

# Development of AD-Like Pathology in Skeletal Muscle

**Keywords:** Alzheimer's disease; Amyloid beta; Phosphorylated tau; Skeletal muscle

## Abstract

Effective therapeutic strategy against Alzheimer's disease (AD) requires early detection of AD; however, clinical diagnosis of Alzheimer's disease (AD) is not precise and a definitive diagnosis of AD is only possible via postmortem examination for AD pathological hallmarks including senile plaques composed of A $\beta$  and neuro fibrillary tangles composed of phosphorylated tau. Although a variety of biomarker has been developed and used in clinical setting, none of them robustly predicts subsequent clinical course of AD. Thus, it is essential to identify new biomarkers that may facilitate the diagnosis of early stages of AD, prediction of subsequent clinical course, and development of new therapeutic strategies. Given that pathological hallmarks of AD including A $\beta$  accumulation and the presence of phosphorylated tau are also detected in peripheral tissues, AD is considered a systemic disease. Without the protection of blood-brain barrier, systemic factors can affect peripheral tissues much earlier than neurons in brain. Here, we will discuss the development of AD-like pathology in skeletal muscle and the potential use of skeletal muscle biopsy (examination for A $\beta$  accumulation and phosphorylated tau) as a biomarker for AD.

## Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disorder of old age that results in massive health care costs in the United States [1,2]. Although gene mutations in amyloid beta precursor protein (A $\beta$ PP) or  $\gamma$ -secretase (presenilin-1 or presenilin-2) can lead to relatively rare familial AD [3], the vast majority (>95%) of AD cases is sporadic with unknown etiology. Currently, pathogenic mechanisms responsible for sporadic AD remain unclear, but are believed to result from complex interactions between aging, genetic factors, and environmental factors [4]. AD is characterized clinically by progressive memory loss and cognitive impairment, and unfortunately there is no effective treatment for AD. Effective therapeutic strategies require the diagnosis of AD at early stage; however, clinical diagnosis of AD is not precise. Definite diagnosis requires examination of postmortem brain tissues for AD pathological hallmarks including senile plaques composed of amyloid beta (A $\beta$ ), neurofibrillary tangles composed of phosphorylated tau, and signs of neurodegeneration [3,5].

It is important to note that pathological hallmarks of AD including A $\beta$  accumulation [6,7] and the presence of phosphorylated tau [8] are not restrict to brain, since they are also detected in peripheral tissues (e.g. skin and skeletal muscle) of AD human subjects. Thus, AD can be considered a systemic disease. Without the protection of blood-brain barrier, systemic factors can affect peripheral tissues much earlier than neurons in brain. Thus, the characterization of the phenotype related to peripheral tissues offers the opportunity to identify new biomarkers that may facilitate the diagnosis of early stages of AD, prediction of subsequent clinical course, and development of new therapeutic strategies. In this perspective, the development of AD-like pathology in skeletal muscle and the potential use of muscle



## Journal of Parkinson's disease & Alzheimer's disease

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**Submission:** 08 March, 2019

**Accepted:** 30 March, 2019

**Published:** 02 April, 2019

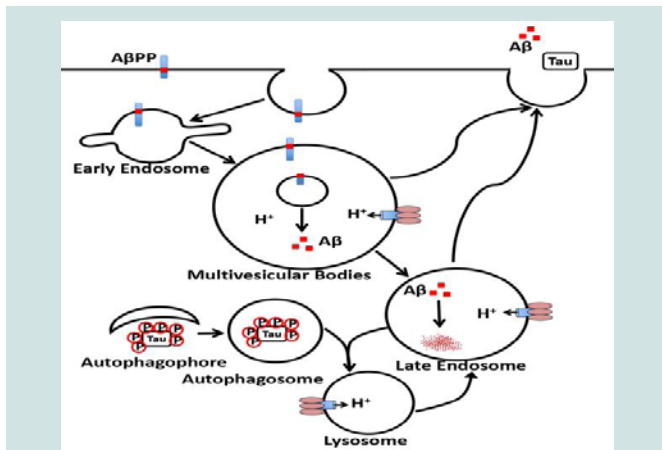
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biopsy (examination for A $\beta$  accumulation and phosphorylated tau) as a biomarker of AD will be discussed.

