

## Early onset Alzheimer's Disease and its Pathogenesis

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### ABSTRACT

Prevalence of Alzheimer's disease (AD) is significantly high in developed countries where life expectancy is relatively higher and its incidence is expected to increase over years. Etiology of this disease is complex; however, in a subtype called Early Onset Alzheimer's Disease (EOAD) which manifests at age below 65 years, genes are the sole cause — mutations in *APP*, *PSEN1* and *PSEN2* genes. Currently, there is no effective cure for AD. Therefore, a thorough understanding of pathogenesis of AD is crucial for designing drug-development studies. However, our knowledge is only in the growing phase in this field. This paper briefly reviews the pathogenesis of EOAD.

**Keywords:** Alzheimer's disease, pathogenesis, mutation

### INTRODUCTION

Alzheimer's disease (AD), which manifests as progressive decline of cognitive abilities, is characterized by formation of beta-amyloid plaques (A $\beta$ ) and neurofibrillary tangles (NFT) in the brain.<sup>1,2</sup> Based on age at onset, the disease has been categorized as early-onset AD (EOAD) and late-onset AD (LOAD) which predominantly manifest at age <60-65 and >=65 years, respectively.<sup>2</sup> While EOAD is caused by mutations, the etiological agents of LOAD can be complex, mutations acting as 'risk factors' only. EOAD is dominantly inherited and is caused by mutations in amyloid precursor protein (*APP*), presenilin-1 (*PSEN1*) and presenilin-2 (*PSEN2*) genes.<sup>3-5</sup> Although the etiology of EOAD is clear-cut, this subtype attributes only up to 5% of total AD cases. *APP*, *PSEN1* and *PSEN2* gene mutations explain about 10-15%, 30-70% and <5% of the total EOAD cases, respectively.<sup>6</sup> It should be noted that EOAD and LOAD can have similar pathological and biochemical changes in the brain.

Pathogenesis of EOAD is mediated by multiple mechanisms. Our understanding about the pathogenesis of this disease is still in its growing phase. A deep understanding through extensive research in this field is crucial for developing effective drugs for EOAD/AD. In this paper, we briefly discuss our current understanding about the pathogenesis of AD with respect to the etiology of EOAD.

### EOAD GENES

#### APP

This gene has 19 exons and the longest isoform encodes a 770 amino acid long protein. Beta-amyloid molecule,

monomer of beta amyloid plaques, is encoded by a region of APP that lies in exons 16 and 17.<sup>7</sup> APP takes part in neuronal cell adhesion, neurite growth, axonogenesis and interaction with elements of extracellular matrix. It also interacts with membrane-bound proteins such as presenilins, Notch, Go-protein, etc., modulating various cellular activities.<sup>8</sup>

#### Presenilins (*PSEN1* and *PSEN2*)

Presenilin-1 (*PSEN1*) gene has 12 exons of which 10 (exons 3 to 12) are coding and the longest isoform encodes a 467 amino acid long protein. Presenilin-2 (*PSEN2*) has 13 exons of which 11 (exons 3 to 13) are coding and the longest isoform encodes a 448 amino acid long protein. Both genes have nine transmembrane (TM) domains and a hydrophilic loop (HL) that faces cytoplasmic membrane, and a high sequence homology (67%). *PSEN1* and *PSEN2* proteins are expressed in endoplasmic reticulum, Golgi bodies and cell membrane.<sup>9</sup> Soon after translation, the full length *PSEN1* undergoes an automatic endoproteolysis to form a functional heterodimer of stable N- and C-terminal fragments (NTF and CTF). Two critical aspartyl residues namely Asp<sup>257</sup> and Asp<sup>385</sup> as well as the expression of *PEN2* (presenilin enhancer-2) are required for endoproteolysis. Presenilins have an array of functions: they are involved in maintaining neural progenitor cells in developing brain; form catalytic component of  $\gamma$ -secretase; are involved in calcium homeostasis; regulate long term potentiation (LTP); take part in NMDA (N-methyl-D-aspartate) receptor functions; induce glutamate release in presynapse of adult brain; and cleave transmembrane proteins such as Notch, Erb-B4 (v-erb-a erythroblastic leukemia viral oncogene homolog 4), etc.<sup>10-13</sup>

## PATHOGENESIS

The invariant events in the pathogenesis of EOAD and/or AD are extracellular deposition of  $\beta$ -amyloid plaques and formation of intracellular neurofibrillary tangles. These events over a time lead to the neurodegeneration that follows development of the disease. The multiple mechanisms of neurodegeneration are outlined in Figure 1.

