Butyrylcholinesterase Protein Ends in the Pathogenesis of Alzheimer's Disease—Could *BCHE* Genotyping Be Helpful in Alzheimer's Therapy?

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Abstract: Late-onset Alzheimer's disease (AD) is clinically characterized by a progressive decline of memory and other cognitive functions leading to the loss of the ability to perform everyday activities. Only a few drugs have been approved to treat AD dementia over the past century since the first AD patient was diagnosed. Drugs increasing the availability of neurotransmitters at synapses in the brain are used clinically in the treatment of AD dementia, and cholinesterase inhibitors (ChEIs) are the mainstay of the therapy. A detrimental effect on cognitive function has been reported in patients with pharmacological inhibition of acetylcholinesterase (AChE) by ChEIs and reduced butyrylcholinesterase (BChE) activity due to the single nucleotide polymorphisms. The BChE K-variant (rs1803274), the most common genetic variant of the *BCHE* gene, was thought to reduce enzyme activity reflecting the lower clinical response to rivastigmine in AD patients. During ChEIs therapy, patients carrying reduced-activity BChE do not present such improved attention like patients with the wild-type enzyme. On the other hand, alterations in the *BCHE* gene causing enzyme activity reduction may delay AD onset in patients at risk by preserving the level of cortical acetylcholine (ACh). Based on our previous results, we conclude that SNPs localized outside of the coding sequence, in 5′UTR (rs1126680) and/or intron 2 (rs55781031) of the *BCHE* gene, but not solely K-variant alteration (p.A539T) itself, are responsible for reduced enzyme activity. Therefore, we suspect that not BChE-K itself, but these coexisting SNPs (rs1126680 and rs55781031), could be associated with deleterious changes in cognitive decline in patients treated with ChEIs. Based on the results, we suggest that SNPs (rs1126680) and/or (rs55781031) genotyping should be performed to identify subjects at risk for lowered efficacy ChEIs therapy, and such patients should be treated with a lower rivastigmine dosage. Finally, our sequence analysis of the N-terminal end of N-BChE revealed evolutionarily conserved amino acid residues that can be involved in disulfide bond formation and anchoring of N-BChE in the cell membrane.

Keywords: Alzheimer's disease; butyrylcholinesterase; pseudocholinesterase; K-variant; rivastigmine; BChE; BuChE

1. Alzheimer's Disease (AD)

Alzheimer's disease (AD), a neurodegenerative disorder characterized by cognitive decline, is the most common form of dementia in elderly individuals. The clinical expression of AD correlates with

synaptic damage accompanied by neuronal loss in the brain, particularly in the hippocampus and cerebral cortex [1–4].

The are many hypotheses regarding the primary cause of AD including cholinergic neuron damage, the accumulation of proteins such as amyloid-β (Aβ) in plaques and hyperphosphorylated-tau in neurofibrillary tangles leading to massive loss of synapses, inflammation, oxidative stress, and others [5–7].

By definition, age is the most potent risk factor for late-onset AD [8], followed by the strongest genetic risk factor, the presence of the *APOE-ε*4 allele [9]. Multiple other genetic and environmental factors seem to be involved in late-onset AD pathogenesis. Many studies have shown that an imbalance between the production and clearance of amyloid-β (Aβ) forming amyloid plaques is probably a significant contributor to neurodegeneration and disease development [10,11].

The biochemical investigation of the brains of patients with AD revealed deficits in the enzyme responsible for the levels of ACh. These studies led to the formulation of the cholinergic hypothesis of AD [1,12]. The oldest hypothesis suggests that degeneration of cholinergic neurons in the basal forebrain and the associated loss of cholinergic neurotransmission in the cerebral cortex initiates the progression of cognitive and behavioral dysfunctions seen in patients with AD [13–15]. The hypothesis became more convincing when cholinesterase inhibitor therapies were shown to be effective in the improvement of main cognitive functions in patients with AD [16].

