>Conclusion</th>
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<tr>
<td>He et al. 2009</td>
<td>MCI across APOE n = 153</td>
<td>Volumetry region based</td>
<td>Amnestic MCI</td>
<td>Amnestic MCI individuals are more likely to have MTL atrophy and to be carriers of an APOE ε4 allele.</td>
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<tr>
<td>Tapiola et al.</td>
<td>Stable versus Progressive MCI</td>
<td>Volumetry region based</td>
<td>Progressive APOE ε4+</td>
<td>While significant atrophy was seen within the MTL in APOE ε4+ carriers with progressive MCI, the presence of an ε4 allele did not predict conversion to AD.</td>
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<td>2008 [48]</td>
<td>across APOE n = 60</td>
<td>Longitudinal</td>
<td>Reduced HC and ERC volume</td>
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<td>Hamalainen et</td>
<td>Stable versus Progressive MCI</td>
<td>Volumetry whole brain</td>
<td>Progressive APOE ε4+</td>
<td>APOE ε4+ converters display global AD-like atrophy in frontal and parietal cortices in comparison to ε4−, 2.5 years prior to diagnosis of MCI.</td>
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<tr>
<td>al. 2008 [95]</td>
<td>(n = 56) across APOE (ε4)</td>
<td>Longitudinal</td>
<td>Atrophy left inferior frontal gyrus, intraparietal sulcus Stable APOE ε4+ Atrophy right amygdala, anterior HC</td>
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<td>Shen et al. 2010</td>
<td>APOE n = 818</td>
<td>Volumetry whole brain</td>
<td>APOE &amp; TOMM40</td>
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<td>GWAS</td>
<td>rs 429358 (ε4 dependence)</td>
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<td>Freesurfer QT: 56 areas VBM QT: 86 areas</td>
<td>associated with whole brain Freesurfer (15 regions) and VBM (4) phenotypes at 10⁻⁶ significance TOMM40 rs2075650 associated with Freesurfer (5) at 10⁻⁷ significance Freesurfer phenotypes APOE associated with widespread phenotypes TOMM40 specifically associated with left and right hippocampi and left amygdala</td>
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<td>Ryan et al. 2011</td>
<td>APOE n = 126</td>
<td>DTI</td>
<td>APOE</td>
<td>While APOE is associated with widespread cortical AD-like changes. TOMM40 appears to be associated mainly with MTL phenotypes. Both APOE and TOMM40 were found among the top 5 SNPs in the GWAS.</td>
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<td></td>
<td>Age range 52–92</td>
<td>region based</td>
<td>Significant differences in ADC and FA with increasing age in frontal WM, lateral parietal WM, centrum semiovale, genu and splenium of CC, temporal stem WM These age-related differences in WM integrity were more prominent in ε4+</td>
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<td>Zhang et al.</td>
<td>APOE n = 409</td>
<td>Volumetry whole brain</td>
<td>Reduced GM volume in left HC in APOE ε4+ No significant differences in basal forebrain</td>
<td>Only left hippocampal volume was significantly reduced in APOE ε4 carriers and no differences were observed in the basal forebrain area.</td>
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<td>2011 [89]</td>
<td>Age range 70–90</td>
<td>region based</td>
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<tr>
<td>Espeseth et al.</td>
<td>APOE ε4+ (n = 37) ε4− (n = 59)</td>
<td>Volumetry whole brain</td>
<td>No group differences in total brain volume, GM volume, WM volume Cortical thickness ε4+ Thicker cortex in bilateral occipital and occipito temporal areas, right parahippocampal gyrus and frontal areas Age related cortical thickness ε4+ Both ε4+ and ε4− have age-related thinning in occipital and insula, but ε4+ also show thinning of MTL</td>
<td>Thicker cortex in APOE ε4+ was found in regions adjacent to those that show accelerated age-related decline, indicating that although well preserved now they may eventually show cortical thinning. APOE ε4 may accelerate thinning in areas that decline with aging (medial prefrontal, pericentral cortex) as well as areas susceptible to Aβ aggregation (occipitotemporal, temporal cortex).</td>
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<td>2008 [86]</td>
<td>Age range 48–75</td>
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<td>Bartzokis et al.</td>
<td>APOE n = 104</td>
<td>DTI</td>
<td>APOE ε4+ showed steeper age-related decline in radial diffusivity in late myelinated regions frontal lobe and genu of the CC</td>
<td>Late myelinated frontal regions appear more susceptible to age-related breakdown in APOE ε4+ carriers. This leads to progressive disconnection of cerebral networks in ε4 carriers and is supportive of an anterior-posterior WM degeneration gradient.</td>
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<td>2006 [96]</td>
<td>Age range 55–75</td>
<td>region based</td>
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accumulation of APP in import pores has been found in AD brains, mainly in the frontal cortex, HC, and amygdala and seen to vary with disease severity. Intriguingly, APOE ε3/ε4 carriers endorse the highest amount of mitochondrial APP, suggestive of a synergistic effect of mitochondrial dysfunction in the presence of APOE [39]. Furthermore, it has been shown that mitochondria have high intracellular Aβ accumulation in AD [114]. It has been pointed out that Aβ accumulation in mitochondria precedes extracellular Aβ deposition, which supports the role of mitochondria in the pathogenesis of AD [38]. Moreover, TOMM40 has recently been associated with CSF biomarkers including Aβ1–42, t-tau, and p-tau [115]. To this end, the mitochondrial cascade hypothesis is receiving increasing support throughout the literature, thereby demonstrating the implications of mitochondrial dynamics in the early pathophysiology of AD. The hypothesis postulates that mitochondrial dysfunction precedes amyloid insult to the brain and that mitochondrial injury is the primary source of pathology in AD [40, 116].

