Jurnal Kedokteran Hewan
P-ISSN : 1978-225X; E-ISSN : 2502-5600
September 2023, 17(3):100-104
DOI: https://doi.org/10.21157/j.ked.hewan.v17i3.31014

THE GENE EXPRESSION OF ADAM17 AS A GENETIC MARKER OF ALZHEIMER DISEASES IN THE BRAIN OF LONG-TAILED MACAQUES (*Macaca fascicularis)*

Lis Rosmanah[1,2], Karin Katina Hasya[3], Uus Saepuloh[2], Wasmen Manalu[3], Adi Winarto[3], Etih Sudarnika[3], and Huda Shalahudin Darusman[1,2,3]*

[1]Primatology Graduate Study Program, Graduate School of IPB University, Bogor, Indonesia
[2]Primate Research Center, IPB University, Bogor, Indonesia
[3]School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia
*Corresponding author: hudada@apps.ipb.ac.id

ABSTRACT

This study aims to identify the expression of the *a disintegrin and* metalloproteinase 17 (ADAM17) gene as a marker of Alzheimer's disease in long-tailed macaques (Macaca fascicularis**)**. This study used six brain samples (hippocampus and cortex regions) of long-tailed macaques which was divided into two groups consisting of aged long-tailed macaques and adult long-tailed macaques. The expression of ADAM17 gene was determined by comparing the relative quantification values between the two age groups, and brain regions consisting of the hippocampus and cortex regions. The results of data analysis showed no significant difference in the expression of ADAM17 gene between brain regions and between age groups of long-tailed macaques. However, numerically the results showed a lower expression of ADAM17 gene in the hippocampus region of aged macaques. Lower expression of ADAM17 gene could be a marker of old animals indicating the stages of Alzheimer's disease.

Key words: ADAM17, Alzheimer's disease, cortex, hippocampus, long-tailed macaques

ABSTRAK

Penelitian ini bertujuan mengidentifikasi ekspresi gen a disintegrin and metalloproteinase 17 (ADAM17) sebagai marka penyakit Alzheimer pada monyet ekor panjang. Dalam penelitian ini digunakan enam sampel otak monyet ekor panjang bagian hipokampus dan korteks yang terdiri atas kelompok monyet usia tua dan kelompok monyet usia dewasa. Ekspresi gen ADAM17 dilakukan menggunakan metode Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) dengan membandingkan nilai kuantifikasi relatif antara dua kelompok usia tua dan muda serta regio hipokampus dan korteks. Hasil analisis data menunjukkan tidak berbeda nyata antara ekspresi gen ADAM17 pada tiap regio dan kelompok usia, namun didapatkan hasil bahwa ekspresi gen ADAM17 lebih rendah ditemukan pada monyet usia tua pada regio hipokampus dan ekspresi gen ADAM17 yang lebih rendah dapat menjadi penanda hewan tua dalam tahap mengindikasikan penyakit Alzheimer.

Kata kunci: ADAM17, penyakit Alzheimer, korteks, hipokampus, monyet ekor panjang

INTRODUCTION

Dementia is a symptom of impairing a person's memory, cognition, communication, and behavior. Dementia is a brain illness that develops progressively with advancing age. The types of dementia are Alzheimer's dementia, vascular dementia, and dementia due to the other common medical conditions (Suriastini *et al.* 2021). Alzheimer's disease is the most common type of dementia. Alzheimer's and dementia are the seventh leading cause of death worldwide in 2019 (WHO 2020). In 2015, people with Alzheimer's dementia were estimated to reach 55 million globally and will increase as many as 10 million new cases per year (WHO 2015). Parents over the age of 65 often have Alzheimer's disease. According to BPS (2020), the number of older people in Indonesia has doubled in the last 50 years. Currently, there are about 26 million of older people in Indonesia, which is 9.92 percent of the total Indonesian population. The rise in the number of older people shows that life expectancy is also increasing, but older people are more likely to get Alzheimer's.