### Aging affects neuron and skeletal muscle similarly

The greatest known risk factor for AD is advancing age. An estimated 12 percent of people over age over 65, and nearly 50% of those aged over 85, are affected by this disorder in the United States [2]. Although it is still a mystery why AD risk rises so dramatically as we grow older, it is known that neurons tend to accumulate biological 'garbage' during aging process, because neurons are long-lived post-mitotic cells that lack the ability to dispose biological 'garbage' via cell division. As such, abnormal intraneuronal accumulation of damaged organelles and protein aggregates is a key event in the pathogenesis of AD [9], for instance, autophagic-lysosomal vacuoles filled with lipofuscin is the most prevalent of age pigments that accumulates in neurons [10], and neurofibrillary tangles is a result of intraneuronal aggregation of phosphorylated tau. Although senile plaques are extracellular depositions of A $\beta$ , it has been shown that A $\beta$  is mainly generated in endolysosomes following A $\beta$ PP internalization and can be accumulated inside neurons [11]. Such intraneuronal accumulation of A $\beta$  plays an early and important role in the pathogenesis of AD preceding the appearance of A $\beta$  plaques in extracellular space [12-14].

Like neurons, skeletal muscle fibers are also long-live post-mitotic cells. During aging, skeletal muscle fibers progressively accumulate damaged organelles and protein aggregates, as evidenced by accumulation of damaged mitochondria with aberrant function in aged skeletal muscle [15-17], accumulation of cytoplasmic p62-polyubiquitin protein aggregates [18,19], and accumulation of lipofuscin in dysfunctional autophagic-lysosome system [20]. More importantly increased A $\beta$  accumulation in skeletal muscle has been demonstrated in AD patients [6]. Recently it has been shown that phosphorylated tau is also present in peripheral tissues [8]. Although accumulation of phosphorylated tau in skeletal muscle has not been reported in AD patients, tau aggregates is present in skeletal muscle in a variety of protein aggregate myopathies [21,22].



**Figure 1:** Autophagic-lysosomal dysfunction contributes to the development of AD pathological hallmarks.

Aβ (generated in multi-vesicular bodies following AβPP internalization) can be degraded in lysosomes. Un-degraded Aβ can either accumulate in endolysosomes as intraneuronal Aβ or undergo exocytotic release to become extracellular Aβ. The formation of autophagosomes and fusion with late endosomes is critical for lysosomal degradation of aggregated tau/p-tau; un-degraded tau can be released extracellularly.

In addition to age-related structural changes, progressive decline in muscle mass and strength (sarcopenia) is a hallmark of the aging process. The prevalence of sarcopenia ranged from 13 to 24% in persons aged 65 to 70 years and was over 50% for those older than 80 years [23], and more recent estimates indicate that approximately 45% of the older U.S. population is affected by sarcopenia [24]. Importantly, such age-related decline in muscle mass and strength has been linked to brain atrophy [25] and cognitive impairment [26]. Moreover, AD patients often exhibit loss of muscle mass and reduced muscle strength, and such deficits in skeletal muscle may be early signs of AD [25, 27, 28]. Thus, skeletal muscle deficits may help predict the onset and progression of AD. Although it is not known how such skeletal muscle deficits are developed in AD, a testable hypothesis is that a common pathogenic process occurs in neuron and skeletal muscle.

