

# Mutations, associated with early-onset Alzheimer's disease, discovered in Asian countries

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**Abstract:** Alzheimer's disease (AD), the most common form of senile dementia, is a genetically complex disorder. In most Asian countries, the population and the number of AD patients are growing rapidly, and the genetics of AD has been extensively studied, except in Japan. However, recent studies have been started to investigate the genes and mutations associated with AD in Korea, the People's Republic of China, and Malaysia. This review describes all of the known mutations in three early-onset AD (EOAD) causative genes (*APP*, *PSEN1*, and *PSEN2*) that were discovered in Asian countries. Most of the EOAD-associated mutations have been detected in *PSEN1*, and several novel *PSEN1* mutations were recently identified in patients from various parts of the world, including Asia. Until 2014, no *PSEN2* mutations were found in Asian patients; however, emerging studies from Korea and the People's Republic of China discovered probably pathogenic *PSEN2* mutations. Since several novel mutations were discovered in these three genes, we also discuss the predictions on their pathogenic nature. This review briefly summarizes genome-wide association studies of late-onset AD and the genes that might be associated with AD in Asian countries. Standard sequencing is a widely used method, but it has limitations in terms of time, cost, and efficacy. Next-generation sequencing strategies could facilitate genetic analysis and association studies. Genetic testing is important for the accurate diagnosis and for understanding disease-associated pathways and might also improve disease therapy and prevention.

**Keywords:** mutation, Asia, presenilin, amyloid precursor protein, genetics

## Introduction

Alzheimer's disease (AD), the most common form of senile dementia, is a major health problem.<sup>1</sup> AD is a complex disease, and several genetic and/or environmental factors can contribute to disease progression. The main hallmarks of AD are the amyloid beta (A $\beta$  or Abeta) peptides and the neurofibrillary tangles. The number of AD patients is rising rapidly as the lifetime expectancy increases. In Asia, the population is growing rapidly, especially in India and the People's Republic of China. Aging of population is also an important current issue. According to 2010 statistics, the elderly constituted 11% of the total population. This percentage is expected to reach 16.5% and 22% by 2030 and 2050, respectively. The fastest increase in the number of elderly individuals has been observed in East Asian countries. More than half (60%) of all patients diagnosed with dementia live in Asian countries. The People's Republic of China, Japan, and India are among the top seven countries with the highest number of dementia patients.<sup>2</sup>

## Genetics of AD

The two main types of AD can be classified as early-onset AD (EOAD) and late-onset AD (LOAD). EOAD or autosomal dominantly inherited AD occurs before the age of 60–65 years. Three main genes are involved in EOAD, *APP*, *PSEN1*, and

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*PSEN2*, encoding amyloid precursor protein, presenilin-1, and presenilin-2, respectively. EOAD is relatively rare, with 5%–10% of all AD cases occurring before the age of 65 years. Mutations in all of these three genes could result in enhanced Abeta production and deposition.<sup>3</sup> APP is a transmembrane (TM) protein, which can play a role in synaptic plasticity. Three enzymes, the alpha-, beta-, and gamma-secretases, have cleavage sites in APP protein. Abnormal cleavage of APP by gamma- and beta-secretases could result in impairment in the Abeta production. It was suggested that Abeta peptide may be important in synaptic vesicle regulation,<sup>4</sup> but the oligomer form of peptide can be involved in neurotoxicity. Abeta42 and Abeta40 peptides and Abeta42/40 ratio have been established as the most important biomarkers for AD. However, even the small alterations in the Abeta42/40 levels could result in pathogenic phenotypes, by enhancing their oligomerization ability and stabilizing their oligomer forms, resulting in toxicity for synapses and nerve cells.<sup>5</sup>

Presenilins (PSEN1 and 2) are the components of gamma-secretase complex. They can be involved in memory, survival of neurons, and synaptic mechanisms. Involvement of PSENs in AD may be heterogeneous. Gamma-secretase dysfunctions have been established as the most common mechanisms in PSEN1–2 mutants, resulting in the impairment of APP cleavage and neuronal toxicity.<sup>1,3</sup> Elevated Abeta42 could be associated with disease progression but not in all AD cases.<sup>6</sup> Some mutations were revealed as “loss-of-function” variants, since they destroy the gamma-secretase activity. These mutations were associated with increased Abeta42/40 levels and impairment in synaptic plasticity.<sup>7</sup> Ben-Gedalya et al<sup>8</sup> revealed that some PSEN1 (especially proline exchange with other amino acids) variants could prevent the interaction of PSEN with the chaperone, called cyclophilin B, resulting in PSEN degradation or

abnormal PSEN aggregation in endoplasmic reticulum. LOAD usually occurs later in life, after 65 years, and its prevalence increases with age. The genetic background of LOAD remains unclear because no exact causative genes have been reported yet. However, genome-wide association studies (GWAS) have identified several genes that might increase the risk of AD onset. The apolipoprotein E (*APOE*) E4 allele appears to be the main risk factor for LOAD, but it does not define all AD cases.<sup>1,3,9</sup>

This review focuses on EOAD and summarizes all mutations found in the three genes associated with EOAD in Asian families and patients. It also briefly summarizes the Asian GWAS, performed on the putative risk factor genes. In addition, the review describes methods that could be useful in genetic testing from the genotyping to next-generation sequencing (NGS).

## APP, PSEN1, and PSEN2 mutations in Asian countries that might contribute to EOAD

Several mutations were discovered in *APP*, *PSEN1*, and *PSEN2* that could contribute to disease progression. Most of these mutations are associated with familial EOAD, in which the inheritance pattern is autosomal dominant and follows Mendelian rules. However, several de novo cases of AD were reported in patients without any family history of dementia. The majority of pathogenic mutations were found in *PSEN1* gene. Several *PSEN1* mutations could be associated with early-onset AD, which occurs at the age <40 years, and with rapid and aggressive dementia progression. Mutations in *APP* and *PSEN2* are quite rare but are possible causative factors for EOAD. Pathogenic mutations could result in disease onset at the age of 40–65 years.<sup>3</sup> In the most Asian countries, as in Europe, genetics of EOAD is not well characterized, and

**Table 1** Mutations in APP, PSEN1, and PSEN2, discovered in Asia

Gene	Location in the gene (exon)	Mutation	Location in PSI protein	Country	References
APP	16	678Asp→Asn	N-term	Japan	Wakutani et al <sup>48</sup>
	17	693Glu→del	N-term	Japan	Tomiyama et al <sup>49</sup>
		710Val→Gly	TM-I	the People's Republic of China and Taiwan	Thajeb et al <sup>36</sup>
		714Thr→Ala	TM-I	Iran	Pasalar et al <sup>86</sup>
		715Val→Met	TM-I	Korea	Park et al <sup>14</sup>
		Val717Ile	TM-I	Japan, Thailand, and the People's Republic of China	Yoshioka et al <sup>50</sup> and Jiao et al <sup>12</sup>
		Ile718Leu	TM-I	the People's Republic of China and Taiwan	Thajeb et al <sup>36</sup>
		Leu720Ser	TM-I	the People's Republic of China and Taiwan	Thajeb et al <sup>36</sup>

(Continued)

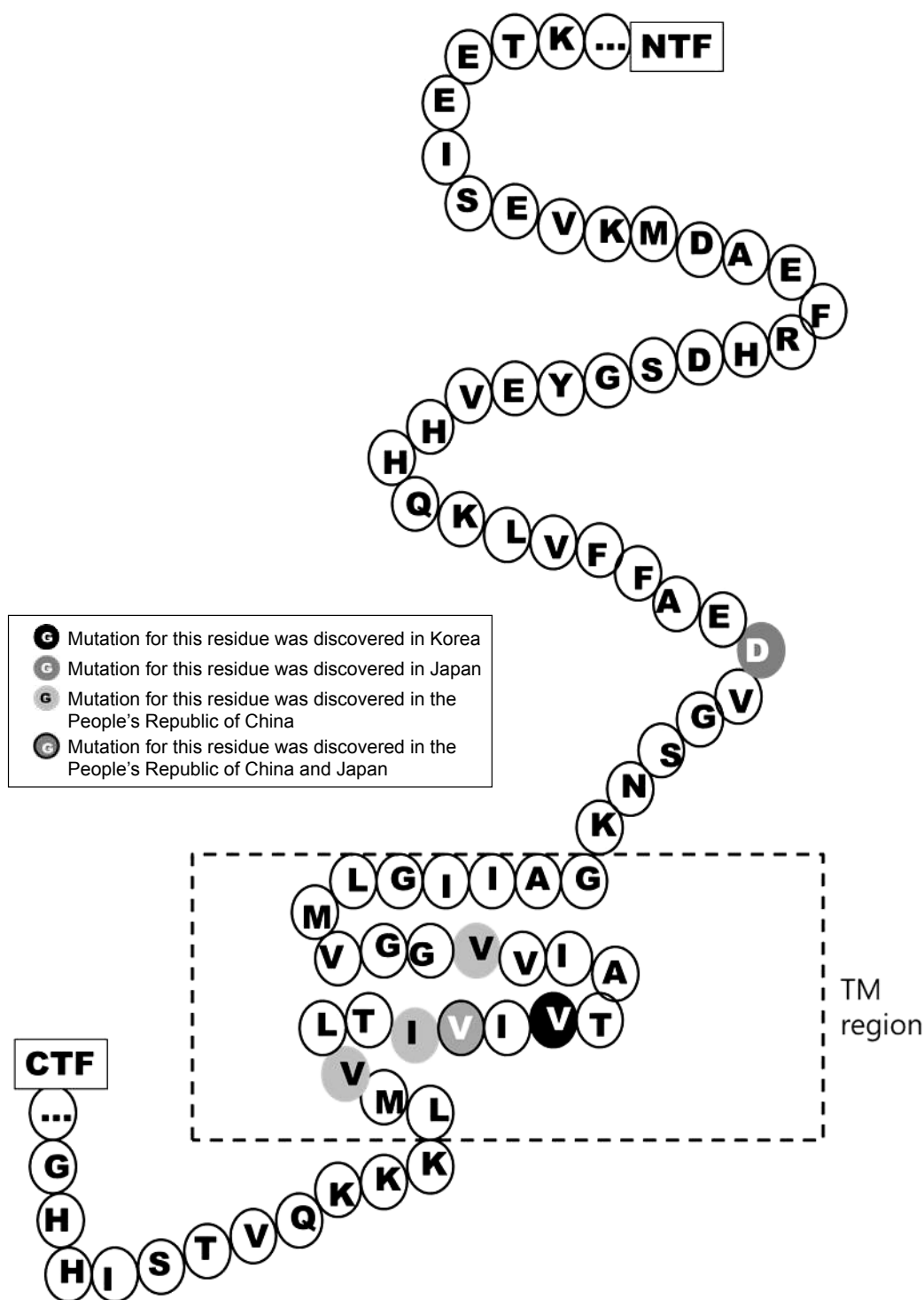
Table 1 (Continued)