The concentration of ACh at synapses of cholinergic neurons is a function of its synthesis and hydrolysis, which is controlled by choline acetyltransferase (Chat) and cholinesterases (ChE), respectively. Mammalian brains contain two sister cholinesterase enzymes: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE mainly participates in cholinergic neurotransmission by hydrolyzing ACh, but BChE can also hydrolyze it [17]. BChE serves as an endogenous bioscavenger and acts as the first line of defense against toxic compounds, that might inhibit AChE activity [18,19]. Besides, BChE can compensate for AChE in the AChE-knockout mouse [20,21].

In the brain, BChE is found in glia and white matter. Nevertheless, the protein is also associated with neurons, particularly in the hippocampus, amygdala, and thalamus [22,23]. BChE was also found in amyloid plaques and neurofibrillary tangles (NFTs), which suggests that the protein may be involved in the pathogenesis of AD [24–26]. Hybrid AChE/BChE tetramers, composed of AChE and BChE dimers, have been found during the development of the brain [27].

2. Donepezil and Rivastigmine

Cholinesterase inhibitors are the first-line drugs in the treatment of AD. They inhibit the hydrolysis of acetylcholine by AChE and BChE and, therefore, prolong ACh activity at cholinergic synapses [28–30]. Currently, the most prescribed FDA-approved cholinesterase inhibitors for the treatment of AD dementia are donepezil, rivastigmine, and galantamine [31]. All these drugs slow down the symptoms of AD dementia but do not have any effect on neurodegeneration. Donepezil is the most commonly prescribed drug for the treatment of cognitive signs of AD dementia. It is reversible mixed competitive and noncompetitive AChE inhibitor that acts mainly on AChE and shows no significant activity against BChE.

Rivastigmine presents an equal affinity for both AChE and BChE enzymes with a pseudo-irreversible mode of inhibition. It is the only ChEI that produces sustained inhibition without any significant increase in the expression of the target enzymes [32]. Non-carbamylating ChEIs (donepezil, galantamine, and tacrine) increases acetylcholinesterase (AChE) protein expression in patients, whereas the carbamylating agent, rivastigmine, acts with no significant change in AChE protein expression [33,34]. The phenanthrene alkaloid galantamine is a reversible, competitive AChE inhibitor and may act as allosteric modulator of nicotinic acetylcholine receptors. Galantamine, similar to donepezil, shows no significant activity against BChE [35].

3. BChE-K Variant

BChE-K substitution (c.1699G>A, p.A539T, rs1803274) is the most common genetic variant detected in the *BCHE* gene. It was presented that K-variant is associated with the lower level of BChE molecules in plasma or with decreased activity caused by the impaired quaternary organization of the tetramer [36]. However, other findings demonstrated that the recombinant K-variant had the same activity and stability as wild-type BChE [37]. We have previously reported that compound SNPs localized outside the coding sequence in 5′UTR and/or intron 2 of the *BCHE* gene, but not solely p.A539T substitution, are responsible for reduced enzyme activity in K allele carriers [38,39].

The BChE-K variant is a long-debated risk factor for AD. It was shown that the K variant was considerably less effective in attenuating the accumulation of Aβ fibrils than BChE wild-type [36,40]. The association between BChE-K variant and AD risk was intensively debated and studied, but no definitive correlation was established since some of the previous results supported the idea [41,42], while other researchers remain doubtful [43,44]. Although many of these studies are based on a meta-analysis, GWAS (genome-wide association study) and cohort study, the results are still inconsistent, and the role of BChE in AD pathology remains unexplained [45,46]. However, our recent study has shown that the BChE-K variant protein with SNP (rs1126680) results in the alteration (p.C-11Y) of 69 amino acid signal peptide, designated N-BChE (the product of K3 haplotype), may play a role in the development of AD [47].

It has been shown that ChEIs therapies have reduced efficacy in dementia patients with BCHE-K variant due to lower enzyme activity. Rivastigmine therapy has been reported to be less effective in AD individuals with *BCHE-K*, especially in the presence of APOE4 [48–50]. AD patients with wild-type BChE receiving rivastigmine showed significantly greater treatment responses than patients receiving donepezil. In contrast, BChE K carriers experienced similar effects of therapy with both agents, although adverse events were more frequent in a group of rivastigmine-treated patients.