No specific treatment for Alzheimer's disease has yet to be discovered. Generally, the treatment of Alzheimer's disease focuses on the alleviating the symptoms and preventing the progression of illness. In the mammalian nervous system, a disintegrin and metalloproteinase (ADAM) are multifunctional transmembrane proteins. There are several protein types of ADAM, one of which is ADAM17 that has been suggested as a potential therapeutic target of Alzheimer's disease (Hsia *et al*. 2019). ADAM17 is a marker of Alzheimer's disease clearance because it has a neuroprotective impact that prevents nerve cell damage (Qian *et al*. 2015). The potential of ADAM17 as a treatment of Alzheimer's disease requires additional investigation.

Long-tailed macaques are widely used in research as model animals. Several findings report the similarity of characteristics of old long-tailed macaques with humans who have Alzheimer's disease (Darusman *et al.* 2014). Long-tailed macaques are widely used as animal models for neurodegenerative diseases such as Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (Park *et al*. 2013). In the brains of old cynomolgus monkeys, especially in monkeys over 20 years old, amyloid beta spontaneously forms intracellularly in the monkey brain (Nakamura *et al*. 1996; Kimura *et al*. 2005). It is necessary to establish acceptable animal models to learn about the process of illness incidence and the body's reaction to therapies against disease (Willard and Shively 2011). This study aims to identify differences in ADAM17 gene expression levels in the brain tissue of long-tailed macaques (*Macaca*

Revised 29-05-2023, Accepted 13-07-2023

fascicularis) based on the comparison of brain regions and age groups.

MATERIALS AND METHODS

Sample Collection

This study used samples in the form of brain tissue archives from 6 female long-tailed macaques, which were divided into two groups, consisting of the group of aged with the ages ≥ 15 years and the group of adults with the age range of 10-12 years (Gartland *et al.* 2020). The samples used were brain tissue from the cortex and hippocampus regions. This research has gone through an ethical study on the use of animals by KPKPHP4 (Commission for Supervision of Animal Welfare in Research, Testing, Education, and Captivity) with IPB number PRC-19-A012.

RNA Extraction and cDNA Preparation

Total RNA was extracted from 2 mm^3 cortex and hippocampus sections of 6 long-tailed macaque using RNeasy Mini Kits (Qiagen, Hilden, Germany) according to the company procedures. RNA concentration was measured using a Nanodrop 1000 spectrophotometer (Thermofisher Scientific, USA). The process of cDNA synthesis was carried out using the reverse transcriptase enzyme (Sensifast cDNA synthesis Kit, Bioline, Meiridian Bioscience, USA) according to the company procedures. The primers used in this study were Forward primer (CATGAAT/GGCAAATGTGAGAAAC) and Reverse primer (TGGACAAGAATGCTGAAAAGGA) for ADAM17 and for beta-actin (ACTB) the Forward primer was ACAGAGCCTCGCCTTTGC and Reverse primer was CACGATGGAGGGGAAGAC. The primers used in this study were modified from Park *et al*. (2015).

Reverse Transcription-quantitative Polymerase Chain Reaction (RT-qPCR) Amplification

The PCR amplification process was performed with a CFX Opus 96 machine (Biorad, USA). Each reaction used a reagent consisting of: 2 µL of cDNA as a template added to 18 µL of a mixture containing 6 µL of Nucleotide Free Water (NFW), 10 µL of Sensifast Sybr mix (Bioline, Meiridian Bioscience, USA), and 1 µL of each of forward and reverse primers for the ADAM17 gene and ACTB, respectively, as a reference gene. The program of qPCR amplification was carried out as follows: 95º C for 2 minutes for pre-denaturation, 95º C for 10 seconds for denaturation, 55º C for 20 seconds for annealing, 65º C for 10 seconds for extension and data collection, and this process was repeated for 40 cycles.