### AD risk factors lead to skeletal muscle dysfunction

Given that the pathogenesis of sporadic AD is influenced by a variety of systemic risk factors, here we will discuss the influence of genetic and lifestyle-related AD risk factors on skeletal muscle and contribute to the development of loss of muscle mass and muscle strength. As shown in (Table 1), a list of genetic AD risk factors have been shown to lead to loss of muscle mass, muscle weakness, or the development of myopathy. These genetic AD risk factors include ApoE4 [29, 30], bridging integrator 1 (BIN1)[31,32], clusterin [33], and dynamin-2 [34]. Similarly, many lifestyle-related AD risk factors can lead to loss of muscle mass, muscle weakness, or the development of myopathy (Table 1). These lifestyle-related AD risk factors include type-2 diabetes [35,36] and type-2 diabetes-related conditions such as obesity [37], hyperinsulinemia [38], and metabolic syndrome [39], elevated homocysteine level [40-42], elevated levels of LDL cholesterol and the use of statins [43]. Due to the protection of the blood-brain barrier for neurons, these systemic metabolic changes can affect skeletal muscle earlier than neuron.

Autophagic-lysosomal dysfunction as a common pathogenesis for the development of AD pathological hallmarks in brain and skeletal muscle

As discussed above, aging affects post-mitotic cells similarly in neuron and in skeletal muscle. In addition, genetic life-style sporadic AD risk factors contribute to the loss of skeletal muscle mass and/or muscle strength. Furthermore, pathological hallmarks of AD including intracellular Aβ accumulation [6,7] and tau aggregates [21,22] are present in skeletal muscle. Thus, a common pathogenic process for the development of AD may occur in neurons as well as skeletal muscle. Here, we postulate that autophagic-lysosomal dysfunction as a common pathogenic mechanism for development of AD pathological hallmarks in skeletal muscle and in brain.

Long-live post-mitotic cells, like neurons and skeletal muscle, lack the ability to dispose biological ‘garbage’ via cell division as occurs in proliferating cells. However, these post-mitotic cells can renew themselves by degrading defective macromolecules and organelles into small molecules that are then either cleared or re-utilized. Short-lived proteins can be decomposed by cytosolic cysteine proteases or proteasomes; whereas most long-lived proteins, cytosolic protein aggregates, and all organelles including proteasome [44] and mitochondria [45] are degraded by lysosomes, which are acidic organelles that contain various lytic enzymes. As such, lysosomes play a key role in protein turnover and cellular homeostasis [46]. Substrates for degradation are delivered to lysosome by two general routes, namely, endocytosis and autophagy. Endocytosis is responsible for up-taking extracellular nutrients as well as the maintenance of membrane integrity. Autophagy, on the other hand, is responsible for removing unwanted cytosolic proteins and “worn out” organelles. Lysosomes are especially important for neurons and skeletal muscle, because they are mainly long-lived post-mitotic cells that require the autophagy-lysosome system in turning over cellular components and obsolete organelles [47,48]. Here, we will discuss the influence of AD risk factors on the development of autophagic-lysosomal dysfunctions (Table 1).

### AD risk factors lead to autophagic-lysosomal dysfunctions

**Aging:** Because neurons and skeletal muscle are post-mitotic cells that rely on lysosomes to dispose biological ‘garbage’, autophagy-lysosome function tends to decline during aging in these cells [49,50]. As such, both skeletal muscle and neurons progressively accumulate damaged organelles and protein aggregates during aging, as evidenced by accumulation of autophagic-lysosomal vacuoles filled with lipofuscin [10,20], accumulation of ubiquitin-positive protein aggregates [18,19,51], and the accumulation of damaged mitochondria with aberrant function [15-17,52,53].

### Genetic AD risk factors

**ApoE4:** ApoE-cholesterol synthesized in situ in brain is a discoidal shaped HDL-like particle composed of phospholipids and unesterified cholesterol [54,55]. Such HDL-like apoE-cholesterol supplies the neuronal need of cholesterol via receptor-mediated endocytosis. As the single strongest genetic risk factor for sporadic AD [56-59], apoE4 has been shown to promote endocytic dysfunction [60] and apoE4 genotype correlates intraneuronal Aβ accumulation in AD patients [61, 62]. In animal models, apoE4, but not apoE3, promotes lysosome