Gene	Location in the gene (exon)	Mutation	Location in PSI protein	Country	References
PSEN1	4	85Leu→Pro	TM-I	Japan	Ataka et al <sup>52</sup>
		96Val→Phe	TM-I	Japan	Kamino et al <sup>53</sup>
		97Val→Leu	TM-I	the People's Republic of China	Fang et al <sup>42</sup>
	5	105He→Cys	HL-I	the People's Republic of China	Jiao et al <sup>12</sup>
		116Thr→Ile	HL-I	Korea	Personal communication*
		123Glu→Lys	HL-I	Japan	Yasuda et al <sup>54</sup>
		136Ala→Gly	TM-II	the People's Republic of China	Fang and Jia <sup>43</sup>
		139Met→Ile	TM-II	Korea	Kim et al <sup>19</sup>
		143Ile→Thr	TM-II	Japan	Arai et al <sup>55</sup>
		154Tyr→Asn	TM-II	Japan	Hattori et al <sup>57</sup>
		163His→Arg	HL-II	Japan and Korea	Kamino et al <sup>53</sup> and Hong et al <sup>22</sup>
	6	163His→Pro	HL-II	Korea	Kim et al <sup>23</sup>
		165Trp→Gly	TM-III	Japan	Higuchi et al <sup>58</sup>
		167Ile→del	TM-III	the People's Republic of China	Jiao et al <sup>12</sup>
		169Ser→del	TM-III	the People's Republic of China	Guo et al <sup>44</sup>
		173Leu→Phe	TM-III	Japan	Kasuga et al <sup>59</sup>
		184Glu→Asp	HL-III	Japan	Yasuda et al <sup>60</sup>
		206Gly→Ser	TM-IV	Korea	Park et al <sup>14</sup>
	7	209Gly→Arg	TM-IV	Japan	Sugiyama et al <sup>24</sup>
		209Gly→Ala	TM-IV	Korea	An et al <sup>11</sup>
		213Ile→Thr	TM-IV	Japan	Kamino et al <sup>53</sup>
		217Gly→Asp	HL-IV	Japan	Takao et al <sup>62</sup>
		226Leu→Phe	TM-V	Korea	Bagyinszky et al <sup>27</sup>
		232Leu→Pro	TM-V	Korea	Personal communication <sup>#</sup>
		233Met→Thr	TM-V	Korea	Park et al <sup>14</sup>
		237Phe→Ile	TM-V	Japan	Sodeyama et al <sup>63</sup>
		248Leu→Pro	TM-VI	the People's Republic of China	Jiao et al <sup>12</sup>
		250Leu→Val	TM-VI	Japan	Furuya et al <sup>64</sup>
		260Ala→Val	TM-VI	Japan	Ikeda et al <sup>65</sup>
	8	266Gly→Ser	HL-VI (a)	Japan	Matsubara-Tsutsui et al <sup>66</sup>
		269Arg→His	HL-VI (a)	Japan	Kamimura et al <sup>47</sup>
		273Glu→Ala	HL-VI (a)	Japan	Kamimura et al <sup>47</sup>
		280Glu→Ala	HL-VI (MA)	Japan	Tanahashi et al <sup>69</sup>
		280Glu→Lys	HL-VI (MA)	Malaysia	Chng et al <sup>88</sup>
		282Leu→Phe	HL-VI (MA)	Japan	Hamaguchi et al <sup>73</sup>
		284Pro→Leu	HL-VI (MA)	Japan	Tabira et al <sup>74</sup>
		285Ala→Val	HL-VI (MA)	Japan	Ikeuchi et al <sup>79</sup>
		286Leu→Val	HL-VI (MA)	Japan	Ikeuchi et al <sup>79</sup>
		Exon9del	–	Japan	Tabira et al <sup>74</sup>
	10	352Arg→Cys	HL-VI (b)	the People's Republic of China	Jiang et al <sup>35</sup>
	11	378Gly→Glu	TM-VII	Japan	Ikeda et al <sup>65</sup>
		381Leu→Val	TM-VII	Japan	Ikeuchi et al <sup>79</sup>
		384Gly→Ala	TM-VII	Japan	Kamimura et al <sup>47</sup>
		392Leu→Val	TM-VII	Japan	Ikeuchi et al <sup>79</sup>
		405Asn→Ser	HL-VII	Japan	Yasuda et al <sup>83</sup>
	12	431Ala→Val	HL-VIII	Japan	Matsushita et al <sup>84</sup>
		434Ala→Thr	HL-VIII	the People's Republic of China	Jiao et al <sup>12</sup>
		440Thr→del	HL-VIII	Japan	Ishikawa et al <sup>85</sup>
PSEN2	4	62Arg→Cys	N-term	Korea	Sleegers et al <sup>31</sup>
		82Lys→Arg	N-term	the People's Republic of China	Shi et al <sup>45</sup>
	5	123Pro→Leu	HL-I	the People's Republic of China	Xia et al <sup>7,46</sup>
		141Asn→Tyr	TM-II	the People's Republic of China	Niu et al <sup>13</sup>
	6	169His→Asn	HL-II	the People's Republic of China	Shi et al <sup>45</sup>
	7	214Val→Leu	TM-IV	Korea	Youn et al <sup>34</sup>

**Notes:** \*Seong-Beom Koh, Eva Bagyinszky, SunOhBae, Seong Soo A An, SangYun Kim, personal communication, January, 2016. #JiYoon Park, Eva Bagyinszky, Vo Van Giau, JyuWhan Shim, YoungChul Youn, SeongSoo An, SangYun Kim, personal communication, January, 2016.

only a few reports are available regarding mutations in EOAD causative genes. However, emerging research projects in Japan, Korea, and the People's Republic of China are assessing the genetic background of patients with early-onset dementia. Table 1 lists all EOAD-associated mutations discovered in Asian countries. The information in this table comes from the two main databases that summarize the mutations found

in EOAD-associated genes. The AD and frontotemporal dementia (FTD) mutation database<sup>10</sup> was edited by Cruts et al and summarized all known mutations associated with the following two main types of dementia: AD and FTD. The Alzheimer's Research Forum (<http://www.alzgene.org/>) is an up-to-date database that summarizes all findings and articles related to AD, including genetic mutations.<sup>10</sup> In APP,



**Figure 1** Mutation residues in APP discovered in Asian countries.

**Abbreviations:** CTF, C-terminal fragment; NTF, N-terminal fragment; TM, transmembrane domain.

35 variants were discovered in the exon 16 and 17, and of them, 9 variants were (also) reported in Asia (Figure 1). Due to the Alzheimer's Research Forum database, 227 variants were discovered in *PSEN1* all around the world. Of them, 51 variants were (also) discovered in Japan, Korea, the People's Republic of China, or Malaysia (Figure 2). In *PSEN2*, 38 missense and frameshift mutations were reported, and until 2014, no pathogenic mutation was found in *PSEN2* in any Asian countries. However, recent studies revealed novel and known variants in *PSEN2* gene in Korean and Chinese patients (Figure 3).<sup>11–13</sup>

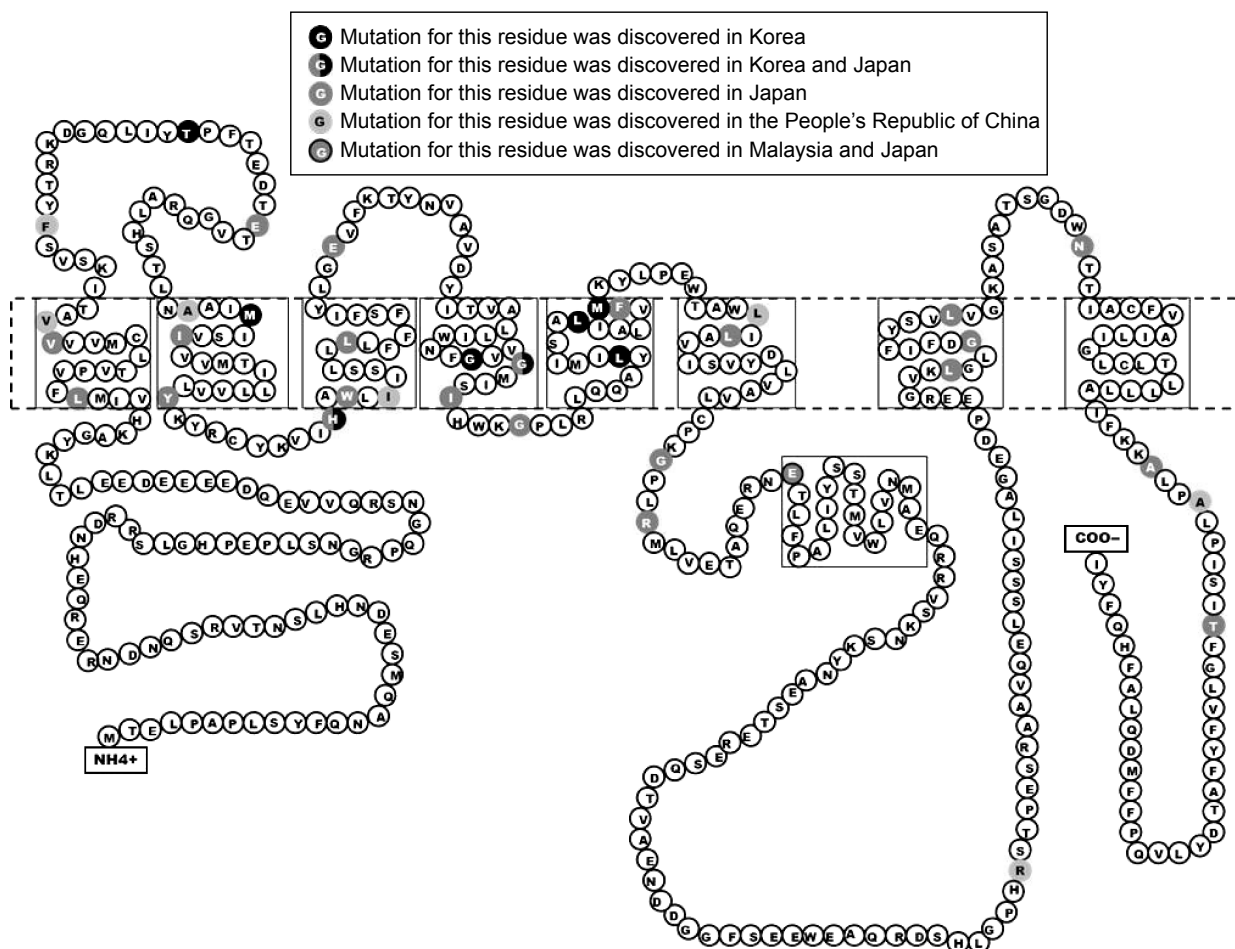
## Mutations discovered in Korea

The genetic background of EOAD in Korea is not well characterized (Table 2). Until 2010, only five mutations were discovered: Val715Met in *APP*,<sup>6</sup> Met139Ile, His163Arg, Gly206Ser, and Met233Thr in *PSEN1*, and no mutations in *PSEN2* have been reported yet. Our research group found four additional novel or known *PSEN1* mutations in Korean AD patients, including Thr116Ile (known), His163Pro (novel), Leu226Phe (known), and Leu232Pro (novel). We also

discovered the following two *PSEN2* mutations in Korea for the first time: Val214Leu (novel) and Arg62Cys (known). Since Korea is one of the fastest “aging countries” in the world, the number of AD, including EOAD, patients will rise fast. In the future, it might be possible to find additional novel/known variants in the EOAD causative genes.

