The Protective A673T mutation of Amyloid precursor protein (APP) in Alzheimer's disease

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Abstract

Alzheimer's Disease is a progressive neurodegenerative disorder characterized by extracellular amyloid beta peptides, and neurofibrillary tangles consisted of intracellular hyperphosphorylated Tau in the hippocampus and cerebral cortex. Most of the mutations in key genes that code for amyloid precursor protein can lead to significant accumulation of these peptides in the brain and cause Alzheimer's Disease. Moreover, Some point mutations in amyloid precursor protein can cause familial Alzheimer's Disease, such as Swedish mutation and A673V mutation. However, recent studies have found that the A673T mutation in amyloid precursor protein gene can protect against Alzheimer's Disease, even it is located next to the Swedish mutation and at the same site as A673V mutation, which are pathogenic. It makes us curious about the protective A673T mutation. Here, we summarize the most recent insights of A673T mutation, focus on their roles in protective mechanisms against Alzheimer's Disease, and discuss their involvement in future treatment.

Introduction

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder causing memory loss, cognitive decline and dementia to millions of elderly individuals worldwide [1]. The two pathological hallmarks of AD are toxic plaque accumulates of extracellular amyloid beta ($A\beta$) peptides, and neurofibrillary tangles consisted of intracellular hyperphosphorylated Tau in the hippocampus and cerebral cortex [2-4]. The key role for $A\beta$ peptides in the pathophysiology of AD based on the amyloid cascade hypothesis in 1992 was the initiating step of AD pathogenesis, leading to subsequent Tau deposition, synaptic damage, neuronal death and cognitive decline [5-7]. Current researches still strongly support this original hypothesis, although its mechanism may be less direct than originally expected and requires further clarity through ongoing studies [8].

$A\beta$ peptides are generated via amyloidogenic processing of amyloid precursor protein (APP) on chromosome 21 (21q21.2-3), catalysed by the sequential cleavage of β - and γ -secretases [9-11]. β -site APP cleaving enzyme 1 (BACE-1), the main β -secretase, cleaves APP between Met671 and Asp672, resulting in the generation of a soluble APP- β ectodomain (sAPP β) and its complementary membrane-retained carboxy-terminal counterpart of 99 amino acids termed C99 or β -CTF. Subsequently, γ -secretase cleaves the resulting carboxy-terminal fragment at promiscuous sites to generate APP intracellular domain (AICD) and $A\beta$ peptides with different C termini, including preference formations of $A\beta_{1-40}$ and $A\beta_{1-42}$, and other minor species [12-14]. $A\beta_{1-42}$ is more prone to $A\beta$ aggregation and promotes fibril formation, triggering neurotoxic effects [15,16]. While BACE-1 can also cleave APP at β' -site, between Thy681 and Gln682, yielding $A\beta_{11-xx}$. $A\beta_{11-42}$ and $A\beta_{11-40}$ generated by this amyloidolytic cleavage showed less toxicity than $A\beta_{1-40}$ and $A\beta_{1-42}$, especially for the neurotoxicity of $A\beta_{11-42}$ [17].

At present, approximately 30 coding mutations in the APP gene have been found to be association with AD [18]. Most of them are pathogenic for AD, because they can either promote $A\beta$ accumulation or specifically augment the $A\beta_{42}/A\beta_{40}$ ratio formation, such as Swedish mutation and A673V mutation

[19,20]. In contrast, a rare APP coding mutation A673T (rs63750847) identified in the Icelandic population was linked to a protection against late-onset AD. After the discovery of this protective mutation in 2012, Icelandic mutation A673T have engaged more and more researchers' attention[21]. Here, we summarize the most recent insights of A673T, focus on population frequencies as well as protective mechanisms, and discuss their involvement in future treatment for AD.

Population frequencies of the APP A673T mutation

Protective A673T mutation was first found in a set of whole-genome sequence data obtained from 1,795 Icelanders in 2012. In addition to the protection against AD, this large Icelandic study also revealed that the 3,673 A673T carriers without Alzheimer's disease had a higher cognitive level than 41 noncarriers based on the Cognitive Performance Scale (CPS), which suggested that the effect of A673T mutation may beyond the boundaries of the protection against AD [21]. Moreover, neuropathologic analysis of the A673T mutation carrier aged 104.8 years in another study showed little beta-amyloid pathology and the score of Consortium to Establish a Registry for Alzheimer's Disease (CERAD) was 0.