A recent neuropathological study, investigating the morphology of mitochondria in AD brains, confirmed the presence of mitochondrial pathology in brain areas typically associated with AD-type pathology [41]. Here mitochondrial alterations of shape and size were observed in AD, in comparison to healthy controls, in the neurons of the HC, neocortex, cerebellum, thalamus, pallidum, red nucleus, and locus coeruleus. As these assessments were conducted in postmortem AD brains, they more likely represent late pathological changes in AD. However, these findings are suggestive of morphological changes in mitochondria, possibly acting causally in the pathogenesis of AD. While mitochondrial morphological changes were not limited to the HC [41], one would expect to see preclinical morphological changes in the MTL, based on previous findings of mitochondrial-induced oxidative stress in preclinical dementia [110] as well as findings of Aβ and mitochondrial interplay [114].

Further support for mitochondrial involvement in AD comes from genetic studies involving the TOMM40 gene. Primarily Roses, and colleagues, the group that discovered the influence of APOE on AD [28], have been able to demonstrate an association between a long poly-T repeat of the TOMM40 gene with earlier age of onset of AD in APOE ε3 carriers [30]. The TOMM40 poly-T length acts either dependently or independently of APOE in the pathophysiology of AD [117]. Moreover, studies focusing on Single-Nucleotide Polymorphisms (SNPs) have found an association between TOMM40 and AD. A recent case-control study, comparing individuals with or without AD, showed a highly significant relationship between a TOMM40 SNP (rs2075650) and AD. Interestingly, a haplotype of TOMM40 rs2075650, rs11556505, and APOE rs429358 held a stronger association with AD than TOMM40 rs2075650 alone [85], supporting Roses and colleagues findings of a synergetic effect of TOMM40 and APOE [30]. Moreover, a recent genetic association study suggested that protein transport across the mitochondrial membrane was implicated in the pathophysiology of AD, and that TOMM40 is a likely contributor to this detrimental transmembrane process within the mitochondria [118].

Although genetic studies on mitochondrial involvement in AD are in their initial stages and replications are warranted, findings are supportive of previous postmortem, animal, and pathological studies in AD suggesting a significant involvement of mitochondrial dysfunction in AD.