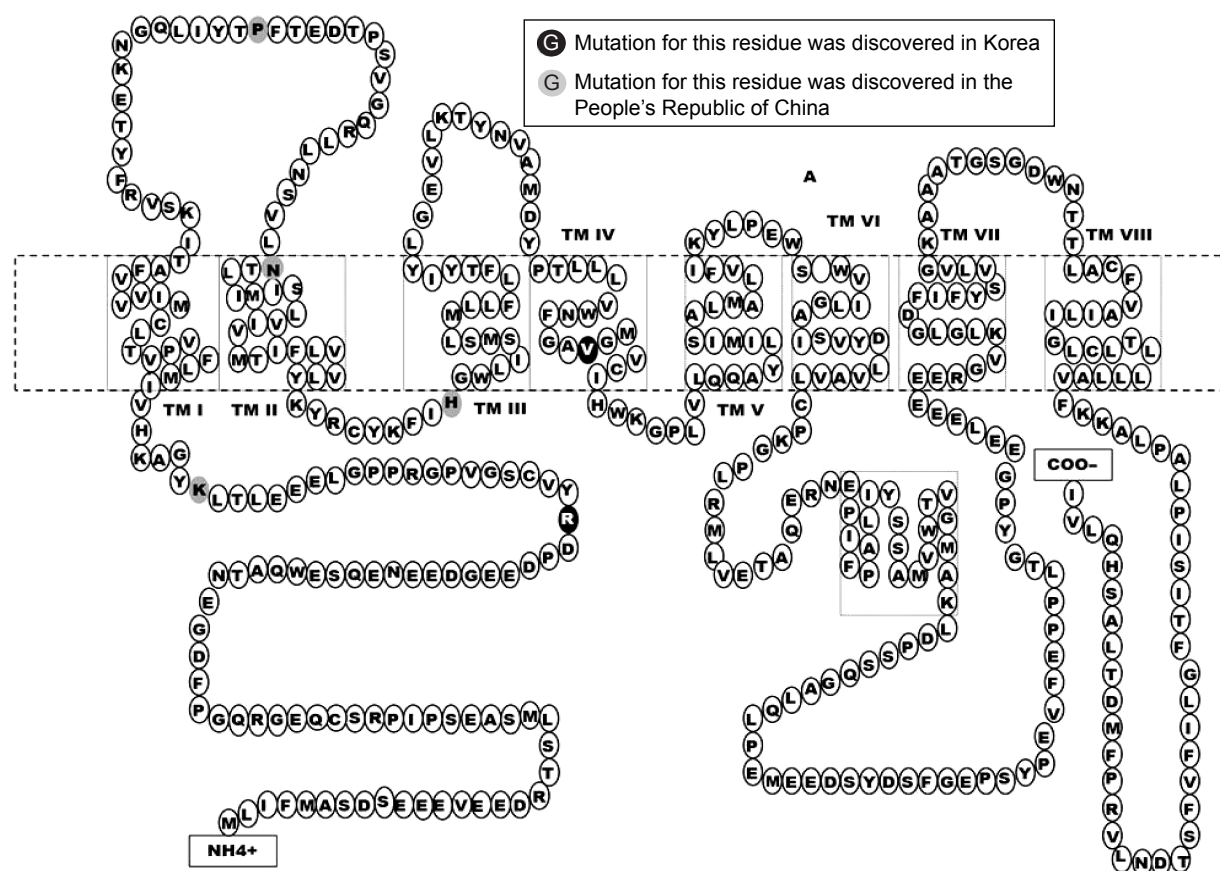
## Mutations in *APP*

Val715Met (GTG→ATG) was discovered in a 41-year-old male patient in Korea who had a positive family history of dementia.<sup>14</sup> The symptoms were memory and visual impairment, bradykinesia, and epilepsy. This is the first and currently the only pathogenic mutation in *APP*, which was found in Korea. Because the mutation was first discovered in a French family (with Italian descendants) with progressive memory decline, the mutation is also called “French APP”. The age of onset was the same in French and Korean patients.<sup>15</sup> The Korean patient might be associated with familial EOAD, because other family members (uncles from the father's side) also developed dementia. *APP* Val715Met was expressed in human embryonic kidney 293 (HEK293)



**Figure 2** Mutation residues in *PSEN1*, discovered in Asian countries.





**Figure 3** Mutation residues in PSEN2, discovered in Asian countries.

cell lines, and a significant decrease in Abeta40 levels (twofold) was observed. Abeta42 levels did not change, but the ratio of Abeta42/Abeta40 increased (1.8-fold). These findings suggested that this mutation might destroy the Abeta40 cleavage site of gamma-secretase.<sup>15</sup>

### Mutations in *PSEN1*

Thr116Ile (ACC→ATC) was identified in a Korean patient (age of onset, 42 years) for the first time in Asia. This patient showed a strong positive family history of disease; her mother was diagnosed with dementia. The patient's three sisters agreed to genetic testing, and they have been screened for Thr116Ile. One of the sisters (37 years of age at the time of analysis) carried the mutation; she had not been clinically diagnosed with AD yet, but symptoms of memory impairment (forgetfulness) have already appeared in her early 30s (Seong-Beom Koh, Eva Bagyinszky, SunOhBae, Seong Soo A An, SangYun Kim, personal communication, January, 2016). Thr116Ile has been described in French and Italian patients before with familial or de novo cases of EOAD, with similar age of onset.<sup>16,17</sup> For Thr116, an additional pathogenic mutation, Thr116Asn, was discovered, which

was also associated with EOAD.<sup>18</sup> Because Thr116 is located in the conservative HL-I region, a Thr→Ile exchange might disturb significantly the PSEN1 protein structure.

Met139Ile (ATG→ATC) was found in a female patient. The mutation has been described before but with a different codon change (ATG→ATA) in a European patient. Codon 139 might be an important residue in *PSEN1* in the TM-II region of PSEN1, given that the following three additional mutations have been described: Met139Lys, Met139Thr, and Met139Val.<sup>19,20</sup> In the Korean patient, memory problems started at the age of 38 years. She had a positive family history of dementia; her mother (deceased at the age of 45 years) and her elder sister had a similar type of memory decline. When *PSEN1* Met139Ile (ATG→ATA) was expressed in COS-1 cell lines, the Abeta42/total Abeta ratio increased.<sup>21</sup> His163Arg (CAT→CGT) is a quite frequently occurring AD-associated mutation, since it has been reported in 15 familial or sporadic EOAD cases in several parts of the world. In Korea, Hong et al described the mutation in a female patient who was hospitalized because of memory disturbances and personality changes at the age of 36 years.<sup>22</sup> Symptoms started in her late 20s. Strong family history of

Table 2 Mutations, discovered in Korea

Gene	Mutation	Was it discovered before?	Clinical phenotype	Age of onset/family history	Functional data	PolyPhen scores	SIFT scores	References
APP	Val715Met	Yes, in French patients "French APP"	EOAD memory and visual impairment	41 years/familial EOAD	Expression in HEK293 cells, revealed decrease in Abeta40 levels (twofold)	0.99 (probably damaging)	0 (damaging)	Park et al <sup>14</sup>
PSEN1	Thr116Ile	Yes, in French and Spanish patients	EOAD, visual impairment	40–47 years/familial EOAD	Not available	0.99 (probably damaging)	0.07 (tolerated)	La Bella et al <sup>16</sup> and personal communication*
	Met139Ile	Yes, in Denmark	EOAD	38 years/familial	Ratio of Abeta42/total Abeta increased in COS-1 cell lines	0.98 (probably damaging)	0.14 (tolerated)	Kim et al <sup>19</sup>
	His163Pro	No	EOAD, parkinsonism	35 years/de novo	Not available	0.98 (probably damaging)	0 (tolerated)	Kim et al <sup>23</sup>
	His163Arg	Yes, in Japanese and European families	EOAD	36–50 years/familial and de novo	Abeta42/Abeta40 ratio doubled in COS-1 cell lines	0.84 (possibly damaging)	0.1 (tolerated)	Hong et al <sup>22</sup>
	Gly206Ser	Yes, in French patients	EOAD	30–35 years/familial	Not available	1 (probably damaging)	0.09 (tolerated)	Park et al <sup>14</sup>
	Gly209Ala	No	EOAD	54 years/familial	Not available	1 (probably damaging)	0 (damaging)	An et al <sup>11</sup>
	Leu226Phe	Yes, in Polish and Spanish patients	EOAD/FTD	30–37 years/de novo	Results elevated Abeta42/Abeta40 ratio in HEK293 cells	1 (probably damaging)	0 (damaging)	Zekanowski et al <sup>28</sup> and Bagyinszky et al <sup>27</sup>
	Leu232Pro	No	EOAD	Unknown	Not available	1 (probably damaging)	0 (damaging)	Personal communication#
	Met233Thr	Yes, in France and Australia	EOAD, rapid progressive memory impairment	34 years/de novo	Elevated (3.2-fold) Abeta42/Abeta40 levels in CHO cells	0.97 (probably damaging)	0.04 (damaging)	Park et al <sup>14</sup>
PSEN2	Arg62Cys	Yes, in the Netherlands	AD, pathogenic nature unclear	40–70 years/unknown	Not available	0.87 (possibly damaging)	0.02 (damaging)	Brouwers et al <sup>33</sup>
	Val214Leu	No	AD	56–70 years/unknown	Not available	0.97 (probably damaging)	0.09 (tolerated)	Youn et al <sup>34</sup>

**Notes:** \*Seong-Beom Koh, Eva Bagyinszky, SunOhBae, Seong Soo A An, SangYun Kim, personal communication, January, 2016. #jiYoon Park, Eva Bagyinszky, Vo Van Giau, Kyuhwan Shim, YoungChul Youn, Seong Soo An, SangYun Kim, personal communication, January, 2016.