Data Analysis

The data obtained were analyzed using Microsoft Excel. The Shapiro-Wilk and Levene tests were used to examine the normality and homogeneity of the data, respectively. The data were then tested using an independent t-test to evaluate the differences between brain regions and between age groups. Data obtained from RT-qPCR were in the form of Cycle Threshold (Ct), which were then processed with the 2-ΔCt formula to obtain Relative Quantification (RQ) values. RQ values were then processed to produce the levels of mRNA expression in the foldchange after being normalized against the ACTB housekeeping gene.

RESULTS AND DISCUSSION

Comparison of The Expressions of *ADAM17* Gene in Long-*tail Macaques* Based on Age Group

Alzheimer's is a degenerative disease that has a more common incidence with the increased age (jahn 2013). The majority of cases of alzheimer's disease occur at late-onset or occur in aged individuals and are rarely found in adult individuals (guerreiro and bras 2015). In this study, tests were carried out on brain samples of long-tailed macaques using the parameter of adam17 gene expression as a marker of alzheimer's disease. Statistical calculations showed no significant difference in the expression of adam17 gene in the adult and aged groups of long-tailed macaques ($p>0.05$, figure 1).

The α disintegrin and metallopeptidase domain 17 (ADAM17) and domain 10 (ADAM10) have an

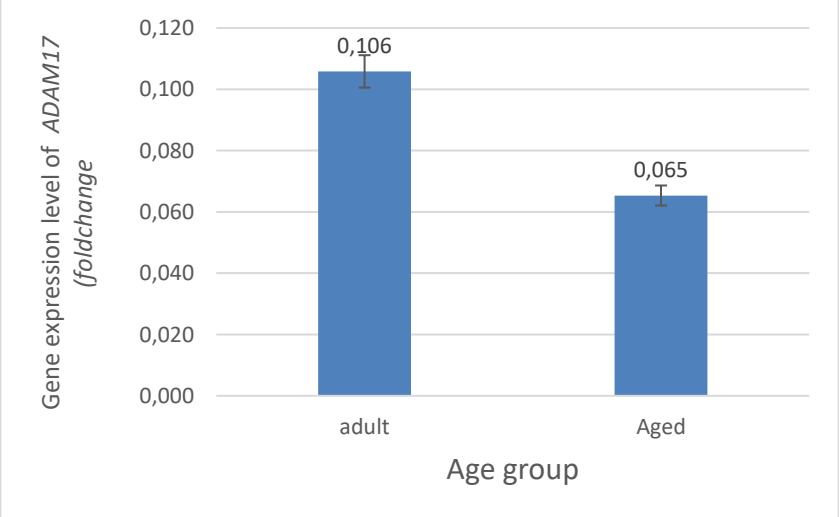

Figure 1. The ratio of ADAM17 gene expression levels by adult and aged groups in long-tailed macaques brain samples

important role as an α secretion involved in the physiological processing of Amyloid Precursor Protein (APP) in the brain. According to the findings, long-tailed macaques in the adult group had the level of ADAM17 gene expression of 0.106, whereas those in the aged group had a level of 0.065. (Figure 1). Compared to the expression of ADAM17 gene in the brains of aged long-tailed macaques, there is a 1.6-fold difference in the expression of ADAM17 gene in the adult group. A study reported that ADAM17 co-localizations were less common in people with Alzheimer's than those without the Alzheimer (Bernstein *et al.* 2009).

A lower level of ADAM17 gene expression may indicate that an animal is getting aged and developing Alzheimer's**.** The low level of ADAM17 as α-secretase causes a lot of APP cleavage to be carried out by β-secretase and λ-secretase, which form Aβ. Aβ accumulation forms a senile plaque, a marker of Alzheimer's disease (Kinney *et al*. 2018). A study reported that aged long-tailed macaques experienced impaired brain function, which was observed through delays in the tests conducted (Darusman *et al.* 2014).

Comparison of ADAM17 Gene Expression in *Macaca fascicularis* Based on Brain Region

Some of the first lesions that can be identified in cases of Alzheimer's disease can be found in the regions associated with memory and learning abilities, such as the hippocampus and cortex (Jahn 2013). Senile plaques and also NFTs (neurofibrillary tangles) are commonly found in these two regions in people with Alzheimer's disease. Statistical calculations showed no significant difference in the expressions of ADAM17 gene between the regions of hippocampus and cortex in the brain (P>0.05, Figure 2).