**Table 1:** AD risk factors lead to skeletal muscle dysfunction.

	Skeletal muscle	Skeletal muscle	Neurons
AD risk factors	Clinical signs of muscle dysfunction	Autophagic-lysosomal dysfunctions	Autophagic-lysosomal dysfunction
Aging	Sarcopenia [23,24] that is linked to brain atrophy [25]and cognitive impairment [26]	Accumulation of lipofuscin [10,20], protein aggregates [18,19,51], and damaged mitochondria[15-17,52,53].	Accumulation of lipofuscin[10], damaged organelles and protein aggregates [9]
<b>Genetic risk factors</b>			
ApoE4	Muscle weakness [29,30]	Potential endosome dysfunction involving insulin receptor [65]?	Endocytic dysfunction [60-186]
BIN1	Centronuclear myopathy [31,32]	Potential lysosome dysfunction involving EHD1 [75]?	BIN1 mutation leads to intracellular protein aggregation [74]
Clusterin	Muscle weakness and myopathy [33]	Aggresome accumulation[33]	Clusterin silencing impairs autophagy [79]
Dynamin-2	Centronuclear myopathy [34]	Lysosome dysfunction [85]	Endocytic trafficking defects [82]and impaired autophagy [83,84]
<b>Lifestyle risk factors</b>			
Type-2 diabetes and related conditions	Loss of muscle mass and muscle strength [35-37]	Autophagic-lysosomes dysfunction via altered insulin singling [96]	Autophagic-lysosomes dysfunction via altered insulin singling [95]
Hyperhomocysteinemia	Muscle atrophy and skeletal muscle malfunction [40-42].	Intracellular protein aggregates resulting from ER stress or derail autophagy [104-106].	Homocysteine inhibits autophagy via mTORC1[187]
Hypercholesterolemia and the use of statins	Statin myopathy [43]	LDL induces endolysosome dysfunction [127]. Statins induce autophagy impairment [153,154], vacuolization [148], increase lipid droplet accumulation[152], and Impaired protein degradation[155].	LDL induces endolysosome dysfunction [125] and autophagy impairment [129,130]

dysfunction and increases intracellular Aβ accumulation in enlarged endosome or lysosomes in neurons [63,64]. Currently, it is not known whether apoE4 leads to autophagic-lysosome dysfunction in skeletal muscle. However, apoE4 knock in mice exhibit impaired insulin signaling in skeletal muscle, which may link to endosome dysfunction and accumulation of insulin receptor in endolysosomes [65].

**BIN1:** As the most important genetic susceptibility locus in sporadic AD after APOE4 [66,67], BIN1 has been implicated in the process of clathrin-mediated endocytosis and intracellular endosome trafficking [68,69]. In addition, BIN1 is a key component in endocytic endosome recycling [70], and thus may be important for the intracellular trafficking of large molecules including proteins and lipids [71]. It has been shown that BIN1 level is decreased in sporadic AD brain [72], and brain specific knockout of BIN1 is deficient in endocytic protein scaffolds and synaptic vesicle recycling [73]. Because impaired endocytic recycling could increase the degradation load of lysosome and subsequent lysosome dysfunction, it is not surprising that BIN1 mutation could lead to intracellular protein aggregation in neurons [74]. Currently, no direct evidence demonstrating that BIN1 mutation leads to autophagic-lysosomal dysfunction in skeletal muscle. However, knocking down EHD1 that regulate BIN1 leads to lysosome dysfunction [75].

**Clusterin:** Also known as apolipoprotein J and the third most associated sporadic AD risk gene [58,59], clusterin is present in lipoprotein particles and regulates cholesterol and lipid metabolism [76]. Beside functioning as a lipid transporter, clusterin also function as a chaperone glycoprotein that inhibits protein aggregation [77]. Recently, it has been shown that AD-associated clusterin mutations are linked to reduced secretion of clusterin [78], and clusterin silencing impairs autophagy in neurons [79]. In skeletal muscle, clusterin has been shown to affect aggresome accumulation [33].

**Dynamin 2:** Dynamin-2, another genetic risk factor for AD [80,81], is important for membrane formation/trafficking and the formation and fission vesicles. Dynamin 2 mutation has been shown

to lead to endocytic trafficking defects [82] and impaired autophagy [83, 84] in neurons. Dynamin 2 Mutation can also lead to lysosome dysfunction in skeletal muscle [85].