**Abbreviations:** AD, Alzheimer's disease; EOAD, early-onset AD; FTD, frontotemporal dementia.

disease was observed in her family; of the 22 family members tested, nine members were affected. The mutation was found in several Japanese familial and sporadic AD cases (five families), with the age of onset ranging from 47 to 50 years. When *PSEN1* His163Arg was expressed in COS-1 cell lines, twofold increase was observed in Aβ42/Aβ40 ratio.<sup>21</sup> The histidine at 163th position in *PSEN1* might be an important residue in the protein. An additional variant, His163Tyr, was discovered, and in 2012, a novel mutation, His163Pro (CAT→CCT), was reported in a Korean female patient with clinically diagnosed EOAD and parkinsonism. She also experienced seizures, myoclonus, and cerebellar ataxia. This might be a de novo case of AD, since no affected family members were found. Even though the mutation was found in only one patient, evidence for its pathogenic nature is strong; it has not been found in healthy controls. The two additional AD causative mutations of His163 could also enhance the evidence on the pathogenic nature of His163Pro. In addition, the mutation is located in the border of TM and loop region, where the rigid proline exchange might disturb protein structure and function.<sup>23</sup> Gly206Ser (GGT→AGT) was reported in Korean and European families, with the age of onset ranging from 30 to 35 years. Patients showed progressive episodic memory and visuospatial impairment, ataxia, acalculia, and aphasia. Gly206 is an important residue in *PSEN1* protein, and the following three additional pathogenic mutations have been reported: Gly206Asp, Gly206Ala, and Gly206Val.<sup>14,17</sup> Gly209Ala (GGA→GCA) was recently discovered in a Korean EOAD patient, with the age of onset at 54 years. This might be a de novo case of EOAD, because no additional affected family members were found. The extra-CH<sub>3</sub> group in alanine could result in extra stress inside the *PSEN1* helix.<sup>11</sup> Further studies about this mutation are needed, because it is located in the TM region, where additional Gly→Ala exchanges were described (the nearest of them is the Gly206Ala), which was also validated as pathogenic mutation. Similarly to G206A, Gly209 might also be an important residue in *PSEN1*, since additional mutations, such as Gly209Arg, Gly209Glu, and Gly209Val, have been described.<sup>24–26</sup> Leu226Phe (CTC→TTC) is a known mutation, discovered in Korea for the first time by our research group.<sup>27</sup> The mutation was detected in an AD patient, with the age of onset at 37 years, and appeared to be de novo case of AD. The age of death was 44 years.<sup>28</sup> This variant has been reported in AD patients in Poland with the age of onset ranging from 30 to 44 years. The Polish proband patient was initially diagnosed with FTD, but postmortem studies revealed AD-associated neuropathology. In the EOAD Korean patient, additional symptoms, such as nonfluent

aphasia and parkinsonism, were detected. An additional mutation (Leu226Arg) has been described for codon 226. In silico modeling was performed with *PSEN1* Leu226Phe. The findings revealed that the interaction between the benzene rings of Tyr225 and Phe226 could disturb the TM-V region of *PSEN1*. In addition, the mutation might enhance hydrophobic interactions between *PSEN1* protein and its putative binding partners.<sup>28–30</sup> Met233Thr (ATG→ACG) was described in six families from France, Australia, and Korea. Mutation appeared in both familial and de novo cases of young onset AD that occurred in the 30s or early 40s. The Korean patient did not have a family history of dementia, with the age of disease onset at 34 years. The patient showed rapid progressive memory impairment; the score of Mini-Mental State Examination dropped drastically 2 years after the first symptoms appeared. Two other mutations, Met233Leu and Met233Val, have been described for codon 233.<sup>14</sup> When the Met233Thr was expressed in Chinese hamster ovary (CHO) cells, the Aβ40/total Aβ ratio decreased sharply (2.2-fold), and the Aβ42/total Aβ ratio increased (3.2-fold).<sup>30</sup> Leu232Pro (CTC→CCC) is a novel mutation recently discovered by our research group. Further investigations, clinical studies, and genetic testing of family members are needed to determine whether the mutation could be involved in AD progression. However, several pathogenic Leu→Pro exchanges have been reported in *PSEN1*, suggesting that this mutation may be involved in EOAD progression, and the nearest is the Leu235Pro. Since the mutation is located in the TM region, the rigid proline might result in significant disturbances. Proline, which was established as a helix breaking molecule, could result in serious torsion in the TM region (JiYoon Park, Eva Bagyinszky, Vo Van Giau, Kyuhwan Shim, YoungChul Yoon, Seong Soo An, SangYun Kim, personal communication, January, 2016).

### Mutations in *PSEN2*

Arg62Cys (CGC→TGC) was discovered in Asia for the first time by our research group. The mutation was identified in a dementia patient. Memory impairment, personality changes, and disorientation appeared at the age of 49 years. He had no family history of dementia (Kyung Won Park, Eva Bagyinszky, SeunOh Bae, Seong Soo An, SangYun Kim, personal communication, January, 2016). Arg62Cys is a known mutation in *PSEN2*, categorized as a “pathogenic nature unclear” variant in the two main AD mutation databases. The mutation was identified in LOAD patients and in healthy controls but with a higher frequency in AD patients. The mutation might be a risk factor for AD. Genetic–environmental interactions might also affect the onset of disease.<sup>31–33</sup> Val214Leu



(GTG→TTG) was also discovered by our research group. Val214Leu was one of the first *PSEN2* mutations identified in Asia. In addition, it is the first mutation identified in the TM-IV region of *PSEN2*. We discovered the mutation in the following two unrelated patients: a 70-year-old patient with AD-type dementia and a 56-year-old patient with memory impairment. The exact family history is unknown in both cases. The absence of Val214Leu in normal controls and the results of an in silico analysis suggested that this mutation could result in abnormalities in the PS2 structure. Because leucine is more hydrophobic than valine, the mutation might result in extra stress inside the TM domain or between the TM and surrounding proteins and could be a possibly pathogenic variant of *PSEN2*.<sup>34</sup>

## Mutations discovered in the People's Republic of China and Taiwan

The genetic background of Chinese (and Taiwanese) AD patients was not well characterized. Initially, three mutations were discovered in *APP* gene and three mutations were discovered in *PSEN1* gene. However, several recent studies reported additional novel mutations.<sup>12,13,35</sup> Most of the variants, discovered in Chinese families and patients, are novel mutations, which have not been described in any other population (Table 3).

### Mutations in *APP*

The following four *APP* mutations have been found in 10 Chinese/Taiwanese patients: Val710Gly (one patient), Ile718Leu (eight patients), and Leu720Ser (three patients, of whom, the mutation occurred with Ile718Leu in two patients). The symptoms of disease started with progressive dementia in all patients, which appeared 2–9 years before the clinical diagnosis of AD. Additional symptoms, such as oral tendency and parkinsonism, were present in some patients. Family history of dementia was negative in all tested patients, with the age of onset ranging from 65 to 82 years. These mutations are located in the TM region of *APP*; they might be critical for *APP* processing and might increase or decrease Abeta42 and Abeta40 levels in the brain.<sup>36</sup>

Val717Ile (GTC→ATC, “London APP”) was reported in several EOAD cases. In Asia, it was identified in five Japanese families and one Thai family.<sup>37</sup> This mutation was recently discovered in Chinese families, with the age of onset between 33 and 45 years, but clinical phenotypes of mutations were different in these families. In the first family, symptoms started with memory impairment and personality changes, followed by progressive memory loss and psychiatric symptoms. In the second family, disease progression was

slower. In the proband patient, memory impairment started at the age of 45 years. Cognitive impairment was observed at the age of 50 years.<sup>32</sup> This mutation was analyzed in several cell lines. CHO and HEK293 experiments suggested that Val717Ile increases the Abeta42/Abeta40 ratio.<sup>12,38</sup> Decreased Abeta40 levels were reported after analyzing this mutation in HEK293, COS cell.<sup>39,40</sup>

### Mutations in *PSEN1*

Val97Leu (GTG→TTG) was described for the first time in a Chinese family with EOAD. Because the mutation is not present in normal controls, it might be a novel mutation involved in familial EOAD.<sup>41</sup> To validate the pathogenic nature of this mutation, *PSEN1* Val97Leu was expressed in human neuroblastoma (SH-SY5Y) cells, and the Abeta concentration was monitored with ELISA and radioimmunity methods. Beta-secretase activity was also monitored. The data showed that intracellular and extracellular Abeta production was higher in the mutant cells, suggesting that the mutation is pathogenic.<sup>42</sup> Phe105Cys (TTT→TGT) was discovered in a Chinese patient, who showed memory impairment at the age of 59 years. Family history was positive, since her sibling carried the same mutation, and the dementia was fully developed in her father (died at the age of 60 years). Patients showed typical amnesic symptoms.<sup>12</sup> Ala136Gly (GCT→GGT) was discovered in a Chinese family. No data are available on the age of onset or on the clinical phenotypes of disease. Experiments were performed to establish the pathogenic nature of the mutation. Ala136Gly was expressed in human neuroblastoma cells. The survival of the mutant cells decreased significantly, suggesting that this mutation could have deleterious effects.<sup>43</sup> Ile167del (delTTA) was initially diagnosed in a Chinese female patient, for whom the memory loss started at the age of 38 years. Later, she developed spastic paraparesis, personality changes, and disorientation. Family history was positive, since her mother died with AD. Her two siblings were also positive for this mutation, and they showed similar symptoms.<sup>12</sup> Ser169del (delTCA.TCT) was described in a Chinese family with EOAD. Symptoms were early memory impairment (42–50 years), followed by cognitive dysfunction, memory decline, apraxia, and disorientation. Two additional mutations, Ser169Pro and Ser169Leu, which are involved in EOAD with rapid disease progression, were described for codon 169. Ser169del might be associated with disturbances in posttranslational modifications, in the protein structure or in the interactions with other proteins because of the missing –OH group.<sup>40</sup> Leu248Pro (CTC→CCC) was found in a female patient, where the memory impairment started