Immunohistochemistry in brain tissue showed no typical senile plaques in the temporal cortex of the carrier's left cerebral hemisphere, and no accumulation of intracortical β -amyloid in the arterial wall, while some meningeal arteries in the parietal cortex were positive for β -amyloid. And the temporal gyrus phosphorylation (p)-Tau immunohistochemistry showed age-related cytoplasmic neurofibrillar tangles and a considerable number of neurophil threads [22]. A673T mutation carrier only have little beta-amyloid pathology and age-related cytoplasmic neurofibrillary tangles and a large number of neurofilaments [23]. In addition, studies had shown that the plasma $A\beta_{40}$ and $A\beta_{42}$ levels of the A673T mutation healthy carrier were reduced by an average of 28% compared to the controls [24].

To determine the frequency of the protective A673T mutation, many countries began to carry out large sample studies (Fig. 1, Table1). In Northern Europe, similar to Icelanders, A673T mutation was also observed in the Danish, Finnish, Norwegian, and Swedish populations, with a relatively high prevalence [22,25]. However, A673T mutation is extremely rare in non-Nordic populations. In 2013, researchers genotyped the A673T mutation in 552 subjects with Alzheimer's disease and vascular dementia, 790 subjects with Parkinson's disease, and 7379 controls, all Asian individuals. A673T mutation was not relevant in all of the subjects [26]. Researchers suggested that this mutation may only present in longevity subjects because of its protectiveness. In 2014, in order to further explore whether the A673T mutation contributes to the Chinese population accurately, researchers genotyped the mutation in 2641 Chinese individuals comprising 1237 healthy longevity subjects with mean age 96.9 years and 1404 matched younger controls with mean age 44.2 years. Nevertheless, the A673T mutation also failed to be observed in all of subject, either longevity subjects or younger controls [27]. These finding suggested that the contribution of A673T mutation is not relevant to Asian population. Similarly, studies in North American Whites with 1674 late-onset Alzheimer's disease cases and 2644 elderly controls were not found any examples of the A673T mutation [28]. However, a Case-control association analysis in US and Swedish individuals found the A673T mutation. In this study, 8943 US AD cases, 10480 US cognitively normal controls, 862 Swedish white AD cases, and 707 Swedish cognitively normal controls were

genotyped for the mutation. Among them, 3 US individuals heterozygous for A673T were identified, and all remaining US cases were homozygous for the alanine (A673) allele [29]. One of 3 US heterozygous individuals was AD with an onset age of 89 years old. It may be due to the incomplete data and the complex pathogenic factors of AD, such as environmental factor. While, the other two heterozygous were controls aged 82 and 77 years at last examination. A673T mutation is a rare mutation and may be confined to specific races/ ethnicities.

The mechanisms of A673T protection against AD

Hereditary mutations can cause Alzheimer's disease, such as PSEN1, PSEN2, APOE4, APP, etc. As one of the risk factors, APP has been discovered with more than 30 AD-related mutations. Most of the common mutations in the APP are pathogenic, such as Swedish mutation next to the A673T mutation, which is a well studied familial AD (FAD) causing mutation and has been widely used as a classic animal model of AD in researches. Similarly, the A673V mutation is pathogenic, which located at the same site as A673T. While the A673T mutation is protective against AD, which makes us curious about the protective mechanism of A673T [18,30,31]. The A673T mutation is an alanine-to-threonine substitution at position 673 of APP [21]. This substitution is adjacent to the β -secretase cleavage recognition site, and lies in the position 2 of the $A\beta_{1-xx}$ peptide cleavage product (A2T).