It was also reported that second ChEI, donepezil can have an adverse effect on cholinergic synapses and therefore on the cognitive function if given to mild cognitive impairment (MCI) patients carrying the K- variant of BChE and particularly among *APOE-ε*4 carriers [51].

It is supposed that pharmacological inhibition of AChE by donepezil and BChE inhibition due to the missense polymorphisms in BChE K-variant carriers leads to an overload of ACh [52], which has a deleterious effect on the cognitive function. On the other hand, alterations in the *BCHE* gene decreasing enzyme activity may delay the onset of disease in patients at risk of AD by preserving the level of cortical ACh.

Studies reporting the influence of BCHE-K genotype on ChEI response are contradictory. Some reports did not find a role of the BChE-K variant on the efficacy of treatment with ChEI [53]. On the other hand, Han et al. concluded that the BChE-K allele was a significant predictor for a poor response to ChEI [50]. We hypothesize that a similar inconsistency was observed in the case of the influence of BCHE-K variant on the enzyme activity. Our recent results have shown that not all BChE-K carriers have lowered enzyme activity, but only individuals with accompanied genetic variants (rs1126680) and/or (rs55781031) designed as the K3 haplotype [39]. Moreover, by analogy, the negative effect of BChE-K carriers on the response to ChEI therapies could only be observed in patients with the K3 haplotype. However, a clinical study should be performed to confirm this hypothesis.

Therefore, we recommend performing molecular testing of *BCHE* SNPs (rs1126680) and/or (rs55781031), instead of BChE-K substitution (rs1803274) in all patients before therapy to identify individuals who are at risk of reduced efficacy of treatment with ChEIs. Moreover, in a group of patients with rs1126680 and rs55781031, the prescription of a lower dosage of rivastigmine or donepezil should be considered to avoid the overload of ACh at synapses [51].

4. The N-Terminal Extended BChE Fragment

Intriguingly, amino acid sequence analysis (BLASTP) revealed that N-terminal extended BChE fragment is abundant in mammals. This extended part of BChE is evolutionarily conserved, suggesting

that it can play an important role during brain development [27]. Moreover, the only difference between human and chimpanzee at N-terminal BChE end is located at the position rs1126680, resulting in a substitution of cytosine to tyrosine. An analysis of more than 50 BChE sequences of the closest living relatives showed that only human has cysteine at the position of the SNP (rs1126680) (Figure 1). In our study, we have shown that only the N-extended BChE with alteration p.C-11Y is produced in homozygotes. However, it cannot be excluded that also the N-extended wild-type protein may exist in human cells [47].
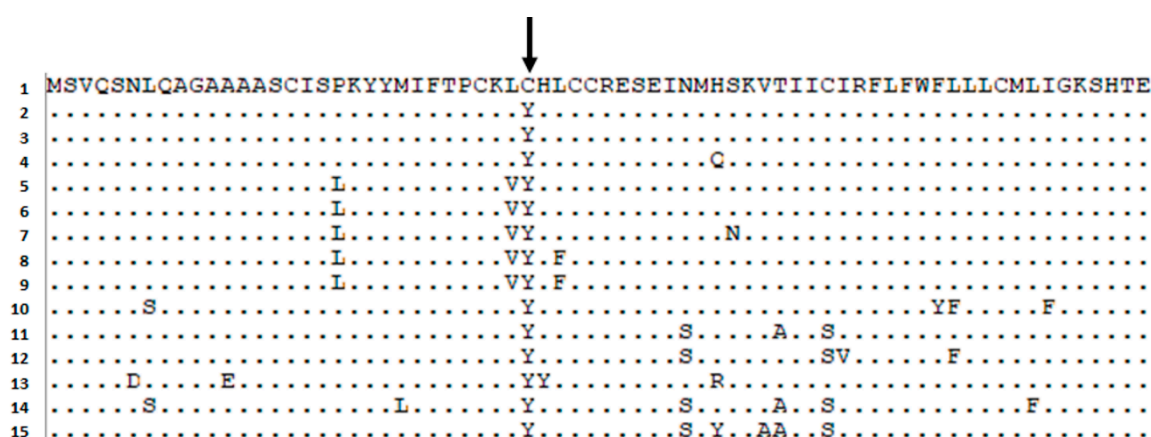