Table 3 Mutations, discovered in the People's Republic of China

Gene	Mutation	Was it discovered before?	Clinical phenotype	Age of onset/family history	Functional data	PolyPhen scores	SIFT scores	References
APP	Val710Gly	No	AD, parkinsonism	65–82 years/familial	Not available	1 (probably damaging)	0 (damaging)	Thajeb et al <sup>36</sup>
	Val717Ile	Yes, in Europe, Japan, Thailand “London App”	EOAD	33–35 years/familial	Increased Abeta42/Abeta40 ratio in CHO and HEK293 cells	0.99 (probably damaging)	0 (damaging)	Jiao et al <sup>12</sup>
PSEN1	Ile718Leu	No	AD, parkinsonism	65–82 years/familial	Not available	0.771 (possibly damaging)	0.1 (tolerated)	Thajeb et al <sup>36</sup>
	Leu720Ser	No	AD, parkinsonism	65–82 years/familial	Not available	1 (probably damaging)	0 (damaging)	Thajeb et al <sup>36</sup>
	Val97Leu	No	EOAD	Unknown	Higher beta-secretase activity in human neuroblastoma cells	0.99 (probably damaging)	0 (damaging)	Fang et al <sup>42</sup>
	Phe105Cys	No	EOAD	59 years/familial	Not available	0.99 (probably damaging)	0 (damaging)	Jiao et al <sup>12</sup>
	Ala136Gly	No	EOAD	Unknown	Survival of mutant neuroblastoma cells dropped	0.98 (probably damaging)	0.09 (tolerated)	Fang and Jia <sup>43</sup>
PSEN2	Ile167del	No	EOAD, spastic paraparesis	38 years/familial	Not available	Not applicable		Jiao et al <sup>12</sup>
	Ser169del	No	EOAD	42–50 years/familial	Not available	Not applicable		Guo et al <sup>44</sup>
	Leu248Pro	No	EOAD	42 years/familial	Not available	1 (probably damaging)	0.06 (tolerated)	Jiao et al <sup>12</sup>
	Arg352Cys	No	EOAD, psychiatric, behavioral symptoms	56–62 years/familial	Not available	0.92 (probably damaging)	0.03 (damaging)	Jiang et al <sup>35</sup>
	Ala434Thr	No	EOAD, hallucinations, delusions	38 years/de novo	Not available	0.99 (probably damaging)	0 (damaging)	Jiao et al <sup>12</sup>
	Lys82Arg	No	AD, language disturbances, depression	53 years/unknown	Not available	1 (probably damaging)	0.003 (damaging)	Shi et al <sup>45</sup>
	Pro123Leu	No	AD, parkinsonism	57 years/familial	Not available	0.99 (probably damaging)	0 (damaging)	Xia et al <sup>746</sup>
	Asn141Tyr	No	EOAD	43–49 years/familial	Not available	0.93 (possibly damaging)	0 (damaging)	Niu et al <sup>13</sup>
	His169Asn	No	AD and progressive nonfluent aphasia	62–68 years/unknown	Not available	0.98 (probably damaging)	0.05 (damaging)	Shi et al <sup>45</sup>
	Val214Leu	Yes, in Korea	AD	64–65 years/unknown	Not available	0.97 (probably damaging)	0.09 (tolerated)	Shi et al <sup>45</sup>

Abbreviations: AD, Alzheimer's disease; EOAD, early-onset AD.

at the age of 42 years. Family history was positive, since her deceased father was diagnosed with AD and her younger brother was also affected by this mutation and showed similar clinical symptoms.<sup>44</sup> Arg352Cys (CGC→TGC) was reported in three members of a Han family. This mutation might not segregate with the disease onset, since this mutation was also found in an unaffected individual. However, the unaffected family member might be presymptomatic and could present dementia-associated phenotypes in the future, with the age of disease onset at 56–62 years, and clinical phenotypes of disease were memory loss and psychiatric- and behavioral symptoms.<sup>35</sup> Ala434Thr (GCT→ACT) was discovered in a 38-year-old patient, who was initially misdiagnosed with schizophrenia, because she showed hallucinations and delusions, followed by memory problems and cognitive disturbances. Family history was positive, because her mother was also diagnosed with AD, but the memory impairment was the main symptom in her.<sup>12</sup>

### Mutation in *PSEN2*

In 2014, a novel *PSEN2* mutation, Asn141Tyr (AAC→TAC), was discovered in a Han Chinese family. The mutation was identified in two affected family members, who were clinically diagnosed with EOAD. The proband was a female patient who developed memory impairment at the age of 43 years. Symptoms started with the inability to handle financial matters and find personal items. Two years later, the memory impairment became worse, and she was unable to perform her job. Several of her family members (grandfather, mother, and two aunts) died with dementia. Her sister developed memory decline at the age of 49 years and later developed speech difficulties and disorientation. In the later disease stages, she developed paranoia, visual hallucinations, and agitation. The location of mutation is similar to the Volga-German mutation (Asn141Ile), and this asparagine to tyrosine change might affect the Abeta42/Abeta40 ratio.<sup>13</sup> Later, additional mutations have been described in Chinese patients. K82R (AAA→AGA) was found in a female patient at the age of 50 years. She developed AD with depression and language impairment. Atrophy was observed in the posterior region and hippocampus, and amyloid deposition appeared in different brain areas, such as frontal and parietal lobes and striatum.<sup>45</sup> Pro123Leu (CAA→CTA) was found in a Chinese family, where the pedigree revealed several affected family members over four generations. At the proband patient, disease started at the age of 57 years, with personality changes and memory problems. Cognitive impairment with parkinsonism and

myoclonic jerks were quite common phenotypes among the patients. However, mutation did not segregate with the disease, since several asymptomatic relatives also carried the mutation.<sup>46</sup> His169Asn (CAT→AAT) was identified in two Chinese individuals, who were unrelated. One of the patients had familial form of AD in her late 60s, when she started with progressive memory loss. Her brother was also diagnosed with AD. The other patient was diagnosed with sporadic FTD at the age of 62 years. His symptoms were initially language impairment and personality changes, later followed by cognitive decline.<sup>45</sup> Val214Leu (GTG→TTG) was also found in two unrelated AD patients, who developed disease phenotype in their 60s. One of the patients had familial form of AD, but segregation could not be confirmed. The second patient developed sporadic early-onset AD.<sup>45</sup>

### Mutations discovered in Japan

The genetics of EOAD in Japan has been relatively well studied, and several mutations have been identified in *APP* and *PSEN1* (Table 4). To date, no *PSEN2* mutations have been found in Japanese patients. However, silent mutations were reported.<sup>47</sup>

### Mutations in *APP*

Asp678Asn (GAC→AAC) or “Tottori APP” was described in a Japanese family, with the age of onset ranging from 59 to 65 years. The proband patient was hospitalized at the age of 65 years; she had amnesia, disorientation, and personality changes (loss of interest in activities and aggression). The symptoms started 6 years before the diagnosis. Seven years after the diagnosis, cortical hippocampal atrophy appeared, and the dementia worsened; the patient lost her ability to perform daily activities and needed assistance. The patient's sister had dementia, which started with amnesic symptoms (inability to recognize her family). Over the next 15 years, she experienced severe cognitive decline (she needed personal care) with oral dyskinesia. The patient's brother developed dementia symptoms when he was in his late 50s. The mother had no signs of dementia (died at 85 years). The father died in an accident at the age of 64 years, and it was unknown if he had any signs of dementia.<sup>48</sup> Glu693del (delAGA) was found in two Japanese families. The mutation might be involved in AD and mild cognitive impairment in homo- and heterozygous stages, respectively. The mean age of onset of AD with this mutation is 44 years. The findings suggest that the mutation makes Abeta more resistant to proteolytic degradation. In addition, Abeta in mutant rats showed a unique aggregation

Table 4 Mutations, discovered in Japan

Gene	Mutation	Was it discovered before?	Clinical phenotype	Age of onset/family history	Functional data	PolyPhen scores	SIFT scores	References
APP	Asp678Asn	No	EOAD	59–65 years/familial	Not available	0.62 (possibly damaging)	I (tolerated)	Wakutani et al <sup>46</sup>
	Glu693del	No	EOAD/MCI	44 years/familial	Enhances the toxic amyloid oligomer formation	Not applicable		Tomiya et al <sup>49</sup>
	Val717Ile	Yes, in Europe, the People's Republic of China, Thailand "London APP"	EOAD	53 years/familial	Increased Abeta42/Abeta40 ratio in CHO and HEK293 cells	0.99 (probably damaging)	0 (damaging)	Yoshioka et al <sup>50</sup>
PSEN1	Leu85Pro	No	Juvenile EOAD	26 years/de novo	Abeta42/Abeta40 ratio increased in HEK293	I (probably damaging)	0 (damaging)	Ataka et al <sup>52</sup>
	Val96Phe	No	EOAD	49–60 years/familial	2.1-fold increased Abeta42/40 ratio in COS-1 cells	I (probably damaging)	0 (damaging)	Kamino et al <sup>53</sup>
	Glu123Lys	No	EOAD, slow disease progression	56–62 years/familial	Not available	0.48 (possibly damaging)	0.46 (tolerated)	Yasuda et al <sup>54</sup>
	Ile143Thr	Yes, in France and Belgium	EOAD, myoclonus, epilepsy	26–45 years/familial	Abeta42/total Abeta increased in COS-1 cells (2.7-fold) and in HEK293 (fourfold) cells	I (probably damaging)	0 (damaging)	Arai et al <sup>55</sup>
	Thr154Asn	No	EOAD, spastic paraparesis	40–60 years/familial	Not available	I (probably damaging)	0 (damaging)	Hattori et al <sup>57</sup>
	His163Arg	Yes, in Korea and Europe	EOAD	43–50 years/five Japanese families, both familial and de novo cases	Abeta42/Abeta40 ratio increased twofold in COS-1 cell lines	0.84 (possibly damaging)	0.1 (tolerated)	Kamino et al <sup>53</sup>
	Trp165Gly	No	EOAD	34–38 years/familial	No functional data	I (probably damaging)	0 (damaging)	Higuchi et al <sup>58</sup>
	Leu173Phe	No	EOAD with depression	40–47 years/familial	Elevated Abeta42 levels and Abeta42/Abeta40 ratio in neuroblastoma cells	0.97 (probably damaging)	0 (damaging)	Kasuga et al <sup>59</sup>
	Glu184Asp	Yes, in UK	EOAD, muscle rigidity	40–44 years/familial	Not available	0.87 (possibly damaging)	0.02 (damaging)	Yasuda et al <sup>60</sup>
	Gly209Arg	No	EOAD	46–53 years/familial	Not available	I (probably damaging)	0 (damaging)	Sugiyama et al <sup>24</sup>
	Ile213Thr	No	EOAD	42–47 years/familial	Increased (1.7-fold Abeta)	I (probably damaging)	0 (damaging)	Kamino et al <sup>53</sup>
	Gly217Asp	Yes, in UK	EOAD with cotton wool plaques, parkinsonism	38–42 years/familial	Not available	I (probably damaging)	0 (damaging)	Takao et al <sup>62</sup>
	Phe237Ile	No	EOAD, spastic paraparesis	35 years/de novo	Not available	I (probably damaging)	0.09 (tolerated)	Sodeyama et al <sup>63</sup>
	Leu250Val	Yes, in Bulgaria	EOAD, myoclonus, seizures	40–51 years/familial	Not available	I (probably damaging)	0 (damaging)	Furuya et al <sup>64</sup>
	Ala260Val	Yes, in Spain and UK	EOAD, pick inclusions	27–46 years/familial	1.5-fold increased Abeta42/total Abeta in COS-1 cells	I (probably damaging)	0 (damaging)	Ikeda et al <sup>65</sup>
	Gly266Ser	No	EOAD, spastic paraparesis, aphasia	35–44 years	Not available	I (probably damaging)	0 (damaging)	Matsubara-Tsutsui et al <sup>66</sup>
	Arg269His	Yes, in France, USA, and UK	EOAD, myoclonus	46–67 years/familial	Not available	I (probably damaging)	0 (damaging)	Kamimura et al <sup>47</sup>