**Lifestyle-related AD risk factors**

**Hyperinsulinemia:** An aggregated relative risk of approximately 1.5 is estimated linking type-2 diabetes with AD, and the relative risk increases considerably by the presence of APOE4 positivity [86-88]. In addition, type-2 diabetes-related conditions such as obesity [89,90], hyperinsulinemia [91,92],and metabolic syndrome [93] also present as risks for sporadic AD. High insulin levels in the blood are a common feature of type-2 diabetes. Upon binding to insulin receptor complex, insulin leads to tyrosine phosphorylation and recruitment of IRS1/2 to the membrane. This leads to activation of class I PtdIns 3-kinase activity generating PtdIns (3,4,5)P3and activating PDK1, Akt phosphorylation, and subsequent phosphorylation of mTOR, which is known to block autophagy [94].Prolonged hyperinsulinemia and subsequent inhibition of autophagy could lead to intracellular accumulation of protein aggregates and damaged organelles. Thus, the development of insulin resistance after chronic exposure of insulin and subsequent autophagy activation can be neuroprotective, especially when cells are faced with proteotoxic stress [95]. Similarly, autophagic-lysosomes dysfunction via altered insulin singling can occur in skeletal muscle [96].

**Hyperhomocysteinemia:** Homocysteine is a sulfur-containing amino acid and intermediate product of the methionine cycle, whose normal levels in the body are maintained by its re-methylation to methionine in a reaction that requires the availability of dietary folate, vitamin B6, and B12. A diet with excessive methionine, diet with a deficit in folate, or genetic alterations in enzymes involved in homocysteine re-methylation or transsulfuration pathway can lead to hyperhomocysteinemia [97]. Epidemiological and clinical studies have revealed that elevated homocysteine level is associated with hippocampal atrophy [98] and represents a modifiable risk factor for developing AD [99-102]. Homocysteine has been shown to induce

ER stress [103], which could result from enhanced ROS production or homocysteinylolation of resident ER chaperon [42]. Upon acute ER stress, the unfolding protein response is activated to restore ER protein-folding homeostasis. A process that involves the transient attenuation of protein synthesis, an increase in protein folding and transport in the ER, an increase in ER-associated protein degradation, and activation of autophagy. Persistent ER stress can derail autophagy, which leads to protein aggregates [104,105]. Similarly, elevated homocysteine level can affect autophagic-lysosomal function in skeletal muscle [104-106].

**Hypercholesterolemia:** Several lines of evidence support the role of elevated plasma cholesterol in the pathogenesis of sporadic AD. First, apoE4 is clearly associated with elevated levels of LDL cholesterol and decreased levels of HDL cholesterol [107,108]. Second, elevated levels of plasma LDL cholesterol, independent of APOE genotypes, are linked robustly to the pathogenesis of AD [109-115]. Third, independent of the APOE genotype, low levels of HDL cholesterol are also associated with increased risk of developing AD [112,144,116,117]. In plasma, apoB-containing LDL is the main lipoprotein particle that mediates the transport of cholesterol and lipids into periphery tissues. LDL-cholesterol is up-taken by receptor-mediated endocytosis, a process where lipoproteins bound to their receptors are internalized, transported to endolysosomes, hydrolyzed to free cholesterol, and from where free cholesterol is transported to various intracellular compartments via a mechanism involving the Niemann-Pick typeC (NPC) proteins type-1 (NPC1) and -2 (NPC2) proteins [118-120]. It has been shown that apoB 100, the exclusive apolipoprotein of LDL, leads to cholesterol being targeted by the lysosome degradation pathway [121,122], thus increased uptake of LDL-cholesterol may lead to cholesterol accumulation in endolysosomes, thereby disturbing endolysosome structure and function [123-125], a phenomenon similar to Niemann-Pick type C disease, a lysosomal lipid storage disorder caused by gene mutations in NPC [126]. Indeed, we have shown, in skeletal muscle fibers from a rabbit model of sporadic AD, cholesterol-enriched diet abnormally enlarged endolysosomes, in which were increased accumulations of free cholesterol and multiple AD marker proteins subject to misfolding and aggregation including A $\beta$ , phosphorylated tau, and ubiquitin [127]. In addition to its direct effect on lysosomes, hypercholesterolemia is associated with hyperactive mTORC1 and mTORC2 signaling [128], which blocks autophagy. Indeed, cholesterol loading with LDL has been shown to block autophagy [129,130]. Based on findings that autophagy regulates intracellular lipid stores [131], it is anticipated that cholesterol-induced autophagy blockage could lead to reduced bioavailability of intracellular cholesterol for the maintenance of membrane integrity in plasma membranes or organelle membrane.