These disturbances are in the form of control and memory disturbances. These results also showed that the level of ADAM17 gene expression was 0.105 in the hippocampus region and 0.066 in the cortex region (Figure 2). The level of ADAM17 gene expression in the hippocampus region was 1.6-fold higher than that of in the cortex region. A study on the detection of ADAM mRNA in rat brains reported that ADAM17 mRNA could be found in the cortex, mesencephalon, medulla, and also in the hippocampus (Kärkkäinen *et al.* 2000). Wang *et al.* (2014) reported that the level of ADAM17 gene expression in the hippocampus was higher than that of in the cortex. ADAM17 is required in the basic biological processes of the brain. ADAM17 binds to integrins to perform ectodomain shedding. As a result, protein synthesis occurs, as well as the activation and inactivation of several important proteins for brain function and development. Some of the processes triggered by ADAM17 include cell migration and differentiation, signaling, and cell regeneration to maintain brain structure (Rybnikova *et al.* 2012). ADAM17 is also important in repairing the damaged structures of axon (Ho *et al.* 2022).

According to Jahn (2013), Alzheimer's disease is characterized by lesions in memory and learning-related brain regions, such as the hippocampus and cortex. The hippocampus is involved in memory formation and storage, navigation, and learning capacity (Clark and Squire 2013; Lisman *et al*. 2017). The cortex is important for integrating sensory data, memory, linguistic abilities, and cognition (Bakkour *et al*. 2013). These results may be related to the first symptoms of Alzheimer's disease, namely disorders caused by impaired hippocampal and cortical functions. The National Institute on Aging and the Alzheimer's Association (NIA-AA) divides this disease into three phases based on the symptoms shown. The first phase is the preclinical phase, where the symptoms have not appeared. The second phase is the Mild Cognitive Impairment (MCI) phase, characterized by memory impairment. In contrast, the third phase is the presence of symptoms that make the sufferer need help from

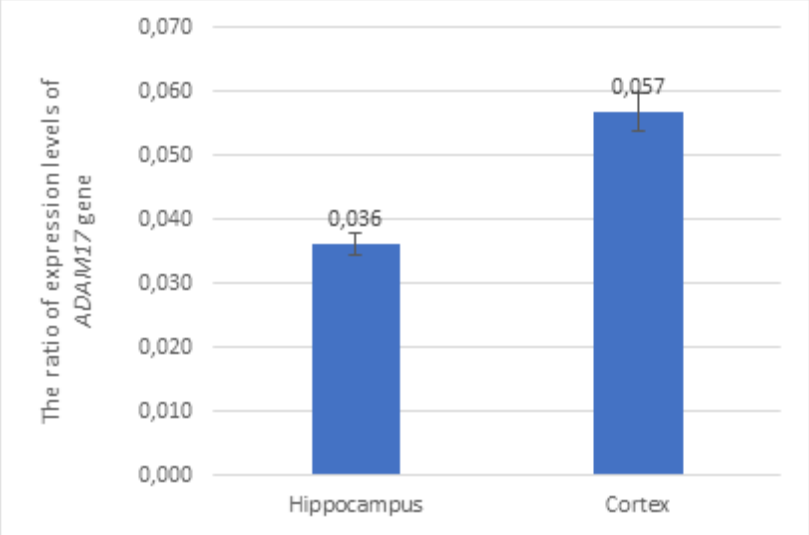

Figure 2. The ratio of ADAM17 gene expression levels in the brain region of the hippocampus and cortex of *Macaca fascicularis*