Glu280Ala	Yes, in Columbia ("Paisa mutation")	EOAD	46–60 years/familial	1.6-fold increased Abeta42/total Abeta ratio in COS-1 cells and 2.4-fold increased Abeta42 levels in HEK293 cells	0.999 (probably damaging)	0 (damaging)	Tanahashi et al <sup>69</sup>
Leu282Phe	No	EOAD	51 years/may be familial	Not available	0.986 (probably damaging)	0 (damaging)	Hamaguchi et al <sup>73</sup>
Pro284Leu	No	AD and spastic paresis	32 years/probable de novo	Not available	1 (probably damaging)	0 (damaging)	Tabira et al <sup>74</sup>
Ala285Val	No, but it was discovered in two Japanese families	EOAD	50 years/familial	Elevated Abeta42/total Abeta ratio in COS-1 and HEK293 cells (1.7- fold and fourfold, respectively)	1 (probably damaging)	0 (damaging)	Ikeuchi et al <sup>79</sup>
Leu286Val	Yes, in Germany	EOAD	47–48 years/familial	Elevated Abeta42/total Abeta and Abeta42/Abeta40 ratio in COS-1 and HEK293 cells	0.99 (probably damaging)	0 (damaging)	Ikeuchi et al <sup>79</sup>
Gly378Glu	Yes, in France	EOAD	37.5 years	3.2-fold increase of Abeta42/ Abeta40 ratio in HEK293	1 (probably damaging)	0 (damaging)	Ikeda et al <sup>65</sup>
Leu381Val	Yes, in Bulgaria	EOAD, spastic paraparesis	29–65 years/familial	1.9-fold increase in Abeta42/ Abeta40 levels in HEK293 cells	0.983 (probably damaging)	0 (damaging)	Ikeuchi et al <sup>79</sup>
Gly384Ala	Yes, in Belgium	EOAD	30–39 years/familial	3.8-fold increase cells in the Abeta42/Abeta40 ratio in COS-1 cells	0.99 (probably damaging)	0 (damaging)	Kamimura et al <sup>47</sup>
Leu392Val	Yes, in Italy and France	EOAD, epilepsy	30–48 years/familial	2.4-fold increased Abeta42/ Abeta40 ratio in COS-1 cells	0.99 (probably damaging)	0 (damaging)	Ikeuchi et al <sup>79</sup>
Gly405Ser	No	EOAD, angiopathy	Unknown	Not available	0.96 (probably damaging)	0.26 (tolerated)	Yasuda et al <sup>83</sup>
Ala431Val	Yes, in Mexico	EOAD	40–48 years/familial	Not available	1 (probably damaging)	0 (damaging)	Matsushita et al <sup>84</sup>
Thr440del	No	EOAD with Lewy bodies	34–35 years/familial	9.2-fold increased Abeta42/ Abeta40 levels in neuroblastoma cells	Not applicable		Ishikawa et al <sup>85</sup>
Exon9del 58304G>A	Yes, in USA	EOAD, spastic paraparesis	47.5 years mean age onset/familial	Abeta42/total Abeta increased and in HEK293 (3.2-fold) cells			Tabira et al <sup>74</sup>

**Abbreviations:** AD, Alzheimer's disease; EOAD, early-onset AD; MCI, mild cognitive impairment.



pattern, forming oligomers instead of fibrils. It was suggested that soluble Abeta oligomers might be more toxic than the fibrillar Abeta aggregates.<sup>49</sup> Val717Ile (GTC→ATC) was also found in a Japanese family.<sup>50</sup> The mean age of onset of AD with this mutation is 53 years. Symptoms start with personality changes and disturbances in memory, emotions, or judgment, but dementia is predominant.<sup>51</sup>

### Mutations in *PSEN1*

Leu85Pro (CTC→CCC) was discovered in a Japanese patient with de novo emergence of juvenile EOAD with spastic paraparesis. The age of onset was 26 years, but the patient had become withdrawn and unmotivated at an earlier age. *PSEN1* Leu85Pro was expressed in HEK293 cells, and Abeta40 and Abeta42 concentrations were measured with ELISA. Western blotting was performed to assess changes in APP expression, but no difference was found between the expression of wild-type *PSEN1* and that of mutant *PSEN1*. The secretion of Abeta42 increased, and the ratio of Abeta42/40 was higher in cells expressing mutant *PSEN1* than in controls.<sup>52</sup> Val96Phe (GTC→TTC) has been described in a Japanese family, with the age of onset ranging from 49 to 60 years. When *PSEN1* Val96Phe was expressed in COS-1 cell lines, the Abeta42/total Abeta ratio increased (1.6-fold) in the mutant cells.<sup>21,53</sup> Glu123Lys (GAG→AAG) was detected in a Japanese family, with the age of onset ranging from 56 to 62 years. Patients showed cognitive decline and memory deficits.<sup>54</sup> Ile143Thr (ATT→ACT) was described for the first time in Belgian familial EOAD patients with cognitive decline, myoclonus, and epilepsy. The mutation was associated with an increased ratio of Abeta42/total Abeta. Abeta accumulation was also high, which suggests that the mutation correlates with *PSEN1* dysfunction, leading to disease progression.<sup>55</sup> The clinical phenotype of Japanese patients with the Ile143Thr mutation was similar to that of the Belgian patients. Codon 143 might be an important locus in *PSEN1*, given that the following additional mutations have been described: Ile143Met, Ile143Phe, Ile143Asn, and Ile143Val, and all of them have been confirmed as pathogenic mutations.<sup>56</sup> Thr154Asn (TAT→AAT) has been described in a Japanese patient diagnosed with EOAD with spastic paraparesis. The first symptoms appeared in her 30s, and dementia developed when she was in her 40s. Her mother had a similar disease progression, but onset occurred later: spastic paraparesis and dementia appeared in her 40s and 60s, respectively. An additional pathogenic mutation at codon 154, Thr154Cys, has been identified.<sup>57</sup> Trp165Gly (TGG→GGG) was discovered in a Japanese family with EOAD, with the age of

onset ranging from 34 to 38 years. No detailed information is available on the patient's clinical symptoms.<sup>58</sup>

Leu173Phe (TTG→TTC) has been described in a Japanese family. The patients exhibited depression and psychiatric symptoms before dementia progression. When Leu173Phe was expressed in neuroblastoma cells, the level of Abeta42 and the Abeta42/Abeta40 ratio increased.<sup>59</sup> Glu184Asp (GAA→GAC) was found in a Japanese family with EOAD (age of onset at 40–44 years). The family members showed typical dementia symptoms, such as memory impairment, muscle rigidity, and disorientation. Disease duration was  $\sim 8.7 \pm 1.5$  years. Three of the four patients with the mutation died at the age of 51 years. Postmortem examinations revealed gliosis, a high degree of neurodegeneration, senile plaques, and neurofibrillary tangles. The degree of amyloid angiopathy in the brain parenchyma and the leptomeninges regions was higher than in a typical AD patient.<sup>60</sup> The mutation has also been described in a Caucasian family (in the UK) with EOAD and a similar age of onset.<sup>61</sup> Gly209Arg (GGA→AGA) has been reported in a Japanese family, with the age of onset at 45–53 years. The symptoms were memory impairment, amnesic aphasia, disorientation, and personality change, without parietal focal symptoms, such as apraxia and agnosia. The mutation results in the exchange of small, hydrophobic glycine with large, polar arginine. The different phenotypes might be the result of different pathomechanisms, other genetic factors, or genetic–environmental interactions.<sup>24</sup> Ile213Thr (ATT→ACT) was described in a Japanese family with EOAD, with the age of onset ranging from 42 to 48 years. When *PSEN1* Ile213Thr was expressed in COS-1 cells, the Abeta42/total Abeta ratio increased (1.7-fold), but no detailed information was available on its pathogenic nature.<sup>21,53</sup> Gly217Asp (GGT→GAT) was described in a Japanese family with EOAD and parkinsonism. Neuropathological changes, such as amyloid plaques, “cotton wool plaques”, amyloid angiopathy, tangles, and gliosis, were also detected.<sup>62</sup> Phe237Ile (TTT→ATT) has been described in a Japanese patient with EOAD and spastic paraparesis without any family history. The first symptoms appeared when he was 31 years. Memory impairment and cognitive dysfunctions developed rapidly. Spastic paraparesis appeared when he was 35 years.<sup>63</sup> Leu250Val (TTG→GTG) was found in a Japanese family and in a Caucasian (Bulgarian) family. In the patients, EOAD appeared with myoclonus (with tonic–clonic seizures), with the age of onset ranging from 40 to 51 years. Additional *PSEN1* mutations, such as Leu153Val, Leu250Ser, and Glu280Ala, were associated with myoclonus, but tonic–clonic seizures appear only in