A β generation

A673T mutation can promote a decreased propensity of the generation level of A β by altering the related secretase and metabolites in A β generation process called amyloidogenic pathway (Fig. 2 a and b). Mechanistically, it has been shown that A673T reduced both of sAPP β and C99 levels by reducing BACE1 cleavage of APP in transfected mouse primary neurons and in isogenic human iPSC-derived neurons, resulting in reduced production of A β . Specifically, the mutation affects the catalytic turnover (V_{max}) of APP rather than APP binding affinity (K_m) for BACE1 [21,32]. Similarly, Kokawa and colleagues reported that the generation level of A β in A673T cells expressing C99 was roughly one-half of which observed in wild-type C99. However, they observed that the level of C99 in A673T cells was indistinctive of which detected in wild-type cells, although a significant reduction of sAPP β was found in A673T cells. One possible explanation for this observation is that A673T APP or its production sAPP β is preferentially processed by η -secretase [33,34]. In addition, their reconstituted β -secretase assay reported that the level of A β_{33} -FLAG production in APP 633–685-FLAG carrying A673T was comparable to that of the wild-type, suggesting that the A673T mutation has no effect on the β -cleavage of APP [33]. While some research showed that A673T can reduce C99 generation and decrease the C99/C89 ratio by changing the cleavage site selection of BACE1 from β -site to β' -site, resulting in decreased production of $A\beta_{1-40}$, but increased $A\beta_{11-40}$ [17]. In order to clarify whether conformational changes of APP caused by mutations (A673T, A673V or KM670/671NL) will affect BACE1 cleavage, we made structural predictions (Fig.3). RaptorX prediction results visualized by Pymol showed extensively obvious differences between the possible 3D structure of the wild-type APP protein and APP protein with different mutations [35-39]. Predictions regarding the secondary structures of wild-type APP protein and APP protein with mutations were carried

out using highly accurate secondary structure prediction tools: Jpred 4 [40]. Analyses revealed that the differences between the helical structure and sheet structure of APP protein with these mutations and wild-type APP protein at all regions of the protein were extensively obvious. The helical structure of APP protein with protective mutation (A673T) and the sheet structure of APP protein with pathogenic mutation (A673V or KM670/671NL) around the mutation region was changed obviously, compared with the wild-type APP protein. It suggested that the conformational change of APP caused by mutation may play a key role in the process of BACE1 cleavage. In addition, in order to explore the effect of mutation in residue 673 of APP, the investigators substituted the alanine at the 673rd amino acid position of APP with a series of amino acids, such as aspartic acid, leucine, and glutamine. Compared with WT, all of these artificial mutations except leucine reduced the level of C99, but increased the level of C89 and C83, the metabolites in APP processing by α -secretase. It suggested that the site 673 of APP may be a key factor in determining APP metabolic pathway (amyloidogenic or non-amyloidogenic pathway) [41]. ELISA of APP metabolites in rats carrying protective mutation showed that A673T can increase APP processing by α -secretase, which can prevent A β production, called non-amyloidogenic pathway, but decrease APP processing by β -secretase [42].

Additionally, a cell-free assay revealed that A β production was more diminished in the microsomal fraction of C99 A2T cells compared to the WT with almost same level of C99 [33]. It suggested that the A673T mutation may affect the C99 processing by γ -secretase. While a CHAPSO-solubilized γ -secretase assay allowing free collision between γ -secretase and C99 A2T in the solution demonstrated no significant difference in A β production of C99 A2T compared with the C99 wild-type, which contradicts the observation above and caused by destruction of C99 A2T intact distribution [33]. It implied that A673T mutation may alter the subcellular components distribution of C99 processing by γ -secretase. Not like our expectations that γ -Secretase was fail to recognize C99 with A2T, γ -secretase can recognize the amino terminus of C99 [43,44]. Moreover, studies showed that γ -secretase components accumulate in lipid raft fractions of membranes after sucrose gradient centrifugation [45]. Although C99 will also be partially distributed to these lipid raft fractions, the subcellular distribution of γ -secretase components will not be affected when C99 carries A2T mutation. While the level of C99 A2T co-distributed with γ -secretase was significantly reduced. The significant reduction of A β may be caused by the altered distribution of C99 with A2T [33]. In addition, measurements in the laboratory had shown that the A β_{42} /A β_{40} ratio of A673T carrier was indistinctive of the controls, although similar reduction was observed in both A β_{42} and A β_{40} with A2T. It suggested that the reduction of the different A β peptides was not mediated by the γ -secretase-related modulation [24]. Moreover, the A673V mutation and Swedish mutation (KM670/671NL) cause FAD by altering APP processing, shifting the preferential β -cleavage site of BACE1 from the major Glu11 site to the minor Asp1 site, increasing the V_{max} of BACE1 for APP, resulting increased β -CTF and A β production compared to WT. Most importantly, the A673V mutation can still produce less A β than the Swedish mutation, although A673V mutation can produce more C99 than the Swedish mutation. It's due to the increase of C99 degradation by the proteasome and decrease of its cleavage by γ -secretase [41].