### Beta-amyloid-mediated neurodegeneration

Extracellular deposition of beta amyloid plaques in the brain is the primary mechanism of neurodegeneration in AD. Formation of  $\beta$ -amyloid plaques in EOAD is a complex process with involvement of APP,  $\beta$ -secretase and  $\gamma$ -secretase. Beta-secretase is an integral membrane aspartyl protease and it has a single cleavage site on APP at residue 671-672.<sup>13</sup> Gamma-secretase, a multiprotein complex that comprises at least one presenilin, is also an aspartyl protease and one of its major functions is to selectively cleave APP.<sup>14</sup> Cleavage of APP at residue 671-672 by  $\beta$ -secretase followed by cleavage at residues 711-712, 713-714, or 714-715 by  $\gamma$ -secretase produces A $\beta$ 40, A $\beta$ 42 or A $\beta$ 43, respectively.<sup>7</sup> Mutations in presenilin genes alter the specificity of APP cleavage by  $\gamma$ -secretase. Presenilin mutations have been shown to reduce the proteolytic activity of  $\gamma$ -secretase and

the less-efficient  $\gamma$ -secretase produces elevated levels of A $\beta$ 42.<sup>15-17</sup> In addition, mutations also alter the conformation of and interaction among the proteins favoring the formation of  $\beta$ -amyloid molecule.<sup>18-19</sup>

AD is characterized by an elevated A $\beta$ 42/A $\beta$ 40 ratio, one of the important biochemical markers for the disease. Elevation in A $\beta$ 42/A $\beta$ 40 ratio can occur by multiple mechanisms: increased A $\beta$ 42 level with unchanged A $\beta$ 40, increased A $\beta$ 42 with decreased A $\beta$ 40, unchanged A $\beta$ 42 with decreased A $\beta$ 40, or decreased A $\beta$ 42 and A $\beta$ 40 levels.<sup>20-21</sup> Such an elevated ratio can also be caused by decreased production of shorter  $\beta$ -amyloids such as A $\beta$ 37, A $\beta$ 38 and A $\beta$ 39 as a result of a few *PSEN1* mutations.<sup>22</sup> Thus, amyloid plaques formed in the brain are a consequence of imbalance in the production of  $\beta$ -amyloid monomers of different lengths. Elevated A $\beta$ 42/A $\beta$ 40 ratio has been found to correlate with or modify the age of disease onset, clinical variability and dementia.<sup>20,23</sup> In contrast, A $\beta$ 40 as well as A $\beta$ 42 monomers at low concentrations are found to possess a neuroprotective role; thus only the aggregated monomers (i.e. oligomers or plaques) or mutated monomers are fibrillogenic and fibrillogenicity increases with the increase in C-terminal length of the beta-amyloid monomer.<sup>20,24</sup>