patients with Leu250Val.<sup>64</sup> Ala260Val (GCT→CCT) has been described in European and Japanese EOAD patients, with the age of onset ranging from 27 to 46 years. In the first Japanese pedigree (three patients), neuropathological studies detected senile plaques, tangles, neuronal loss, and Pick-like inclusions. In the second pedigree, the disease onset occurred later (mean, 51 years), but disease progression was faster.<sup>65</sup> When *PSEN1* Ala260Gly was expressed in COS-1 cells, the Abeta42/total Abeta ratio increased (1.5-fold).<sup>21</sup> Gly266Ser (GGT→AGT) was reported in six patients in two generations of a Japanese family. Patients were diagnosed with AD, spastic paraparesis, and aphasia. The affected family members showed rapid disease progression after the age of 50 years. Diagnosis was complicated because the patients did not show typical AD symptoms, since tauopathy and prion disease-like symptoms were present. The patients were negative for mutations in the prion (*PRNP*) and Tau (*MAPT*) genes, suggesting that they had an AD variant.<sup>66</sup> Arg269His (CGT→CAT) has been associated with EOAD and LOAD in American, European, and Japanese families. The mean age of disease onset is 50.5 years. The mutation was described by Gómez-Tortosa et al<sup>67</sup> in an early dementia patient, with amyloid deposition particularly strong in the temporal cortex. Larner et al reported Arg269His in a patient with a positive family with LOAD. The examined patient exhibited the first symptoms of memory disturbances when he was 66 years of age.<sup>68</sup> Her sister was also diagnosed with AD. Their grandmother, mother, and several aunts (5/6) were also diagnosed with AD or dementia. A few additional mutations were found in patients with LOAD. The study suggested that patients with LOAD should be tested for the main EOAD risk factor genes.<sup>68</sup> Glu280Ala (GAA→GCA) or “Paisa mutation” has been detected in Japanese, European, and Columbian patients,<sup>69</sup> with the age of onset ranging from 30 to 45 years. In Japan, Glu280Ala has been detected in familial AD cases.<sup>69</sup> In Columbian families, it segregated with the disease. Glu280Ala might be independent from APOE alleles.<sup>70</sup> *PSEN1* Glu280Ala was expressed in different cell lines, including COS-1,<sup>21</sup> N2a,<sup>71</sup> and HEK293.<sup>72</sup> The experiments established the pathogenic nature of the mutation. In COS-1 cells, the ratio of Abeta42/total Abeta (1.6-fold) increased. In HEK293 cells, the level of Abeta42 (2.4-fold) and the ratio of Abeta42/Abeta40 (2.1-fold) increased; no change was detected in the Abeta40 levels. In N2a cells, the amount of Abeta42 (2.7-fold) and the ratio of Abeta42/Abeta40 (2.4-fold) increased significantly, and a small increase in the level of Abeta40 (1.3-fold) was detected.<sup>21,71,72</sup> Leu282Phe (CTT→TTT) was identified in a Japanese family, with the

main age of onset at 51 years. Patients were diagnosed with progressive memory impairment. Given the family history, this might be a case of familial AD. The following two other mutations have been found at codon 282: Leu282Arg and Leu282Val. Both are associated with EOAD.<sup>73</sup>

Pro284Leu (CCA→CTA) was identified in a Japanese patient with AD and spastic paresis. The first movement problems started at the age of 32 years and became more serious. She died when she was 54 years. Postmortem studies found several cotton wool plaques and neurofibrillary tangles or amyloid angiopathy in her brain. Kuru-like plaques also appeared, but the prion-staining was negative.<sup>74</sup> Ala285Val (GCT→GTT) has been described in two Japanese families, with the mean age of onset at 50.5 years. Patients did not show any sign of myoclonus, seizures, or paratonia. Increased Tau levels were found in the cerebrospinal fluid (CSF) of patients.<sup>75</sup> Ikeda et al have also reported this mutation.<sup>65</sup> The age of onset was similar, but disease progression was rapid. When *PSEN1* Ala285Val was expressed in COS-1 cells, the Abeta42/total Abeta ratio increased (1.7-fold).<sup>21</sup> When expressed in HEK293 cells, the Abeta42/total Abeta ratio increased (fourfold), and the Abeta40/total Abeta and Abeta38/total Abeta ratios decreased (1.3-fold and twofold, respectively).<sup>76</sup> Leu286Val (CTC→GTC) has been described in a Japanese patient and in a Caucasian (German) family with EOAD, with the mean age of onset at 47 and 48 years, respectively.<sup>77,78</sup> When *PSEN1* Leu286Val was expressed in COS-1 and HEK293 cell lines, increases in the Abeta42/total Abeta ratio (1.5-fold) and Abeta42/Abeta40 ratio (2.1-fold) were detected, respectively.<sup>21,77</sup> Gly378Glu (GGA→GAA) was initially described in France<sup>78</sup> and in Japan,<sup>79</sup> with the mean age of onset at 37.5 years, and all patients were diagnosed with AD. Cerebral amyloid angiopathy also appeared in some patients. When *PSEN1* Gly378Glu was expressed in HEK293 cells, the Abeta42/Abeta40 ratio increased (3.2-fold).<sup>74</sup> Leu381Val (CTT→GTT) was first described in a Bulgarian family. The patients were diagnosed with AD and spastic paraparesis, with the mean age of onset at 30.5 years. Abeta-positive cotton wool plaques appeared in the brain.<sup>76</sup> In Japan, the mutation was found by Ikeuchi et al.<sup>79</sup> When *PSEN1* Leu381Val was expressed in HEK293 cells, the Abeta42/Abeta40 ratio increased (1.9-fold).<sup>79</sup> Gly384Ala (GGA→GCA) was initially discovered in a Caucasian family, with the age of onset ranging from 30 to 39 years.<sup>56</sup> In Japan, the mutation has been described in a family, with the age of onset ranging from 31 to 37 years. Autopsy confirmation suggested that the patients had AD: they had senile plaques and tangles inside their brain.<sup>69</sup>

The mutation has been analyzed in several studies. When PSEN1 Gly384Ala was expressed in COS-1 cells, significant increases (3.8-fold) in the Abeta42/Abeta40 ratio were detected.<sup>21</sup> When expressed in CHO cells, the Abeta42/total Abeta ratio increased (3.8-fold),<sup>30</sup> while in HEK293 cells, Abeta40 and the Abeta42/Abeta40 ratio decreased and increased significantly, respectively.<sup>76,80,81</sup>

Leu392Val (CTG→GTG) has been reported in several cases, with the mean age of onset at 42.5 years.<sup>17,79,82</sup> Transfection into COS-1 cells increased the Abeta42/Abeta40 ratio (2.4-fold).<sup>16</sup> An increase in the Abeta42/Abeta40 ratio (2.9-fold) was also detected after transfection into HEK293 cells.<sup>79</sup> Asn405Ser (AAC→AGC) was found in one patient whose family history was unknown. The patient showed rapid and aggressive AD progression. The mutation was absent in healthy controls. The patient has several senile plaques and tangles in the brain (temporal cortex), but cerebral amyloid angiopathy also appeared in several areas. The mutation caused disturbances in the motor neuronal systems, leading to spastic paraparesis.<sup>83</sup> Ala431Val (GCA→GTA) was found in a Japanese patient who was first diagnosed with mild cognitive impairment, followed by AD onset in the next 16 months. Tau and phospho-Tau levels increased in the CSF, and metabolic deficits were detected in several parts of the brain, including the posterior and medial temporal regions.<sup>84</sup> T440del (delACC) was found in a 52-year-old male patient with early-onset dementia and parkinsonism. The family history was positive: the father and grandfather also suffered from a similar type of dementia. A serious degree of neuronal loss was detected in the brain; cotton wool plaques, cerebral amyloid angiopathy, and Lewy bodies were also present.<sup>85</sup>

Deletion of exon 9 is associated with a point mutation, 58304G→A, located in the splice acceptor consensus site in intron 8. This variant can affect the PSEN1 transcript splicing,

by in-frame skipping of exon 9 and the junction of exon 8 and 9. This mutation was discovered in Japanese and Caucasian patients, resulting in EOAD with spastic paraparesis, with the mean age of onset at 47.5 years. Cotton wool plaques were also detected in the brain of patients. Mutation was expressed in HEK293 cell lines, where elevated Abeta42 levels and Abeta42/40 ratio were detected.<sup>74</sup>

## Mutations discovered in other Asian countries

With the exception of Korea, the People's Republic of China, Taiwan, and Japan, only a few reports are available on EOAD-associated mutations in Asian countries. Two *APP* mutations were discovered in Thailand and Iran, and recently, a novel *PSEN1* mutation was reported in a Malaysian family (Table 5).

In Iran, an *APP* mutation, Thr714Ala (ACA→GCA or "Iranian APP"), has been reported in an autosomal dominant case of AD, with the mean age of onset at 55 years, but no detailed information was available regarding the clinical phenotypes.<sup>83</sup> This mutation has also been reported in two other families. One of the patients, from Denmark, had atypical signs of AD; epilepsy also appeared. His father was a suspected AD patient, but the diagnosis was unclear. The age of onset ranged from 47 to 55 years. It has also been detected in a Polish (Caucasian) family, with the age of onset at 44–45 years. The symptoms were memory impairment, disorientation, changes in behavior, and aphasia.<sup>86,87</sup> Val717Ile (GTC→ATC; London APP) has been identified in one Thai family, but no detailed information on the patients was available.<sup>37</sup>

No additional reports are available regarding EOAD-associated mutations in other East Asian countries. Studies of Malaysian patients have started recently. *PSEN1* Glu280Lys

**Table 5** Mutations, discovered in other Asian countries

Gene	Mutation	Was it discovered before?	Clinical phenotype	Age of onset	Functional data	PolyPhen2 scores	SIFT scores	References
APP	Thr714Ala	No, but it was discovered in Poland later	EOAD, epilepsy	47–55 years/familial	Not available	1 (probably damaging)	0.01 (damaging)	Finkch et al <sup>37</sup>
	Val717Ile (Thailand)	Yes, in Europe, the People's Republic of China, Japan	EOAD	54 years/unknown	Increased Abeta42/Abeta40 ratio in CHO and HEK293 cells	1 (probably damaging)	0 (damaging)	Pasalar et al <sup>86</sup>
PSEN1	Glu280Lys (Malaysia)	No	EOAD, parkinsonism	48–57 years/familial	Not available	0.99 (probably damaging)	0 (damaging)	Chng et al <sup>88</sup>

**Abbreviation:** EOAD, early-onset Alzheimer's disease.

(GAA→AAA) is a novel mutation, recently discovered by our research group in three siblings from Malaysia who were diagnosed with early-onset dementia. Strong evidence indicates that this mutation is involved in AD or dementia; it was found in three patients who likely had a positive family history of dementia. The existence of two additional pathogenic mutations at codon 280 (Glu280Gly and Glu280Ala) indicates that Glu280 is an important residue in PSEN1.<sup>88</sup>