Statins, a class of HMG-CoA reductase inhibitors that block cholesterol biosynthesis and lower plasma cholesterol levels, have been proposed as potential agents for the treatment and/or prevention of AD [132]. Although some beneficial effects have been reported in some case-controlled epidemiological studies [133,134], recent data and meta-analysis from randomized clinical trials indicates that statins have little or no beneficial effects against AD [135-138]. In fact, adverse effects of statins on memory and cognitions have been reported [139-143]. Currently, it is not clear how statins might lead to

memory and cognitive impairment. However, it is known that statins block cholesterol biosynthesis in the ER, and such an effect would decrease cholesterol transport to plasma membranes thus leading to membrane cholesterol deficits, synaptic disruption, and inability to repair membranes once injured [144-146]. In addition, as a consequence of blocking ER cholesterol synthesis, statins increase the expression of LDLRs and enhanced receptor-mediated endocytosis of cholesterol [147]. Such an effect could increase cholesterol burden in endolysosomes and subsequent lysosome dysfunction, which could promote intraneuronal accumulation of damaged organelles and protein aggregates. Statins also affect skeletal muscle function, and in fact myopathy is one of the common side effects of statins. It is estimated that 15% of reported adverse reactions were associated with objective muscle weakness in statin users [43]. Currently, it is not clear how statins lead to myopathy. However, it is known that by blocking cholesterol biosynthesis in the ER, statins decrease cholesterol transport to plasma membranes and thus decrease membrane cholesterol in skeletal muscle [148-150]. In addition, statins increase levels of LDLR and enhance LDL endocytosis in skeletal muscle [151], and such an effect could increase lipid droplet accumulation [152], autophagy impairment [153,154], vacuolization [148], and protein degradation impairment [155].

#### **Autophagic-lysosomal dysfunction contributes to the development of AD pathological hallmarks**

Senile plaque, the deposition of A $\beta$  in brain, is a pathological hallmark of AD. However, A $\beta$  accumulation is not restricted to brain since they are also detected in peripheral tissues (e.g. skeletal muscle) of AD human subjects [6,7]. Intracellular accumulation and extracellular deposition of A $\beta$  starts with specific proteolytic cleavage of A $\beta$ PP, a ubiquitously expressed type-I transmembrane protein with largely uncharacterized physiological functions. A $\beta$ PP is synthesized in the endoplasmic reticulum and it is transported to the Golgi/trans-Golgi network apparatus where it undergoes post-translational modifications and maturation. Once inserted into plasma membranes via secretory vesicles, A $\beta$ PP can traffic into endosomes via clathrin-dependent endocytosis, whereupon it can either be recycled back to the cell surface or it is delivered to lysosomes for possible degradation [156,157]. Endolysosomes appear to play a critical role in amyloidogenic processing of A $\beta$ PP [156,158,159], in part, because the rate-limiting enzyme BACE- and  $\gamma$ -secretase are almost exclusively located in endosome where the acidic pH is optimum for their activities [160-163]. The fate of endosome-derived A $\beta$  is further influenced by A $\beta$  degradation catalyzed by lysosome-resident cathepsins [164]. Once formed, A $\beta$  can accumulate in endolysosomes as intraneuronal A $\beta$  or it can undergo exocytotic release into extracellular spaces where diffuse A $\beta$  plaques can form. Thus, A $\beta$  generation can be enhanced by such factors as those that promote A $\beta$ PP internalization [165], those that enhance protein levels and/or activities of BACE-1 and/or  $\gamma$ -secretase, and those that prevent A $\beta$ PP recycling back to the cell surface [166], and those that impair A $\beta$  degradation in lysosomes [167].