A β aggregation and Microglial uptake

A673T mutation promote a decreased propensity for A β aggregation [32] (Fig. 2c). This single amino acid substitution in A β peptide alters its own properties. Using thioflavin-T (ThT) incorporation and fluorescence, A β_{1-42} containing the A2T mutation showed a reproducibly lower level of aggregation compared with the wild-type. By contrast, the aggregation kinetics of A β_{1-40} expressing A2T mutation was indistinctive of which observed in wild-type. When A β_{1-42} and A β_{1-40} were mixed together in a 1:9 ratio, an approximate representation of their heterogeneity in human cerebrospinal fluid, A β containing the A2T mutation showed slightly slower aggregation kinetics compared with the wild-type [46]. While A2V carriers showed increased aggregation kinetics for A β_{1-40} peptides than the WT, not A β_{1-42} peptides [32]. These above effects of A2T on A β provide another angle to understand the pathogenesis of AD. Maybe the risk of AD depend upon APP metabolites, not just A β . Most importantly, the relative protective effect of the A673T mutation does not change or even more robust when other mutations coexist, such as A673T mutation in ApoE4 carriers et al. [47-49]. And the pathological conditions caused by these pathogenic mutations may be counteracted by A673T mutation [42]. In order to clarify whether conformational changes of A β caused by mutations will affect the aggregation level of A β , we have made structural predictions of wild-type A β_{1-40} or A β_{1-42} protein and A β_{1-40} or A β_{1-42} protein with A2T mutation or A2V mutation (Fig. 4). RaptorX prediction results visualized by Pymol showed possible 3D structure of the A β and A β with different mutations [35-39]. The THR-2 form a hydrogen bond with GLU-3 in A β_{1-42} A2T peptide. While wild-type A β_{1-42} peptide and A β_{1-42} protein with A2V mutation do not form hydrogen bond at the same position. And they all form a hydrogen bond in ASP-1. While only the wild-type A β_{1-40} peptid form a hydrogen bond in ASP-1. A β_{1-40} protein with A2T mutation or A2V mutation do not form hydrogen bond at the same position. Jpred 4 prediction results showed that the probable secondary structure changes of A β_{1-40} and A β_{1-42} caused by mutations were extensively subtle [40]. The helical structure and sheet structure of A β_{1-40} had hardly changed. While the helical structure of A β_{1-42} protein with mutations around the mutation region were changed, compared with the wild-type A β_{1-42} protein. The sheet structure of A β_{1-42} protein with mutations were not changed. These prediction results may explain the aggregation level of A β_{1-42} with mutations, further supporting the belief that aggregates have more β -strand structures and less α -helix content than the native [50].

Microglial uptake is considered to be an important clearance mechanism for soluble A β in the brain [51]. One may expect that A2T mutation would promote the microglial uptake of A β , especially for more toxic A β_{1-42} peptides. While contrary to our assumption, A β_{1-42} peptides containing the A2T mutation was internalized to a much greater extent than A β_{1-40} , which was approximately half of that observed in wild-type. The A β clearance rate of microglia may affected by the slightly slower aggregation kinetics caused by A2T mutation. While the A β with A2V mutant were internalized at high levels both as A β_{1-40} and A β_{1-42} species, because its higher aggregation state, which may further clarify the mechanisms of microglia uptake.