Figure 1. Evolutionary conservation of the N-terminal extended 70 aa. BChE fragment. Position of p.C-11Y (rs1126680) (or p.C31Y counted from the start of the protein) is marked by a black arrow. BLASTP search of BChE proteins of the 14 closest living relatives: 1—(*Homo sapiens*) EAW78592.1; 2—(*Pan troglodytes*) XP_516857.2; 3—(*Pan paniscus*) XP_003817746.1; 4—(*Gorilla gorilla gorilla*) XP_018880258.1; 5—(*Chlorocebus sabaeus*) XP_007970394.1; 6—(*Colobus angolensis palliatus*) XP_011800018.1; 7- (*Piliocolobus tephrosceles*) XP_023069278.1; 8—(*Rhinopithecus bieti*) XP_017745933.1; 9—(*Rhinopithecus roxellana*) XP_010353594.1; 10—(*Nomascus leucogenys*) XP_003256448.1; 11—(*Cebus capucinus imitator*) XP_017358747.1; 12—(*Callithrix jacchus*) XP_002807699.3; 13—(*Pongo abelii*) XP_009237773.2; 14—(*Saimiri boliviensis boliviensis*) XP_003925023.1; 15—(*Aotus nancymaae*) XP_012302784.1.

A change of tyrosine to cysteine in the protein sequence may introduce potential covalent disulfide bond that could influence the structure of the N-terminal end of BChE. Our secondary structure predictions revealed the presence of three putative alpha-helical regions in the extended N-BChE end that can be linked by three disulfide bonds (Figure 2). Moreover, we assume the N-terminal extension of the BChE may play a role as an anchor that can bind the protein into cellular membranes as it was shown for the N-terminally extended N-AChE [54].
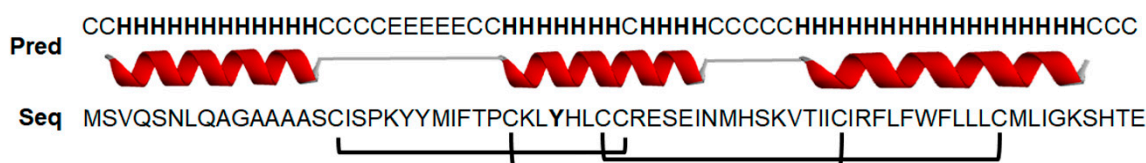

Figure 2. Prediction of the secondary structure of the N-terminal extended 70 aa. BChE fragment with p.C-11Y (rs1126680) (or p.C31Y from the start of the protein) using PSIPRED server [55,56]. H = helix, E = strand, and C = coil. The cysteine residues can form disulfide bridges leading to link and stabilization of 3 helical regions (black parentheses). Disulfide bonds formation between the cysteine residues were predicted using DISULFIND server [57].

References

1. Davies, P. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **1976**, *308*, 1403. [CrossRef]

2. Serrano-Pozo, A.; Frosch, M.P.; Masliah, E.; Hyman, B.T. Neuropathological Alterations in Alzheimer Disease. *Cold Spring Harb. Perspect. Med.* **2011**, *1*, a006189. [CrossRef] [PubMed]

3. Ball, M.J. Neuronal loss, neurofibrillary tangles and granulovacuolar degeneration in the hippocampus with ageing and dementia. *Acta Neuropathol.* **1977**, *37*, 111–118. [CrossRef] [PubMed]