Neurofibrillary tangle composed of phosphorylated tau is another pathological hallmark of AD. Microtubule-associated protein tau is a component of neurons, but it is also found in other non-neuronal tissues including the skeletal muscle [168]. In fact, accumulation

of tau protein in skeletal muscle disorders has been reported in vacuoles and inclusions [169]. Given that tau aggregates can be degraded by cathepsin D in autophagosomes-lysosomes [170-174], impaired lysosome degradation could lead to tau aggregation and the development of neurofibrillary tangle. In support, tau is accumulated in autophagic vacuoles in rat models of vacuolar myopathy induced by chloroquine [175]. Increased accumulation of cholesterol in lysosomes and subsequent lysosome dysfunction has been linked to the development of neurofibrillary tangle in brains of patients with Niemann-Pick type C disease [126,176-180]. More importantly, transcriptional activation of autophagy-lysosome biogenesis helps clear aggregated tau [181]. Together, autophagic-Lysosomal dysfunction contributes to the development of AD pathological hallmarks (Figure 1).

### Perspective

Effective treatment strategies against AD require the detection of AD at early stage; However, clinical diagnosis of AD is not precise, and a definitive diagnosis of AD relies on postmortem examination for AD pathological hallmarks. Although a variety of biomarker has been developed, none of them robustly predicts subsequent clinical course of AD. Thus, it is essential to identify new biomarkers that may facilitate the diagnosis of early stages of AD, prediction of subsequent clinical course, and development of new therapeutic strategies. A perfect biomarker candidate for AD may be muscle biopsy examination for AD pathological hallmarks. Because A $\beta$  accumulation and tau aggregates are detected in skeletal muscle. In addition, AD is associated with loss of skeletal muscle mass and strength, and such loss of muscle function can be caused by system AD risk factors including aging, genetic factors, and lifestyle-related factors. More importantly, a variety of AD risk factors could lead to autophagic-lysosomal dysfunction, which could be a common pathogenesis for the development of AD pathological hallmarks including A $\beta$  deposition and tau aggregation in brain and skeletal muscle. Without the protection of blood-brain barrier, these systemic factors can affect skeletal muscle earlier than neurons, thus muscle biopsy examination for AD pathological hallmarks would be a new biomarkers that may facilitate the diagnosis of early stages of AD, prediction of subsequent clinical course, and development of new therapeutic strategies.

So far mounting evidence from basic science side supports the idea that muscle biopsies is a useful tool for pre-mortem neuropathological diagnosis of AD; however, the actual proof showing a path forward is lacking. In a way, AD is lagging behind; in Parkinson's disease, clinical studies have started to test the feasibility of using colonoscopy biopsies [182] and skin biopsy [183,184] as a biomarkers for early diagnosis of Parkinson's disease. A 10 year follow-up of a prospective cohort with initial muscle biopsy is needed to test the feasibility of using muscle biopsies as a tool for pre-mortem neuropathological diagnosis of AD. We suggest that muscle biopsy from distal limbs and examination for AD pathological features such as A $\beta$  accumulation, tau pathology, and autophagic-lysosomal dysfunction should be conducted in matched control patients and in patients with mild cognitive impairment, an early stage of AD. Although the muscle biopsy itself is a fairly straight forward outpatient procedure with little risk, a successful muscle biopsy requires optimal cryo-processing of

the fresh specimen in order to preserve viable macromolecules for routine histochemistry and immunohistochemistry assays [185].

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### Acknowledgement

This study was funded by the National Institute of Mental Health (R01MH100972 and R01MH105329).