### 6. TOMM40 and Structural Integrity

Postmortem studies on mitochondrial morphology in the HC [41] and the presence of APP in mitochondrial import pores in the HC of AD patients [39] suggest that mitochondrial dysfunction may follow Braak staging of neuropathology [8], with degeneration commencing in the MTL. By assessing the mitochondrial influence on brain integrity in AD, this temporal association can be further evaluated. Current cross-sectional studies, focusing on the differential influence of TOMM40 polymorphisms on the brain, offer promising insight to this link between genes and neuropathology in AD.

Johnson and colleagues [98] assessed the influence of TOMM40 poly-T length on structural brain integrity and cognition among APOE ε3 carriers. Analyses were restricted
to areas known to be vulnerable in AD including the amygdala, HC, parahippocampal gyrus, posterior cingulated, and precuneus. APOE ε3 carriers were divided according to length variations of the TOMM40 polymorphisms, homozygous short (SS), homozygous very long (VL), and heterozygotes (S/VL). TOMM40 length variation was found to influence episodic memory, which strongly depends on HC integrity, exemplifying the genetic involvement of TOMM40 on AD-type cognitive deficits. On the brain level, the poly-T length seems to influence the integrity of the medial ventral precuneus and posterior cingulate [98], which have been shown to be the site of early amyloid burden in AD [55]. This confirms previous findings, where the influence of TOMM40 poly-T length on AD onset has been shown [31] and supports the notion of mitochondrial influence in areas of the brain that are vulnerable to AD. Hence, it appears that healthy middle-aged individuals, who are APOE ε3 homozygotes with a long poly-T of the TOMM40 gene, show an AD-like profile with regards to cognitive performance as well as structural brain changes.

GWAS with HC volume as the phenotype supported the influence of the TOMM40 gene on structural integrity of areas implicated in AD. The authors found that three TOMM40 risk alleles (rs157580, rs2075650, and rs11355650) were overrepresented in the AD population as assessed by case-control analysis [27]. Shortcomings of focusing on one region in the brain were overturned, and a recent GWAS used whole brain imaging phenotypes in an attempt to understand the association between TOMM40 and structural integrity [85]. Notably, this analysis resulted in a significant association of the TOMM40 gene (rs2075650) with left amygdala and bilateral HC volume. Furthermore, comparison of healthy versus AD individuals revealed that TOMM40 was among the top 5 SNPs associated with whole brain imaging phenotypes. This points to the selective influence of TOMM40 on structural integrity in brain areas vulnerable to AD and supports previous findings of high APP burden in mitochondrial import pores in the HC and amygdala [85].

In an effort to examine the influence of TOMM40 in an independent cohort, we used data from nondemented individuals (age range: 60–90 years), from the Swedish National study on Aging and Care in Kungsholmen (SNAC-K) [119]. We assessed the genetic influence of the TOMM40 (rs2075650) gene on GM volume of the HC and episodic memory performance [120]. We expected to observe an APOE-independent negative influence of TOMM40 G (risk allele) on both cognitive performance and volume. Based on previous studies where APOE-independent TOMM40 influence was assessed [117], we stratified our TOMM40 sample across APOE. While we found no independent effect of TOMM40 on HC or ERC volume per se, we did observe that the positive association between HC volume and episodic memory was driven by the presence of at least one TOMM40 G allele in APOE ε4 carriers. This finding indicates that carriers of a TOMM40 G allele may be more dependent on HC volume for accurate episodic memory performance. This study suggests alterations within the mitochondrial system in TOMM40 G allele carriers, perhaps resulting in early morphometric alterations in mitochondrial shape and size. These alterations are not influencing structure, but rather the function of the HC, as assessed by episodic memory performance. It is possible that we are observing functional alterations at early AD stage that are not yet accompanied by significant volumetric changes in aging. As the timeline shifts to neurodegeneration, these functional changes may result in substantial structural changes, supported by postmortem findings of morphometric alterations in the mitochondria of the HC in AD [41]. Further support is provided by TOMM40 influence on brain integrity and cognition that are vulnerable in MCI and AD, as well as the overrepresentation of TOMM40 risk alleles in AD population [27, 98]. Functional changes within the HC might therefore be a primary sign of mitochondrial degeneration in preclinical AD.