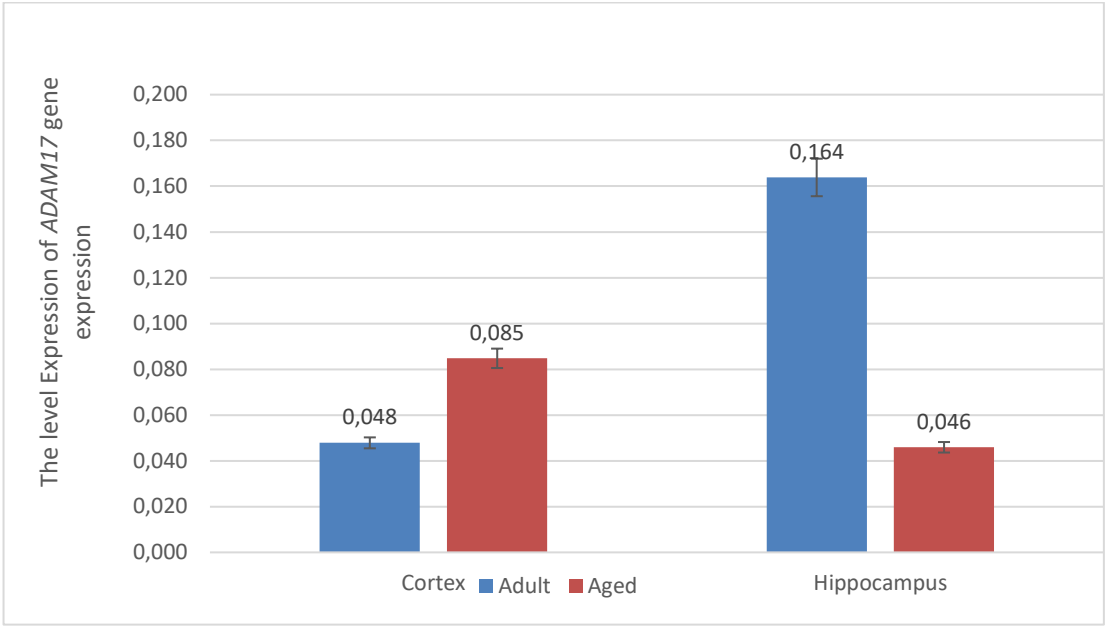

Figure 3. The ratio of ADAM17 gene expression in *Macaca fascicularis* brain based on the aged and adult age groups and the regions of cortex and hippocampus

others to carry out daily life (McKhann *et al.* 2011). The Alzheimer's Association (2018) states that early stages of Alzheimer's disease are characterized by difficulties acquiring new information, topographical disorientation, irregular word usage, and increased irritability.

Comparison of *ADAM17* Gene Expression in *Macaca fascicularis* Based on The Age and Brain Region

The levels of *ADAM17* gene expression were also compared based on the aged and adult age and regions of the hippocampus and cortex of the brain. This comparison was made to evaluate the differences in the levels of *ADAM17* gene expression in each region of brain for each age group of the long-tailed macaques. Statistical calculations for the two regions did not show a significant difference, with P-values for the hippocampal region of 0.159 and the cortex region of 0.410 (Figure 3).

Data analysis revealed that the expression of the *ADAM17* gene in the hippocampus region of adult was 3.6-fold higher than its expression in the hippocampus region of aged individuals, with an expression level of 0.164 and 0.046 in the hippocampus regions of adult and aged monkey, respectively (Figure 3). In contrast to the hippocampus region, the level of *ADAM17* mRNA expression in the aged group in the cortex region was 1.8-fold change in *ADAM17* gene expression in the adult group (Figure 3).

ADAM17 has a role in the process of learning and the storage of memories. This could explain how ADAM17 is highly expressed in the hippocampus of adult long-tailed macaques. ADAM17 contributes to memory formation via its function in the onset of Long-Term Depression (LTD). ADAM17 is reported to have a function to process membrane proteins that are important for synapse formation and plasticity, including neuronal pentraxin, which regulates the occurrence of receptor endocytosis for LTD in neurons in the hippocampal and cerebellum regions (Cho *et al.* 2008; Zunke and Rose-John 2017).