4. West, M.J.; Coleman, P.D.; Flood, D.G.; Troncoso, J.C. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. *Lancet* **1994**, *344*, 769–772. [CrossRef]

5. Hyman, B.T.; Trojanowski, J.Q. Editorial on Consensus Recommendations for the Postmortem Diagnosis of Alzheimer Disease from the National Institute on Aging and the Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer Disease. *J. Neuropathol. Exp. Neurol.* **1997**, *56*, 1095–1097. [CrossRef]

6. Whitehouse, P.J.; Price, D.L.; Struble, R.G.; Clark, A.W.; Coyle, J.T.; DeLong, M.R. Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science* **1982**, *215*, 1237–1239. [CrossRef]

7. Ballatore, C.; Lee, V.M.Y.; Trojanowski, J.Q. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. *Nat. Rev. Neurosci.* **2007**, *8*, 663–672. [CrossRef]

8. Hebert, L.E.; Scherr, P.A.; Beckett, L.A.; Albert, M.S.; Pilgrim, D.M.; Chown, M.J.; Funkenstein, H.H.; Evans, D.A. Age-Specific Incidence of Alzheimer's Disease in a Community Population. *JAMA* **1995**, *273*, 1354–1359. [CrossRef]

9. Liu, C.-C.; Kanekiyo, T.; Xu, H.; Bu, G. Correction: Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. *Nat. Rev. Neurol.* **2013**, *9*, 184. [CrossRef]

10. Bekris, L.M.; Yu, C.-E.; Bird, T.D.; Tsuang, D.W. Genetics of Alzheimer Disease. *J. Geriatr. Psychiatry Neurol.* **2010**, *23*, 213–227. [CrossRef]

11. Mawuenyega, K.G.; Sigurdson, W.; Ovod, V.; Munsell, L.; Kasten, T.; Morris, J.C.; Yarasheski, K.E.; Bateman, R.J. Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science* **2010**, *330*, 1774. [CrossRef] [PubMed]

12. Bowen, D.M.; Smith, C.B.; White, P.; Davison, A.N. Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. *Brain* **1976**, *99*, 459–496. [CrossRef] [PubMed]

13. Bartus, R.; Dean, R.; Beer, B.; Lippa, A. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **1982**, *217*, 408–414. [CrossRef] [PubMed]

14. Hampel, H.; Mesulam, M.-M.; Cuello, A.C.; Farlow, M.R.; Giacobini, E.; Grossberg, G.T.; Khachaturian, A.S.; Vergallo, A.; Cavedo, E.; Snyder, P.J.; et al. The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain* **2018**, *141*, 1917–1933. [CrossRef]

15. Francis, P.T.; Palmer, A.M.; Snape, M.; Wilcock, G.K. The cholinergic hypothesis of Alzheimer's disease: A review of progress. *J. Neurol. Neurosurg. Psychiatry* **1999**, *66*, 137–147. [CrossRef]

16. Summers, W.K.; Majovski, L.V.; Marsh, G.M.; Tachiki, K.; Kling, A. Oral Tetrahydroaminoacridine in Long-Term Treatment of Senile Dementia, Alzheimer Type. *New Engl. J. Med.* **1986**, *315*, 1241–1245. [CrossRef]

17. Mesulam, M.; Guillozet, A.; Shaw, P.; Quinn, B. Widely Spread Butyrylcholinesterase Can Hydrolyze Acetylcholine in the Normal and Alzheimer Brain. *Neurobiol. Dis.* **2002**, *9*, 88–93. [CrossRef]

18. Johnson, G.; Moore, S.W. Why has butyrylcholinesterase been retained? Structural and functional diversification in a duplicated gene. *Neurochem. Int.* **2012**, *61*, 783–797. [CrossRef]

19. Lockridge, O. Review of human butyrylcholinesterase structure, function, genetic variants, history of use in the clinic, and potential therapeutic uses. *Pharmacol. Ther.* **2015**, *148*, 34–46. [CrossRef]