Overall, the studies that are available today point to a selective influence of TOMM40 polymorphisms on structural changes in AD vulnerable areas such as the HC, precuneus and posterior cingulate cortex. To our knowledge, no studies have been conducted on the genetic influence of TOMM40 on WM changes in the brain. While the majority of findings concerning TOMM40 implicate GM changes, recent findings from our laboratory suggest that mitochondrial dysfunction might influence hippocampal functioning as well, as assessed using cognitive testing. These findings are supportive of a prominent mitochondrial dysfunction in AD and are promising for the utilization of mitochondrial biomarkers for the accuracy of early detection of preclinical AD.

7. The Mitochondrial Disconnection Model

As an attempt to recapitulate and expand on findings in the field, we propose the mitochondrial disconnection model (see Figure 2). This model is an adapted representation of the mitochondrial cascade in AD, and its downstream influence on structural changes. We propose that this cascade has a primary influence on GM structural integrity of the MTL, leading to disconnection and isolation of the MTL as a result of deterioration of connecting WM tracts.

In the adapted mitochondrial disconnection model TOMM40 acts via APOE-independent and -dependent pathways [31, 117]. Via APOE-independent pathways, TOMM40 regulates Aβ influx to the mitochondria via the Tom40 outer membrane pore. This notion is in line with postmortem studies that have found APP lodged in the Tom40 channels [39] as well as genetic studies suggesting that protein transport across the mitochondrial membrane, that is governed by the TOMM40 gene, is implicated in the pathophysiology of AD [118]. Via APOE-dependent pathways, there may be an interaction between APOE and TOMM40, which in turn may influence the Aβ influx. APOE is essential in the clearance and deposition of Aβ [79, 121–123] and has also been shown to increase extracellular Aβ availability [39, 43]. This increase in APOE-induced Aβ availability may allow for a larger proportion of Aβ to flow into the mitochondria via Tom40 import pores [117]. APOE ε3/ε4 carriers have the highest amount of mitochondrial APP, resulting in impaired mitochondrial functioning, suggestive of the importance of
Figure 2: The mitochondrial disconnection model is an extension of the TOMM40-induced mitochondrial cascade in Alzheimer’s disease (adapted from [31, 117]). TOMM40 governs the Tom40 complex on the outer mitochondrial membrane, allowing the influx of amyloid beta (Aβ) into the organelle. TOMM40 influence occurs either independently or dependently of APOE. Nevertheless, TOMM40-induced influx of Aβ to the cell starts downstream apoptotic processes via Reactive Oxygen Species (ROS), inducing cell death. We hypothesize that this results in early functional and structural alterations within the Medial Temporal Lobe (MTL), primarily in the hippocampus (yellow). Subsequent disconnection of the MTL, via deterioration of White Matter pathways such as the cingulum (green), fornix (red), and uncinate fasciculus (blue) follow. Disconnection of the MTL may induce secondary functional and structural alteration in distal areas possibly as a result of primary mitochondrial-induced cell death. (Brain graphic: courtesy of Michel Thiebaut de Schotten from the Natbrainlab, King’s College, London, UK.).

mitochondrial dynamics, even in APOE ε3/ε4 carriers [39]. There is support in the literature for both APOE-independent and -dependent pathways. Moreover, the TOMM40-induced mitochondrial cascade is unlikely to be autonomous of APOE, considering that APOE and TOMM40 are genetically linked via LD. Nevertheless, we propose that even in the APOE-dependent pathway, the role of TOMM40 is primary, as it influences mitochondrial protein transport via the Tom40 import pore.

Irrespective of the pathway through which the mitochondrial cascade commences, the flow of Aβ into the organelle induces apoptotic processes. The latter functions by increasing ROS within the mitochondria and has detrimental effects on cell survival within the MTL. As neurons contain hundreds of mitochondria, apoptotic processes may occur in a gradient fashion and might not influence neuronal structure initially. Morphometric changes have been seen in the mitochondria of the HC in AD, but these take place in the later stages of the disorder [41]. It is possible that early TOMM40-induced mitochondrial changes likely influence HC function, rather than its volume, evidenced by the triad between TOMM40, HC volume, and episodic memory in our aforementioned ongoing study [120]. This points to the importance of combining genetic, structural, and cognitive biomarkers to assess preclinical AD, as structural brain changes alone might not be sufficient for early and accurate prediction of preclinical mitochondrial alterations in AD. We suggest that functional changes in the HC, as a result of early mitochondrial alterations, could be utilized as an additional biomarker for preclinical AD. A timeline of mitochondrial degeneration commencing with early functional changes followed by structural changes within the brain can be hypothesized.