Metalloproteinases that function as secretase enzymes, including ADAM17, ADAM10, and ADAM9, have been implicated in shedding low-density lipoprotein receptor-related protein 1 (LRP1) and boosting Aβ transport out of the brain (Shackleton *et al.* 2016). ADAM17 also cleaves pro-inflammatory molecules that enhance Aβ phagocytosis (Qian *et al.* 2015). Both of these processes aid the removal of Aβ from brain tissue.

CONCLUSION

The expression of *ADAM17* gene in long-tailed macaques showed no significant difference between the adult and aged groups and between the hippocampus and cortex brain regions. The expression of *ADAM17* gene is mostly found in the hippocampus region of the adult macaques, and the expression of the *ADAM17* gene in the cortex region is mostly found in the aged group.

ACKNOWLEDGMENTS

The author would like to thank the Director of LPPM IPB, Yuliana MSi, Elis Dwi Ayuningsih, SSi, drh. Silvia Arin Prabandari, MSi, drh Dyah Setiawati, Kartika Sari, and Rahmat Supriatna for technical assistance in

research preparation. This study was supported by the Ministry of Finance of the Republic of Indonesia in the LPDP Productive Research and Innovation (RISPRO) program (ID Contract PRJ/29/LPDP/2019).

REFERENCES

Alzheimer's Association. 2018. Stages of Alzheimer's Disease https://www.alz.org/alzheimersdementia/stages.

Bakkour A, Morris JC, Wolk DA, Dickerson BC. 2013. The effects of aging and Alzheimer's disease on cerebral cortical anatomy: Specificity and differential relationships with cognition. *Neuroimage*, 76(1):332-344.

Bernstein HG, Stricker R, Lendeckel U, Bertram I, Dobrowolny H, Steiner J, Bogerts B, Reiser G. 2009. Reduced neuronal co-localisation of nardilysin and the putative α-secretases ADAM10 and *ADAM17* in Alzheimer's disease and down syndrome brains. *AGE*, 31(1):11-25.

BPS. 2020. Statistik Penduduk Lanjut Usia 2020. Badan Pusat Statistik, Jakarta.

Cho RW, Park JM, Wolff SB, Xu D, Hopf C, Kim JA, Reddy RC, Petralia RS, Perin MS, Linden DJ, Worley PF. 2008. mGluR1/5-dependent long-term depression requires the regulated ectodomain cleavage of neuronal pentraxin NPR by TACE. *Neuron*, 57(6):858-871.

Clark RE, Squire LR. 2013. Similarity in form and function of the hippocampus in rodents, monkeys, and humans. *Proceedings of the National Academy of Sciences*, California 110(2):10365-10370.

Darusman HS, Call J, Sajuthi D, Schapiro SJ, Gjedde A, Kalliokoski O, Hau J. 2014. Delayed response task performance as a function of age in cynomolgus monkeys (*Macaca fascicularis*). *Primates*, 55(2):259-267.

Gartland KN, Brand CM, Ulibarri LR, White FJ. 2020. Variation in adult male-juvenile affiliative behavior in Japanese macaques (*Macaca fuscata*). *Folia Primatologica*, 91:610-621.

Guerreiro R, Bras J. 2015. The age factor in Alzheimer's disease. *Genome Medicine*, 7(1):1-3.

Ho XY, Coakley S, Amor R, Anggono V, Hilliard MA. 2022. The metalloprotease ADM-4/ADAM17 promotes axonal repair. *Science Advances*, 8(11):1-14.

Hsia HE, Tüshaus J, Brummer T, Zheng Y, Scilabra SD, Lichtenthaler SF. 2019. Functions of 'A disintegrin and metalloproteases (ADAMs)'in the mammalian nervous system. *Cellular and Molecular Life Sciences*, 76(16):3055-3081.

Jahn H. 2013. Memory loss in Alzheimer's disease. *Dialogues in Clinical Neuroscience* 15(4): 445-454.