Cellular toxicity of A β

Cell toxicity may be associated with aggregation propensity [52]. It is believed that A β peptides are toxic to neurons, and even can induce cell death at high concentrations *in vitro*. In a luminescent viability assay, cellular toxicity of A β peptides containing the A2T mutation and wild-type were indistinguishable from each other. Cytotoxicity of the A β peptides with A2T mutant was not dramatically altered. It seems somewhat counterintuitive, as the A β peptide containing protective mutation might be expected to be less toxic. Similarly, the A2V mutant carriers showed slightly less toxic than the wild-type *in vitro*. And A β oligomers are cytotoxic to cultured neurons [53,54]. It is possible that fewer toxic oligomeric A β are present in A2V peptides. In addition, the inhibition of mitochondrial axonal trafficking by amyloid beta protein (A β) is related to the early pathophysiology of Alzheimer's disease (AD). Mitochondrial motility plays an important role in supporting synaptic activity and calcium buffering. Changes in mitochondrial dynamics may lead to synaptic dysfunction, neuronal loss and memory decline. Data from the tissues and cells of AD patients and animal models of FAD show that mitochondrial axon transport is affected in the early stage of the disease before memory impairment or amyloid plaques appear. Exogenous A β peptide initiated the signaling cascade involved in the inhibition of plasma membrane axon transport. The binding to the plasma membrane of neurons is sufficient to cause a rapid and significant inhibition of axonal transport. A2T can affect mitochondrial axonal transport, thereby protecting against AD [55] (Fig. 2e). Studies have found that the aggregation state of A β have a great effect on mitochondrial axonal trafficking. A β oligomers are the culprit in trafficking inhibition, and peptides with a higher aggregation tendency also have a greater inhibitory effect on this process. The sequence of N-terminal amino acid can influence the formation of A β oligomers [56]. A β oligomers containing the A2T mutation form approximately 50% less oligomers than wild-type [57] (Fig. 2f). Moreover, it is difficult to predict the protective trafficking impact of A673T mutation on pathological amyloid accumulation. Because the resulting aggregation of A β expressing A2T mutation remains substantial, especially in heterozygous carriers, although A β peptide with A2T showed lower level of aggregation than WT. Furthermore, the binding of the A β peptide to the plasma membrane is sufficient to induce trafficking inhibition, in which the peptides with reduced plasma membrane binding and internalization have little effect on mitochondrial motility. A β peptide with A2T has a very low plasma membrane binding and internalization rate in neurons, which may explain its relatively low toxicity and protection against mechanisms for AD [55]. Similarly, the binding affinity to synaptic receptors of A β oligomers containing the A2T mutation is >4-fold lower than wild-type [57]. A673T mutation may affect the transmission of A β oligomers among various nerve cells, such as neurons, microglia, astrocytes, even the endothelial cells.

In addition, studies have shown that the level of transforming growth factor β 2 (TGF β 2) in hippocampal and cortical neurons of patients with sporadic AD is up-regulated, and the activation of APP mediated intracellular death signal induced by TGF β 2 may be one of the causes of sporadic AD [58]. TGF β 2-induced neuronal death is intracellularly mediated by c-Jun N-terminal kinase (JNK) which is independent of A β neurotoxicity [59]. APP may be the receptor for the death-inducing ligand of TGF β 2. When protective mutations appear on APP, intracellular death signal via APP induced by TGF β 2 will cease. TGF β 2 did not induce neuronal death in SH-SY5Y cells expressing A598T, corresponding to the protective mutation A673T APP isoform APP770, but induced in SH-SY5Y cells expressing wt-APP [60]. A673T has

a protective effect on JNK mediated intracellular death signal via APP induced by TGF β 2. It is highly likely that the conformational change of APP prevents the functional interaction between APP and heterotrimeric GTP-binding protein Go which is the first signal molecule of intracellular death signal mediated by APP. Moreover, this protective mutation can prevent the phosphorylation of JNK induced by TGF β 2. JNK protein kinase family is one of the three mitogen activated protein (MAP) kinase families. JNK's, particularly JNK3 (MAK10), mainly located in neurons and was identified to phosphorylate Tau protein at Thr205 and Ser422 readily [61,62]. JNK phosphorylation is one of the prime suspects for driving hyperphosphorylation of Tau protein, which is the important pathology of AD. TGF β 2 did not induce JNK phosphorylation in SH-SY5Y cells expressing A598T [61,62]. These results may provide important hints for the protective mechanism of A673T mutation related to Tau protein synthesis and A β -independent neurotoxicity against AD, and provide ideas for the study of protein kinase catalytic activity similar to JNK phosphorylation, such as GSK3 β and CDK5, which can also effectively induce Tau protein phosphorylation causing AD [51,63] (Fig. 2d).

Conclusions: Challenges And Perspectives

In the treatment of AD drugs, the study of anti-A β drugs has always been dominant [64]. In basic research and clinical trials, the most relevant experimental interventions are immunotherapy [65], β -secretase inhibitors [66], γ -regulators [67] and many A β aggregation inhibitors. However, in recent years, some secretory enzyme inhibitors and immunotherapy experiments have failed continuously, such as the failure of Bapineuzumab and Solanezumab for moderate AD, and the related anti-A β research has been significantly reduced [68-73]. There are many explanations for the failure of these clinical trials, including: 1. The A β hypothesis may be wrong. Up to now, the positive or negative effects of A β on the occurrence and development of AD have not been determined. 2. The onset of AD is caused by a variety of complex factors, and its mechanism is not clear yet. Therefore, it may not be effective to treat AD only for a single pathological process. Recently, one study found that the anti-cancer drug Gleevec and its related compounds can mimic the action of the protective mutation A673T. They can make APP difficult to be hydrolyzed by BACE protein without inhibiting BACE enzyme activity or other BACE substrate processing. In addition, Gleevec can also induce the formation of a specific set of APP C-terminal fragments, which were also observed in cells expressing APP protective mutations and cells exposed to conventional BACE inhibitors. These compounds have also been shown to reduce the number of APP for amyloid processing exposed to BACE and γ -secretase by increasing protein transport from plasma membrane or endoplasmic reticulum to lysosome. These results provide evidence for the A β hypothesis and provide important hints for the protective mutation A673T as a therapeutic target.