Nevertheless, initial TOMM40-governed mitochondrial insult has been found to take place in the HC [85]. Based on previous findings that disconnection and isolation of the HC
plays an important role in the early pathophysiology of AD [59–61], we hypothesize that WM changes of directly HC-connected WM tracts including the fornix, cingulum, and uncinate fasciculus follow in the mitochondrial cascade (see Figure 2). Whether this occurs via primary Wallerian degeneration or balanced retrogenesis remains to be elucidated. WM changes have been shown to be both primary and secondary to GM alterations in AD. The balance between primary or secondary WM degeneration within a certain region may be dependent on the gradient of mitochondrial dysfunction within that area. This balance might not be the same throughout the brain, as mitochondrial dysfunction has been primarily observed in MTL structures such as the HC and amygdala [39]. Future longitudinal studies will have to discern the temporal order of events and whether WM changes in preclinical AD are dependent on mitochondrial dysfunction in the GM. Overall, a succession of mitochondrial dysfunctional events in the pathophysiology of AD is supported not only by our ongoing study, but also by studies showing mitochondrial damage in normal aging [124, 125]. The degree of how widespread mitochondrial injury is in the brain may be determined by the neurodegenerative status of the individual and may follow Braak staging of pathology. That would explain why we observe TOMM40-induced influence on structural integrity of areas implicated in the early stages of AD. Based on these findings, we propose that widespread GM atrophy, seen in the later stages of AD, results from mitochondria-induced MTL disconnection via corticofugal pathways. Disconnection of the MTL may induce secondary functional and structural alteration in distal areas [61].

The proposed model is a representation of mitochondria-induced disconnection as an early and accurate biomarker for preclinical AD (see Figure 1). By this we expand on Jack and colleagues [23] dynamic biomarker timeline and propose that mitochondrial dysfunction initiates the pathophysiological cascade in AD. Findings in support of this timeline include the selective influence of TOMM40 on AD onset, HC volume, and cognition over and beyond that of APOE alone [27, 31, 85]. Moreover, mitochondrial Aβ aggregation precedes extracellular Aβ aggregation [38] supporting the mitochondrial cascade hypothesis rather than the amyloid cascade hypothesis, as the primary event in the biomarker timeline of AD. However, Jack and colleagues [23] pointed out that Aβ depositions are also observed in asymptomatic individuals, suggesting that the amyloid pathological process might be part of the process of aging. While amyloid depositions precede the clinical outcome of AD, the high presence of Aβ in healthy individuals suggests that other factors are at play. Moreover, the amyloid cascade does not fall into line with the Braak staging of pathology, where tau pathology was proposed to precede amyloid aggregation and commence within more basal midbrain structures [6, 7]. It has been shown that both tau and amyloid have synergic effects on mitochondrial dysfunction [126], suggesting that a biomarker timeline based on mitochondrial pathology might be more accurate in AD and would reconcile with Braak staging of pathology that has been well supported by neuroimaging studies.

8. Conclusion

There is increasing evidence for a primary mitochondrial involvement in the pathophysiology of AD, as mitochondria have been found to regulate cell death. Several studies have highlighted the importance of altered mitochondrial dynamics in the preclinical stages of AD, as well as mitochondrial involvement in structural brain changes within the MTL. Perhaps more importantly, mitochondrial dysfunction appears to be primary to extracellular Aβ aggregation. These findings demonstrate the necessity to direct attention away from the amyloid cascade hypothesis towards the mitochondrial cascade hypothesis. Moreover TOMM40 should be considered as a possible genetic modifier of the biomarker timeline in AD. The distinction between amyloid and mitochondrial cascades is not arbitrary with consideration to potential biomarkers and treatments of AD. While “mitochondrial protectors” as a potential treatment for AD are currently under investigation [116], further studies are needed in order to assess mitochondrial dynamics in preclinical AD. Genetic polymorphisms such as TOMM40 have the potential not only to assess individuals at risk, but also to serve as biomarkers in combination with current known structural and cognitive ones. We propose the mitochondrial disconnection model as a means by which the mitochondrial dynamics can be assessed in preclinical AD.