Kärkkäinen I, Rybnikova E, Pelto-Huikko M, Huovila APJ. 2000. Metalloprotease-disintegrin (ADAM) genes are widely and differentially expressed in the adult CNS. *Molecular and Cellular Neuroscience.* 15(6):547-560.

Kimura N, Yanagisawa K, Terao K, Ono F, Sakakibara I, Ishii Y, Kyuwa S, Yoshikawa Y. 2005. Age-related changes of intracellular Aβ in cynomolgus monkey brains. *Neuropathology and Applied Neurobiology*,31(2):170-180.

Kinney JW, Bemiller SM, Murtishaw AS, Leisgang AM, Salazar AM, Lamb BT. 2018. Inflammation as a central mechanism in Alzheimer's disease. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, 4(1):575-590.

Lisman J, Buzsáki G, Eichenbaum H, Nadel L, Ranganath C, Redish AD. 2017. The role of the hippocampus in navigation is memory. *Journal of Neurophysiology*. 117(4):1785-1796.

McKhann GM, Knopman DS, Chertkow H, Hyman BT, Jack Jr CR, Kawas CH, Klunk WE, Koroshetz WJ, Manly JJ, Mayeux R. 2011. The diagnosis of dementia due to Alzheimer's disease: Recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's Dement*, 7(3): 263-269.

Nakamura S, Kiatipattanasakul W, Nakayama H, Ono F, Sakakibara I, Yoshikawa Y, Goto N, Doi K. 1996. Immunohistochemical characteristics of the constituents of senile plaques and amyloid angiopathy in aged cynomolgus monkeys. *Journal of Medical Primatol*ology, 25(4):294-300.

Park SJ, Kim YH, Nam GH, Choe SH, Lee SR, Kim SU, Kim JS, Sim BW, Song BS. 2015. Quantitative expression analysis of APP pathway and tau phosphorylation-related genes in the ICV STZ-induced non-human primate model of sporadic Alzheimer's disease. *International Journal of Molecular Sciences*,16(2):2386-2402.

Qian M, Shen X, Wang H. 2016. The distinct role of ADAM17 in APP proteolysis and microglial activation related to Alzheimer's disease. *Cellular and Molecular Neurobiology*, 36(4):471-482.

Rybnikova E, Gluschenko T, Galeeva A, Tulkova E, Nalivaeva NN, Makova NZ, Turner AJ, Samoilov M. 2012. Differential expression of ADAM15 and ADAM17 metalloproteases in the rat brain after severe hypobaric hypoxia and hypoxic preconditioning. *Neuroscience Research*, 72(4):364-373.

Shackleton B, Crawford F, Bachmeier C. 2016. Inhibition of ADAM10 promotes the clearance of Aβ across the BBB by reducing LRP1 ectodomain shedding. *Fluids and Barriers of the CNS*, 13(1):1-9.

Suriastini NW, Turana Y, Witoelar F, Sikoki BS, Wicaksono TY, Mulyanto ED. 2016. Angka prevalensi demensia, perlu perhatian kita semua. *Majalah Kesehatan Indonesia*, 2(2):39-44.

Wang L, Hu J, Zhao Y, Lu X, Zhang Q, Niu Q. 2014. Effects of aluminium on β-amyloid (1-42) and secretases (APP-cleaving enzymes) in rat brain. *Neurochemical Research* 39(7):1338-1345.

WHO (World Health Organization). 2015. 10 Facts on Dementia. http://www.who.int/features/factfiles/d ementia/en/.

WHO (World Health Organization). 2020. The top 10 causes of death. https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.

Willard SL, Shively CA. 2012. Modeling depression in adult female cynomolgus monkeys (*Macaca fascicularis*). *American Journal of Primatology*, 74(6):528-542.

Yiannopoulou KG and Papageorgiou SG. 2020. Current and future treatments in Alzheimer disease: an update. *Journal of central nervous system disease*. DOI: 10.1177/1179573520907397.

Zunke F, Rose-John S. 2017. The shedding protease ADAM17: Physiology and pathophysiology. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1864(11): 2059-2070.