In the 1960s, American molecular biologist Lederberg J proposed the initial concept of gene therapy, which laid the foundation for the development of gene therapy [74]. In 1995, the first successful clinical case of gene therapy about severe combined immunodeficiency syndrome (SCID) was reported and considered as an important milestone in the development of gene therapy [75]. Since that, gene therapy has developed rapidly, especially after the emergence of gene editing technology. Already today, gene therapy is used in the clinic to treat Duchenne muscular dystrophy (DMD) [76]. Luxturna, Glybera

Strimvelis and other three drugs for the treatment of rare diseases were approved for marketing [77-79]. However, the development of gene therapy is not always smooth sailing. From the death of the subject due to the fatal immune response in a clinical trial of adenovirus gene therapy conducted by Dr. James Wilson of the University of Pennsylvania in 1999 to the gene-editing babies event in 2018, gene therapy always arouses widespread discussion and controversy internationally, especially for the ethics of editing the human genome [80-82]. These challenges and opportunities inspire researchers to go in new directions, and drive development of gene therapy for human health. Acquired immunodeficiency syndrome (AIDS), an infectious disease caused by HIV, may be cured following CCR5 Δ 32/ Δ 32 allogeneic haemopoietic stem-cell transplantation [83,84]. In addition, multiple lines evidence revealed that the potential of gene therapy to alter neurological diseases [85-87].

The death of AD is an inevitable outcome generally occurring within 5–12 years of symptom onset, and the dementia due to AD is throughout the disease course [88]. Caregivers and the public health sector face enormous burden [1]. There is a dire need for effective research and development that may prevent or slow the rate of AD progression, but unfortunately none are currently available over the past 20 years. Though numerous mid-to-late-stage clinical trial have failed to demonstrate benefits, researchers are still actively developing and testing novel therapeutic strategies. While it is unclear how early A673T mutation is needed to provide protection from AD, these mechanistic studies of A673T mutation further validate efforts to reduce the generation and aggregation level of A β , the pathological hallmarks of AD, as therapeutic approaches to treat AD. Recently, various stem cells (ESCs, MSCs, NSCs, and iPSCs) therapy and gene therapy for AD have been introduced as a suitable therapeutic source [89-93]. One may expect that A673T mutation would alleviate or even treat AD by inducing A673T mutation of stem cells based on methods such as the CRISPR system and transplanting them into hypothalamus because of suppressed A β production. At present, Taisia Rolova and colleagues have generated a human iPSC line (UEFi003-A) carrying heterozygous A673T variant [94]. Furthermore, A673T was a natural mutation, which is a mutation existing in natural people found among Icelanders. Most importantly, there is no research showing that A673T mutation can cause other diseases until now. A673T based AD therapies may represent potential strategies in the future. We hope to present the most recent knowledge of A673T inspiring researchers to go in new study directions of A673T mechanism and driving development of future diagnostic tools and treatments for AD.

Declarations

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Competing interests

The authors declare that they have no competing interests.

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Author' contributions

All authors contributed to the article. Xu Gao and YongDa Wang had the idea for the article. The first draft of the manuscript was written by Qing Xia and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Table