20. Mesulam, M.-M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E.; Lockridge, O.; Duysen, E. Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine. *Neuroscience* **2002**, *110*, 627–639. [CrossRef]

21. Li, B.; Stribley, J.A.; Ticu, A.; Xie, W.; Schopfer, L.M.; Hammond, P.; Brimijoin, S.; Hinrichs, S.H.; Lockridge, O. Abundant Tissue Butyrylcholinesterase and Its Possible Function in the Acetylcholinesterase Knockout Mouse. *J. Neurochem.* **2002**, *75*, 1320–1331. [CrossRef] [PubMed]

22. Darvesh, S.; Grantham, D.L.; Hopkins, D.A. Distribution of butyrylcholinesterase in the human amygdala and hippocampal formation. *J. Comp. Neurol.* **1998**, *393*, 374–390. [CrossRef]

23. Darvesh, S.; Hopkins, D.A. Differential distribution of butyrylcholinesterase and acetylcholinesterase in the human thalamus. *J. Comp. Neurol.* **2003**, *463*, 25–43. [CrossRef] [PubMed]

24. Mesulam, M.; Geula, C. Butyrylcholinesterase reactivity differentiates the amyloid plaques of aging from those of dementia. *Ann. Neurol.* **1994**, *36*, 722–727. [CrossRef]

25. Geula, C.; Mesulam, M.-M. Cholinesterases and the Pathology of Alzheimer Disease. *Alzheimer Dis. Assoc. Disord.* **1995**, *9*, 23–28. [CrossRef] [PubMed]

26. Gómez-Ramos, P.; Bouras, C.; Morán, M.A. Ultrastructural localization of butyrylcholinesterase on neurofibrillary degeneration sites in the brains of aged and Alzheimer's disease patients. *Brain Res.* **1994**, *640*, 17–24. [CrossRef]

27. Chen, V.P.; Xie, H.Q.; Chan, W.K.B.; Leung, K.W.; Chan, G.K.L.; Choi, R.C.Y.; Bon, S.; Massoulié, J.; Tsim, K.W.K. The PRiMA-linked Cholinesterase Tetramers Are Assembled from Homodimers. *J. Boil. Chem.* **2010**, *285*, 27265–27278. [CrossRef] [PubMed]

28. Lane, R.M.; Enz, A.; Potkin, S.G. Targeting acetylcholinesterase and butyrylcholinesterase in dementia. *Int. J. Neuropsychopharmacol.* **2005**, *9*, 101. [CrossRef]

29. Jann, M.W.; Shirley, K.L.; Small, G.W.; Jann, D.M.W. Clinical Pharmacokinetics and Pharmacodynamics of Cholinesterase Inhibitors. *Clin. Pharmacokinet.* **2002**, *41*, 719–739. [CrossRef]

30. Grutzendler, J.; Morris, J.C. Cholinesterase Inhibitors for Alzheimer's Disease. *Drugs* **2001**, *61*, 41–52. [CrossRef]

31. Szeto, J.Y.Y.; Lewis, S.J.G. Current Treatment Options for Alzheimer's Disease and Parkinson's Disease Dementia. *Curr. Neuropharmacol.* **2016**, *14*, 326–338. [CrossRef] [PubMed]

32. Ferris, S.; Karantzoulis, S.; Somogyi, M.; Meng, X. Rivastigmine in moderately severe-to-severe Alzheimer's disease: Severe Impairment Battery factor analysis. *Alzheimer's Res. Ther.* **2013**, *5*, 63. [CrossRef] [PubMed]

33. Colović, M.B.; Krstić, D.Z.; Lazarević-Pašti, T.D.; Bondžić, A.M.; Vasić, V.M. Acetylcholinesterase Inhibitors: Pharmacology and Toxicology. *Curr. Neuropharmacol.* **2013**, *11*, 315–335. [CrossRef] [PubMed]

34. Darreh-Shori, T.; Soininen, H. Effects of Cholinesterase Inhibitors on the Activities and Protein Levels of Cholinesterases in the Cerebrospinal Fluid of Patients with Alzheimers Disease: A Review of Recent Clinical Studies. *Curr. Alzheimer Res.* **2010**, *7*, 67–73. [CrossRef]