Glossary

Allele: One of two versions of a gene, an allele is a DNA coding that occupies a position on a given chromosome.

Amyloid cascade hypothesis: Proposes that the primary pathogenic event in AD is alterations in Amyloid Precursor Protein (APP) leading to the aggregation of the amyloid beta (Aβ) peptide.

Diffusion tensor imaging (DTI): Imaging acquisition that generates three-dimensional representation of the degree and direction of water diffusion (Brownian motion) in each voxel (tensor). Images are derived from computing 3 eigenvalues (λ1, λ2, λ3) within each tensor.

Fractional anisotropy (FA): DTI measure derived from the ratio of eigenvalues in each voxel, providing information about the directionality of diffusion along a scale of 0 (isotropic diffusion) to 1 (anisotropic diffusion) where 0 represents completely random diffusion indicative of damaged along white matter fibers.

Freesurfer: Software allowing assessment of volumetric and cortical thickness measures of the brain.

Genomewide association study (GWAS): Study by which the whole genome within a population is assessed in an attempt to identify common genetic associations with diseases such as AD.
Linkage disequilibrium (LD): Defines a genetic region that has had minimal recombination through ancestral history, making genes within such regions linked and dependent of each other.

Mean Diffusivity (MD): A DTI measure derived from the mean of the three eigenvalues that reflects magnitude of water diffusion within a voxel, without providing directionality. Increased MD is an indicator of tissue degeneration.

Missing heritability: The notion that a large proportion of the heritability of complex diseases such as AD remains unknown. Current GWAS have been able to identify genes with small effect sizes, leading us to ask where in the genome the remaining heritability is contained and why we cannot observe them with current techniques.

Mitochondria: Are often referred to as the powerhouse of the cells, as they produce adenosine triphosphate (ATP), the main energy source of the cell. Mitochondria have an outer and inner membrane, with the outer being permeable via active import channels allowing the passage of proteins that are essential for ATP. Mitochondria are mainly independent organelles, as they contain their own DNA. While they are the major energy provider to the cells, they are also critical for cell survival, cell division, and neuronal death.

Mitochondrial cascade hypothesis: Proposes that mitochondria are the primary source of pathology in AD, driving AB plaque and neurofibrillary tangle formation.

Radial diffusion: Radial diffusivity ($\lambda_2 + \lambda_3$) represents perpendicular diffusion across fiber pathways. Reduced radial diffusion has been associated with myelin damage.

Reactive oxygen species (ROS): A term that describes a variety of byproducts that are formed during the metabolism of oxygen, otherwise known as free radicals. In mitochondria they are formed as a result of respiration, and a disruption in this balance by increased ROS within mitochondria negatively influences cell survival.

Region of interest (ROI): Imaging procedure that involves manual outlining of an a priori brain region for volumetric analysis.

Retrogenesis: The hypothesis that brain areas that were early to develop are also the first to show AD-type pathology.

Single nucleotide polymorphism (SNP): Stands for a difference in DNA sequence, occurring at a single nucleotide (A, T, C, or G) on a paired chromosome in an individual and denoted by RS (related sequence).

Translocase of outer mitochondrial membrane (TOM): A major import channel on the outer mitochondrial membrane that allows for the influx of proteins to the intermembrane space of the organelle. Tom40 is the main component of this channel serving as the only channel by which proteins enter mitochondria.

Voxel Based Morphometry (VBM): Automatic procedure that allows whole-brain voxelwise analysis of tissue density and volume.

Wallerian Degeneration: Is a model for axonal degeneration. Initial neuronal damage is hypothesized to result in distal axonal degeneration.

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