Table 1 Population frequencies of the APP A673T mutation by country

Country	Sample	NO.	Homozygotes /Heterozygotes	Method	Reference
Iceland	1,795 Icelanders	3	Homozygotes	Single track assay SNP genotyping Illumina SNP Chip Genotyping Whole Genome Sequencing and SNP Calling	[21]
Denmark	3487 unrelated Danes	1	NA	Allelic discrimination using the predesigned Taqman single nucleotide polymorphism genotyping assay C 89522366_10 (Life Technologies)	[25]
Norway	544 patients with schizophrenia or bipolar disorder and 204 controls, all Norwegians	3	NA	Single track assay SNP genotyping Illumina SNP Chip Genotyping Whole Genome Sequencing and SNP Calling	[21]
Sweden	283 schizophrenia samples and 310 controls, all with European ancestry	5	NA	Single track assay SNP genotyping Illumina SNP Chip Genotyping Whole Genome Sequencing and SNP Calling	[21]
Finland	8943 US AD cases, 10480 US cognitively normal controls, 862 Swedish AD cases, and 707 Swedish cognitively normal controls	3	Heterozygotes	Infinium HumanExome V1 Beadchip (Illumina, Inc) TaqMan genotyping (Life Technologies).	[29]
	200 schizophrenia patients and 200 controls—all Finnish sample	4	NA	Single track assay SNP genotyping Illumina SNP Chip Genotyping Whole Genome Sequencing and SNP Calling	[21]
	515 Finnish subjects [aged 85 or older]	1	NA	Neuropathologic analyses	[22]
USA	8943 US AD cases, 10480 US cognitively normal controls, 862 Swedish AD cases, and 707 Swedish cognitively normal controls	3	Heterozygotes	Sanger method Infinium HumanExome V1 Beadchip (Illumina, Inc) TaqMan genotyping (Life Technologies).	[29]
	1674 late-onset Alzheimer's disease cases and 2644 elderly control subjects, all North American Whites	0	NA	TaqMan SNP genotyping assay (C 89522366_10; Life technologies, Grand Island, NY, USA)	[28]
China	552 with Alzheimer's disease and vascular dementia, 790 with Parkinson's disease,	0	NA	Applied Biosystems 7500 Fast Real- time PCR TaqMan SNP Genotyping Assays.	[26]

and 7379 controls, all Asian individuals

1237 healthy longevity subjects (mean age 96.9 years) and 1404 matched younger controls (mean age 44.2 years) , all Chinese

Sequencing of exon 16 in 10% of the samples
Illumina HumaExome BeadChip array with sequencing of random samples
Sequencing on an ABI3730xl Genetic Analyzer (ABI, USA) [27]
Using high-resolution melting (HRM) on a lightcycler 480 High Resolution Melting Master (Roche, Switzerland)
Sequencing of exon 16 in 5% of the HRM sample using an ABI3730xl Genetic Analyzer.

Figures

Figure 1

Population frequencies of the APP A673T mutation by country. Created with R package 'ggplot2' 'maps'.
Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

The mechanisms of A673T protection against AD a A673T can reduce C99 generation and decrease the C99/C89 ratio by increasing APP processing by α -secretase and change the cleavage site selection of BACE1 from β -site to β' -site. b A673T can reduce both of sAPP β and C99 levels by reducing the catalytic turnover (V_{max}) of APP rather than APP binding affinity (K_m) for BACE1. A673T can alter the distribution of C99 in the raft which was codistributed with γ -secretase components, resulting in reduced production of A β . c A673T promote a decreased propensity for A β aggregation. The internalization of microglia highly correlated purely with the aggregation result. d A673T has a protective effect on JNK mediated intracellular death signal via APP induced by TGF β 2. e A β peptide with A2T has a very low plasma membrane binding. A673T can affect mitochondrial axonal transport, thereby protecting against AD. f A673T mutant can reduce the formation of A β peptide oligomers which are cyto-toxic to cultured neurons.

Figure 3

Structure prediction of wild-type APP peptide and the APP protein with A673T mutation, A673V mutation or KM670/671NL mutation. The molecular visualization system Pymol show possible 3D structure of the APP protein with different mutations in the left. Created with <http://raptorx.uchicago.edu/>. Jpred 4 prediction results in the right show the probable secondary structure changes of APP caused by mutations in red frames. Created with <http://www.compbio.dundee.ac.uk/jpred4/>.

Figure 4

The molecular visualization system Pymol showed possible 3D structure of wild-type Aβ peptide (pink) and Aβ peptide with A2T mutation (blue) or A2V mutation (green) in the left (a. Aβ1-40 peptide, b. Aβ1-42 peptide). Created with <http://raptorx.uchicago.edu/>. Jpred 4 prediction results in the right (a. Aβ1-40 peptide, b. Aβ1-42 peptide) showed the probable secondary structure changes of Aβ caused by mutation in red frame. Created with <http://www.compbio.dundee.ac.uk/jpred4/>.

Supplementary Files

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- [TableandFigure.pdf](#)