## Asian GWAS of LOAD

As mentioned before, the genetic background of LOAD is not well defined. Several genes have been proposed as risk factors for LOAD. In addition, nongenetic factors (environmental and lifestyle) could also play a role in AD that develops after the age of 65 years.<sup>89</sup> Several meta-analyses and GWAS have been performed to identify genes and sequence variants that increase the risk for LOAD. These studies tested several mutations and polymorphisms by using high-throughput assays.<sup>90</sup>

*APOE* has three main alleles, with variations at codons 112 and 158. In the normal allele, called the E3 allele, codon 112 encodes cysteine, and codon 158 encodes arginine. The other two *APOE* alleles, E2 and E4, contain cysteine and arginine, respectively, at both sites. Several studies have confirmed the strong association between the E4 allele and AD.<sup>91</sup> ApoE protein is the main cholesterol transporter in the brain; by binding to the Aβ peptide, it might contribute to AD progression. ApoE might inhibit Aβ clearance and induce neuroinflammation. The *APOE* E4 allele might be involved in LOAD, but it might not define all LOAD cases.<sup>91</sup>

Association studies were performed to find additional risk factor genes for AD. In Korea, Chung et al have performed GWAS; they found an association between AD and variants in *PICALM*, *CR1*, and *BIN1*.<sup>92,93</sup> In the People's Republic of China, several reports have described the relationship between different genes and LOAD. Jin et al have studied the *CR1* gene and found an association between a polymorphism (rs6656401) and AD in the Han Chinese population.<sup>94</sup> Liu et al have analyzed variants of several genes in Han Chinese subjects.<sup>95</sup> Associations were found between LOAD and variants of three genes, *MOBP* (rs1768208), *EIF2AK3* (rs7571971), and *MAPT*, in the presence of the *APOE* E4 allele.<sup>94</sup> Tan et al have studied the MS4A and CD33 loci in the northern Han population and suggested that both loci might be involved in LOAD.<sup>96</sup> Liu et al have performed GWAS on rs744373 in *BIN1* and rs3851179 in *PICALM* in East Asians,<sup>97,98</sup> and both genes showed a significant association with AD. Shang et al found an association

between a variant (rs597668) of *EXOC3L2* and AD.<sup>99</sup> Several additional studies have reported associations between AD and variants of different genes, such as *PLD3*,<sup>100</sup> *SORCS1*,<sup>101</sup> *NLRP3*,<sup>102</sup> and *ARRB2*.<sup>103</sup> A few genetic studies have also been published in Japan on GWAS and different genes that might be involved in LOAD. *PICALM* and *SORL1*<sup>104,105</sup> might contribute to LOAD. However, other Japanese studies have failed to find an association between AD and other genes, such as *TREM2*,<sup>106</sup> *CLU*,<sup>107</sup> *GAB2*,<sup>108</sup> and *PGBD1*.<sup>109</sup>

## Methods for genetic testing: from PCR-based methods to NGS

Genetics plays an important role in AD onset; several genes were suggested to be involved in disease onset. To identify the genes and mutations involved in AD progression, we should know the direct sequence of the causative and risk factor genes for the disease. Direct sequencing should be performed for the mutations in the following three main causative genes: APP, PSEN1, and PSEN2. Genomic DNA can be extracted from blood, tissues, and bone marrow, and specific DNA isolation kits have been developed for it. To identify the variants in them, PCR and standard sequencing methods can be performed.<sup>3</sup> However, the mutations in these genes are relatively rare, and there may be similarities between the phenotypes of different neurodegenerative disorders. For the different types of early-onset dementia (such as frontotemporal dementia, prion diseases, and Parkinson's disease), the phenotypes may be similar especially in the earlier stages. Pathological overlap may be possible, which makes the disease diagnosis more difficult.<sup>110</sup>

AD is one of the most complex disorders, in which several genes were identified to be involved, especially in the late-onset disease form. Even APOE is the main susceptibility factor for LOAD, but it does not define all cases of AD, which occur after the age of 65 years. As mentioned earlier, the genetic background of LOAD is currently unclear. Standard sequencing is still a widely used technology, but several problems are available in this approach, such as the cost, the error rates are relatively high, and it is time-consuming. Several genes and polymorphisms have been identified as risk factors for AD by GWAS.<sup>3</sup> Recently, NGS approaches have been developed, which could provide a more accurate, fast, and cost-effective process to discover additional AD causative or risk factor genes. Several types of technologies are available, such as sequencing by synthesis (SBS), SBS by novel nucleotide analogs, homopolymer sequencing, double-ended sequencing, polony sequencing, single-nucleotide sequencing, and sequencing by hybridization or ligation.<sup>111</sup> These



approaches are useful in the searching for genes with Mendelian inheritance pattern, and they are also able to identify the common variants, which might be associated with the sporadic forms of AD.<sup>112</sup> NGS can also be useful to analyze the transcriptomic and epigenetic changes, associated with the disease, and they might be helpful to understand the disease-associated pathways.<sup>113</sup>

## Estimation of role of novel mutation

To verify the pathogenic nature of mutations in the EOAD causative genes, Guerreiro et al designed an algorithm. Mutations can be categorized as definitely pathogenic, probably pathogenic, possibly pathogenic, or nonpathogenic (putative risk factor) variants. In this study, segregation analysis was performed, and the affected family members should also be tested for the mutation. They also examined the residue, whether any additional mutation was found for it. Association studies are needed, since it was also important to define the pathogenic nature. Mutations should be screened in large number of healthy individuals. If the mutation appears in them, it might refute its pathogenic nature; however, it could be possible that these individuals could develop disease phenotype in the future.<sup>114</sup> In Korea, the Korea Centers for Disease Control and Prevention (KCDC; <http://www.cdc.go.kr>) performed whole genome sequencing in 622 healthy individuals and screened them for pathogenic mutations. The Exome Aggregation Consortium database (<http://exac.broadinstitute.org/>) was designed for the same reason; they performed exome sequencing on >60,000 healthy individuals. This database is an easy-to-use database, which could be used for clinical and population genetic studies.

Guerreiro et al<sup>115</sup> also suggested checking whether the mutations could affect the Abeta levels. Several mutations in *PSEN1* and *PSEN2* were tested in cell models, such as COS-1, N2A, and HEK293 cells, and coexpressed with *APP* to determine whether this mutation could result in increased Abeta42, decreased Abeta40 levels, or increased Abeta 42/Abeta40 ratio.<sup>21,53</sup> These cell models could accurately determine the role of mutation in the disease progression. However, these cell model studies are expensive and time-consuming and were not performed for all discovered mutations (Tables 2–5).

In silico modeling is important, especially at the novel mutations, since it would be helpful to get hypothetical insight on the role of abnormal protein in disease progression. Recently, online prediction programs, such as PolyPhen2 and SIFT, were developed to estimate the damaging nature of mutations, by comparing several structure and function-based properties of proteins.<sup>116–118</sup> In addition, three-dimensional

modeling could also be helpful to estimate the role of mutations in disease progression. However, depending on the in silico predictions is not sufficient for full clinical prognosis, but these predictions could enhance the estimation of the pathogenic nature of mutations.<sup>119</sup>

## Conclusion

Several neurodegenerative disorders can be distinguished, such as AD, FTD, amyotrophic lateral sclerosis, Parkinson's disease, dementia with Lewy bodies, Huntington's disease, and prion diseases. Currently, there is no effective treatment for neurodegenerative dementia, but potential therapeutic approaches might be successful in early disease stages. The main problem is that disease diagnosis, especially differential diagnosis, before the appearance of clinical symptoms is complicated. There is no unique marker for each disorder. However, combining proteomic and genetic markers might improve disease diagnosis. Determining the genetic background of dementia would also improve the accuracy of disease diagnosis. Several genes have been described as causative or risk factor genes for dementia.<sup>3,115</sup>

Several genetic studies on EOAD patients were performed in Japanese, Korean, and Chinese populations. Similar studies have not been investigated yet in South Asian countries (Thailand and Malaysia). Hence, our investigators may start multinational efforts for screening AD-related mutations in collaborations with them. Comparing the EOAD-associated mutations between Caucasian and Asian patients, >30 novel Asian mutations were found in *APP*, *PSEN1*, and *PSEN2* (<http://www.alzforum.org/mutations>). In *APP* gene, novel mutations were discovered in Japanese or Chinese/Taiwanese patients, such as Asp678Asn (Tottori *APP*), Ile718Leu, and Leu720Ser. Val715Met (French *APP*) and the Val717Ile (London *APP*), which were initially discovered in British or French Caucasians, were also detected in Korean and Japanese EOAD patients, respectively.<sup>12,14,36,48</sup> In *PSEN1*, several novel mutations have been discovered in Korean, Malaysian, and Chinese EOAD patients, such as His163Pro, Leu232Pro, Leu248Pro, and Glu280Lys.<sup>12,23,41,88</sup> *PSEN1* H163R, the most frequently described AD causative mutation among French, German, and Turkish Caucasians, has appeared in Korea and Japan.<sup>22,47,53,69</sup> Additional mutations, such as Leu226Phe, Thr116Ile, Met233Thr (Korea), Ile143Thr, and Arg269His (Japan), were also reported in Korea and Japan.<sup>16,17,27,28,55,66–68</sup> In the People's Republic of China, most of the *PSEN1* variants, such as Ile167del, Ser169del, and Leu248Pro, were newly discovered.<sup>12,43,44</sup> *PSEN2* mutation was rare in



Asian countries; however, novel Val214Leu, His169Asn, and Asn141Tyr mutations were found. PSEN2 Arg62Cys mutation, which was initially discovered in Belgian Caucasians, was found among Korean.<sup>31–34,46</sup>

Because the overall population and aging population in most Asian countries are increasing, genetic testing of patients with AD and other types of dementia is important in the diagnosis of dementia. Several genes could be directly or indirectly involved in the onset of AD. These genes should be tested, and other candidates should be identified in order to obtain a clearer understanding of the cascade associated with disease progression. It was suggested that neurodegenerative disorders arise from a common molecular pathway. Clinical diversity might be the result of different genetic mutations that directly or indirectly affect disease progression or protect against the disease. However, determining the initial disease-associated process is also a key issue for diagnosis. Screening to identify potential candidate genes associated with the disease is important.<sup>112,120</sup> NGS technologies can facilitate the discovery of novel disease-associated genes and identify candidate genes involved in the onset of EOAD or LOAD. Simultaneous analysis of different genes may improve differential diagnosis, which is essential in the development of preventative and therapeutic strategies. Genetic profiling could also help the disease risk prediction, since it might allow the start of prevention strategies in the presymptomatic stages.<sup>110</sup>

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## Disclosure

The authors report no conflicts of interest in this work.

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