35. Lilienfeld, S. Galantamine—A novel cholinergic drug with a unique dual mode of action for the treatment of patients with Alzheimer's disease. *CNS Drug Rev.* **2002**, *8*, 159–176. [CrossRef]

36. Podoly, E.; Shalev, D.E.; Shenhar-Tsarfaty, S.; Bennett, E.R.; Ben Assayag, E.; Wilgus, H.; Livnah, O.; Soreq, H. The Butyrylcholinesterase K Variant Confers Structurally Derived Risks for Alzheimer Pathology. *J. Biol. Chem.* **2009**, *284*, 17170–17179. [CrossRef]

37. Altamirano, C.V.; Bartels, C.F.; Lockridge, O. The butyrylcholinesterase K-variant shows similar cellular protein turnover and quaternary interaction to the wild-type enzyme. *J. Neurochem.* **2000**, *74*, 869–877. [CrossRef]

38. Jasiecki, J.; Jońca, J.; Żuk, M.; Szczoczarz, A.; Janaszak-Jasiecka, A.; Lewandowski, K.; Waleron, K.; Wasag, B. Activity and polymorphisms of butyrylcholinesterase in a Polish population. *Chem. Interactions* **2016**, *259*, 70–77. [CrossRef]

39. Jasiecki, J.; Żuk, M.; Krawczyńska, N.; Jońca, J.; Szczoczarz, A.; Lewandowski, K.; Waleron, K.; Wasąg, B. Haplotypes of butyrylcholinesterase K-variant and their influence on the enzyme activity. *Chem. Biol. Interact.* **2019**, *307*, 154–157. [CrossRef]

40. Diamant, S.; Podoly, E.; Friedler, A.; Ligumsky, H.; Livnah, O.; Soreq, H. Butyrylcholinesterase attenuates amyloid fibril formation in vitro. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8628–8633. [CrossRef]

41. Lehmann, D.J.; Johnston, C.; Smith, A.D. Synergy between the genes for butyrylcholinesterase K variant and apolipoprotein E4 in late-onset confirmed Alzheimer's disease. *Hum. Mol. Genet.* **1997**, *6*, 1933–1936. [CrossRef] [PubMed]

42. Lehmann, D.J.; Nagy, Z.; Litchfield, S.; Cortina Borja, M.; Smith, A.D. Association of butyrylcholinesterase K variant with cholinesterase-positive neuritic plaques in the temporal cortex in late-onset Alzheimer's disease. *Hum. Genet.* **2000**, *106*, 447–452. [CrossRef] [PubMed]

43. Singleton, A.B.; Smith, G.; Gibson, A.M.; Woodward, R.; Perry, R.H.; Ince, P.G.; Edwardson, J.A.; Morris, C.M. No association between the K variant of the butyrylcholinesterase gene and pathologically confirmed Alzheimer's disease. *Hum. Mol. Genet.* **1998**, *7*, 937–939. [CrossRef] [PubMed]

44. Lee, D.; Liu, H.; Liu, T.; Chi, C.; Hong, C. No association between butyrylcholinesterase K-variant and Alzheimer disease in Chinese. *Am. J. Med Genet.* **2000**, *96*, 167–169. [CrossRef]

45. Wang, Z.; Jiang, Y.; Wang, X.; Du, Y.; Xiao, D.; Deng, Y.; Wang, J. Butyrylcholinesterase K Variant and Alzheimer's Disease Risk: A Meta-Analysis. *Med Sci. Monit.* **2015**, *21*, 1408–1413. [CrossRef]

46. Ramanan, V.K.; Risacher, S.L.; Nho, K.; Kim, S.; Swaminathan, S.; Shen, L.; Foroud, T.M.; Hakonarson, H.; Huentelman, M.J.; Aisen, P.S.; et al. APOE and BCHE as modulators of cerebral amyloid deposition: A florbetapir PET genome-wide association study. *Mol. Psychiatry* **2014**, *19*, 351–357. [CrossRef]