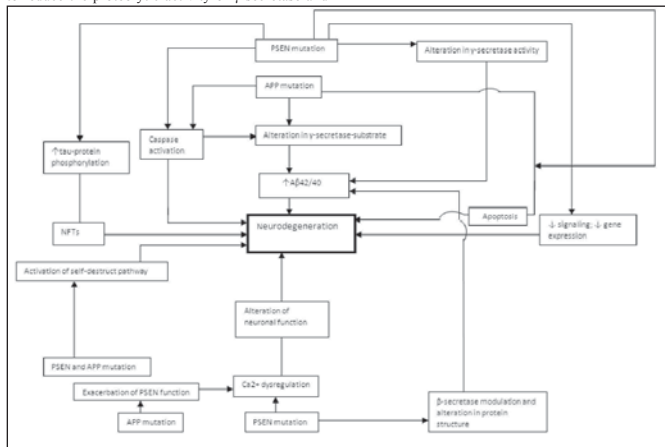


Fig. 1. Major mechanisms in EOAD pathogenesis

There are reports about how elevated A $\beta$ 42 levels are destined to form beta amyloid plaques. Photochemical cross-linking studies have revealed that A $\beta$ 42 monomers form pentamer/hexamer whereas A $\beta$ 40 monomers exist as a mixture of monomer, dimer, trimer, and tetramer. The pentamer/hexamer molecules, also called as paranuclei, have high entropies and undergo oligomerization with the help of some APP residues (Phe<sup>19</sup>, Ala<sup>21</sup>, Ile<sup>41</sup>, and Ala<sup>42</sup>). A continuous oligomerization will lead to the formation of protofibrils and then to fibrils. The fibrils in combination with other proteins and cellular materials then become 'insoluble  $\beta$ -amyloid plaques'—the driver of the pathological effects in AD.<sup>25-26</sup>

The  $\beta$ -amyloid plaques have several effects such as i) induction of chronic neuroinflammation and vascular alterations, ii) extensive synaptic loss, iii) impact on blood flow and iv) disturbance in neurocommunication. These effects ultimately result in neurodegeneration and atrophy of brain tissue, characteristic of AD.<sup>6,24,27</sup>

Apart from insoluble amyloid plaques, oligomers formed during intermediate stages are also believed to take part in the pathogenesis of EOAD/AD. They have been shown to impact metabolic processes; provoke deleterious reactive oxygen species; reduce blood flow in the brain; induce mitochondrial apoptotic toxicity; cause synaptic damage; and inhibit long-term potentiation (LTP) and angiogenesis.<sup>27</sup> They may also diffuse into cerebral blood vessels inducing angiopathy and hemorrhage as observed in Flemish form of EOAD.<sup>25</sup> Furthermore, they are also believed to initiate pathological changes subclinically and they correlate with disease severity. Thus some of the effects of soluble oligomers and  $\beta$ -amyloid plaques can be overlapping.

### Neurofibrillary-tangles (NFT) mediated neurodegeneration

Microtubule proteins are found in neuronal cells as well as in dividing cells, and provide the tract to transport neuronal signals, organelles and chromosomes.<sup>28</sup> Microtubule is stable because of outer binding protein called tau ( $\tau$ ). When the tau-protein is phosphorylated (3-4 times higher levels of phosphate are found in AD brain compared to normal brain), it detaches from the microtubule and becomes unstable.<sup>29-30</sup> Upregulation of Wnt pathway—a pathway involved in gene transcription with the participation of glycogen synthase kinase-3 $\beta$  and  $\beta$ -catenin—leads to the hyperphosphorylation of tau protein.<sup>31</sup> Elevated level of A $\beta$ 42 is a key starter in this pathway. Thus, the dysregulation of Wnt pathway as a result of presenilin mutations has been considered as the central mechanism that interlinks  $\beta$ -amyloid- and NFT-mediated neurodegeneration.<sup>32</sup> The unstable tau-protein breaks down into small fragments and becomes

aggregated. This aggregation leads to formation of paired helical filaments (PHFs) which then polymerize to form neurofibrillary tangles (NFTs).<sup>33</sup> The PHFs and NFTs induce cytoskeleton dysfunction and synaptic loss which ultimately leads to neurotoxicity and death of neurons.<sup>34</sup>

### Calcium dysregulation-mediated neurodegeneration

Destabilization of neuronal calcium also attributes to the pathogenesis of AD.<sup>35</sup> Presenilins have been shown to be involved in controlling the calcium homeostasis in the endoplasmic reticulum (ER). In vitro studies suggest that several presenilin mutations destabilize the calcium homeostasis by activating the release of calcium from the ER to the cytosol.<sup>36</sup> The effects caused by an increased cytosolic calcium level include activation of  $\beta$ -secretase activity which leads to increased production of A $\beta$ , alteration of the synaptic transmission and function which leads to neurite degeneration, alteration of the expression of genes that have roles in neurodegeneration, and alteration of apoptosis and excitotoxicity of neurons.<sup>36-38</sup>