47. Jasiecki, J.; Limon-Sztencel, A.; Żuk, M.; Chmara, M.; Cysewski, D.; Limon, J.; Wasąg, B. Synergy between the alteration in the N-terminal region of butyrylcholinesterase K variant and apolipoprotein E4 in late-onset Alzheimer's disease. *Sci. Rep.* **2019**, *9*, 5223. [CrossRef]

48. O'Brien, K.K.; Saxby, B.K.; Ballard, C.G.; Grace, J.; Harrington, F.; A Ford, G.; O'Brien, J.T.; Swan, A.G.; Fairbairn, A.F.; Wesnes, K.; et al. Regulation of attention and response to therapy in dementia by butyrylcholinesterase. *Pharmacogenetics* **2003**, *13*, 231–239. [CrossRef]

49. Blesa, R.; Bullock, R.; He, Y.; Bergman, H.; Gambina, G.; Meyer, J.; Rapatz, G.; Nagel, J.; Lane, R. Effect of butyrylcholinesterase genotype on the response to rivastigmine or donepezil in younger patients with Alzheimer's disease. *Pharmacogenet. Genom.* **2006**, *16*, 771–774. [CrossRef]

50. Han, H.J.; Kwon, J.C.; Kim, J.E.; Kim, S.G.; Park, J.-M.; Park, K.W.; Park, K.C.; Park, K.H.; Moon, S.Y.; Seo, S.W.; et al. Effect of Rivastigmine or Memantine Add-on Therapy Is Affected by Butyrylcholinesterase Genotype in Patients with Probable Alzheimer's Disease. *Eur. Neurol.* **2015**, *73*, 23–28. [CrossRef]

51. Sokolow, S.; Li, X.; Chen, L.; Taylor, K.D.; Rotter, J.I.; Rissman, R.A.; Aisen, P.S.; Apostolova, L.G. Deleterious Effect of Butyrylcholinesterase K-Variant in Donepezil Treatment of Mild Cognitive Impairment. *J. Alzheimer's Dis.* **2017**, *56*, 229–237. [CrossRef] [PubMed]

52. Hartmann, J.; Kiewert, C.; Duysen, E.G.; Lockridge, O.; Greig, N.H.; Klein, J. Excessive hippocampal acetylcholine levels in acetylcholinesterase-deficient mice are moderated by butyrylcholinesterase activity. *J. Neurochem.* **2007**, *100*, 1421–1429. [CrossRef] [PubMed]

53. Scacchi, R.; Gambina, G.; Moretto, G.; Corbo, R.M. Variability of AChE, BChE, and ChAT genes in the late-onset form of Alzheimer's disease and relationships with response to treatment with Donepezil and Rivastigmine. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **2009**, *150*, 502–507. [CrossRef] [PubMed]

54. Meshorer, E.; Toiber, D.; Zurel, D.; Sahly, I.; Dori, A.; Cagnano, E.; Schreiber, L.; Grisaru, D.; Tronche, F.; Soreq, H. Combinatorial Complexity of 5' Alternative Acetylcholinesterase Transcripts and Protein Products. *J. Boil. Chem.* **2004**, *279*, 29740–29751. [CrossRef] [PubMed]

55. Jones, D.T. Protein secondary structure prediction based on position-specific scoring matrices. *J. Mol. Biol.* **1999**, *292*, 195–202. [CrossRef] [PubMed]

56. A Buchan, D.W.; Jones, D.T. The PSIPRED Protein Analysis Workbench: 20 years on. *Nucleic Acids Res.* **2019**, *47*, W402–W407. [CrossRef] [PubMed]

57. Ceroni, A.; Passerini, A.; Vullo, A.; Frasconi, P. DISULFIND: A disulfide bonding state and cysteine connectivity prediction server. *Nucleic Acids Res.* **2006**, *34*, W177–W181. [CrossRef]