### Caspase-, neurotrophin- and Notch signaling-mediated neurodegeneration

Caspase activation which leads to apoptosis of neurons plays a role in EOAD pathogenesis. There are in vivo evidences of presenilin and APP mutations which result in activation of caspase-6.<sup>39</sup> Caspase-12 can sensitize neurons to DNA damage-induced death.<sup>40</sup> In addition, activation of caspase-2 and -8 may also induce presenilin or  $\gamma$ -secretase which results in increased A $\beta$ 42 production.<sup>41</sup> However, some *PSEN1* mutations (p.Tyr115Cys, p.Ile143Phe, and p.Gly384Ala) were not shown to alter the caspase-mediated cleavage; thus, caspase activation appears to be specific for only certain mutations.<sup>42</sup> Similarly, *PSEN1* mutations are found to affect neurotrophin receptor proteins (such as p75NTR: p75 neurotrophin receptor) resulting in apoptosis of neurons.<sup>43</sup> Notch signaling is a pathway that is involved in regulation of gene expression. Studies have shown that a complete presenilin knock-out, mutagenesis of critical aspartyl residues in *PSEN1* and  $\gamma$ -secretase inhibitors block the Notch signaling. As the Notch cleavage is presenilin-dependent, mutations in presenilins are likely to result in dysfunction of the Notch signaling and this deficit in Notch signaling may explain neurodegeneration.<sup>44-45</sup>

### Self-destruct pathway-mediated neurodegeneration

This is a relatively newly-perceived mechanism that is speculated to be involved in AD pathogenesis. In the mouse model experiment, after deprivation of nerve

growth factors, N-terminal APP (formed as a result of beta cleavage) was shown to trigger a self-destruct pathway in embryonic neuronal cells by binding to neuronal receptor called DR6. Because DR6 is highly expressed in regions of the brain affected by AD, it is possible that interaction of N-APP with DR6 might play a role in the pathogenesis of AD. However, information about N-APP and DR6 interaction is scarce and the role of EOAD mutations on this interaction has not been investigated. If this interaction gets well-elucidated in adult-brain cell-types in future, the effects of EOAD mutations on N-APP and its pathway of interaction will be possible to study.<sup>46</sup>

## CONCLUSION

A deeper understanding of the pathogenesis of AD is essential for the development of an effective cure and/or treatment. Unfortunately, etiology is well-known for only EOAD which explains only 5% of AD cases. With ever-growing incidence of the disease, developing drugs for AD based on the knowledge of etiology, pathogenic mechanisms of mutations and the overall pathogenesis of EOAD can be very helpful. We recommend that research should be continued to elucidate the key underlying factors, mechanisms and/or pathways of the pathogenesis of EOAD/AD and find the key steps in the pathogenesis.

## Alzheimer's disease in Nepal

In Nepal, the detailed status of Alzheimer's disease (AD) is unknown due to lack of systematic research. Moreover, status of the EOAD and LOAD sub-phenotypes are even more obscure. However, we can expect the latter being reported in hospital records. Although there are none or limited studies to reveal the prevalence and number of people with dementia in Nepal, the burden could be very significant; four psychiatrists were expected to see over 200 patients in two days from a mental health camp held in Janakpur district in 2008.<sup>47</sup> Dementia being the hallmark of AD, we can expect the disease to be a significant clinical burden in Nepal. Three major hurdles for the diagnosis of AD are the 'social viewpoint' of AD-related symptoms (doctors say that people link AD-like behaviors with madness or the normal aging process), lack of facilities to provide confirmatory diagnosis and the high cost of diagnosis. With growing life expectancy and changes in lifestyle in Nepal, both provoking the increase in incidence of AD, we have reached a stage where governmental and non-governmental organizations need to pay more attention. In addition, since EOAD is more severe than LOAD and EOAD cases may die at productive ages, development of molecular diagnostics facilities for this subtype should be helpful for clinicians for case diagnosis, treatment and counseling of patients.

## ACKNOWLEDGEMENT

We want to thank WHO (World Health Organization) for providing access to research publications via HINARI for developing countries like Nepal.

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