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Research Article

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Spontaneous Gene Expression of Alzheimer's Disease Biomarker BACE1 in Cerebrospinal Fluid of The Long-Tailed Macaque (*Macaca fascicularis*)

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ABSTRACT

Background: Alzheimer's disease is a neurodegenerative disorder characterized by progressive decline in memory and cognitive function. Long-tailed macaques are used as experimental models due to their anatomical and physiological similarities to humans, making them relevant for studying molecular markers associated with Alzheimer's disease.

Aims: This study aimed to analyze the expression of the β-site Amyloid Precursor Protein Cleaving Enzyme 1 (BACE1) gene in the brain as a molecular marker of Alzheimer's disease in long-tailed macaques.

Methods: Brain tissue samples were obtained from six female long-tailed macaques divided into adult and old age groups. The hippocampus and prefrontal cortex regions were collected for analysis. Total RNA was extracted and processed using Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR). The mRNA expression levels of the BACE1 gene were quantified and normalized against beta-actin (ACTB) as a housekeeping gene. Statistical analysis was performed to compare gene expression between age groups and brain regions.

Results: Data analysis showed no significant differences in BACE1 mRNA expression between adult and old age groups or between the hippocampus and prefrontal cortex regions. However, there was a tendency toward higher mRNA expression levels in the hippocampus compared to the cortex and in the adult group compared to the old group.

Conclusion: Although no significant differences were detected, the observed tendency suggests potential regional and age-related variations in BACE1 expression in long-tailed macaques, which may contribute to further investigations of Alzheimer's disease molecular mechanisms.

INTRODUCTION

Alzheimer's Disease (AD) is a common neurodegenerative disease that is recognized by cognitive impairment and synaptic damage (Shi et al., 2022). AD is initiated by the amyloid β (Aβ) peptide aggregation. Aβ is the result of the deviation of the amyloid protein precursor (APP) cleavage, which in healthy conditions is mediated by the β-secretase enzyme. This deviation involves the protease enzyme named β-site APP cleaving enzyme (BACE). The BACE genes are often targeted in AD treatment as their inhibition is believed to suppress the Aβ generation (Gorman, 2006). The BACE mRNA expression is in line with the aging process, and its enzymatic activity increases along with the development of AD in the

brain, specifically in its cortical region (Fukumoto et al., 2004).

Studies of AD have been conducted on animals like mice and primates. The primates are often chosen as the research subjects as they share similarities with humans, both in terms of physiology and morphology (Phillips et al., 2014). The AD occurrence in humans shares similar behaviour with the occurrence in primates, specifically long-tailed macaques (*Macaca fascicularis*) in its elderly stage. The similarity lies in the formation of Aβ aggregation in the brains (Darusman et al., 2013; Oikawa et al., 2010). However, the similarity of the molecular biomarker of AD between both species still needs to be studied further.

This research aimed to study further the expression of the BACE1 gene as one of the biomarkers of AD in the brain of long-tailed macaques. The study focused on two different regions of the brain, which are the hippocampus and cortex regions, as well as studying the difference in expression levels in both regions. The result of the study was then compared to observe the similarity of the expression level in the human brain. Hence, the similarity of the molecular biomarker pathway in both species can be predicted.

METHODS AND MATERIALS

Ethical Approval

This research used the samples of non-human primate Long-tailed macaques in which had obtained ethical approval from the Institutional Animal Care and Use Committee (IACUC) of Bogor Agricultural University Primate Research Center with the ethical number IPB-PRC-19-A012. The long-tailed macaques are separated by their age into two groups, consisting of the elderly group (≥15 years) and the adult group (10 – 12 years).

RNA Extraction and cDNA Synthesis

The RNA extraction was conducted using the brain organ of long-tailed macaques (approximately 2 – 6 mm) and RNAeasy Mini Kits (Qiagen, Germany). The obtained samples were then homogenized with lysis buffer, centrifuged, and the supernatants were taken and added with 70% alcohol. The mixtures were centrifuged at a speed of 10.000 rpm for 15 seconds. The supernatants containing the RNA were transferred and washed with the wash buffer and centrifugated to obtain the purified RNA. The obtained RNAs were reverse-transcribed to obtain the gDNA. using QuantiTect® Reverse Transcription Kit (Qiagen, Germany). The reverse transcriptase enzyme was added to the RNA and incubated at 42°C for 15 minutes, then continued at 95°C for 3 minutes.

qPCR Amplification

The amplification was conducted with CFX Opus 96. Each reaction consists of 2 µL cDNA as the template and 18 µL of a mixture containing 6 µL Nucleotide Free Water (NFW), 10 µL SsoFast EvaGreen Supermix, and 1 µL of each forward and reverse primer. The primers used were aimed at the BACE1 gene and beta-actin (ACTB) for a separate quantification. The ACTB acted as the housekeeping gene. The primer templates used in this research are based on the previous research of Park et al. (2015), which is shown in Table 1. The qPCR program was set: predenaturation at 95°C for 2 minutes, denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 65 °C for 10 seconds. The denaturation to annealing process was repeated for 40 cycles.

Data Analysis

The Cycle Threshold (Ct) obtained from the qPCR amplification was analysed with Microsoft Excel and the R Studio application. The normality of the data was tested through the Shapiro-Wilk test, and the homogeneity of the data was tested through the Levene test. The data was then tested with a paired t-test to distinguish the difference between the brain regions and an independent t-test to distinguish the difference

Table 1. Primers used for the gene amplification of BACE1 and ACTB in qPCR

Gene Abbreviation	Gene Name	Primer* Forward (F)/Reverse (R)
ACTB	*Beta-Actin*	F: CAGAGCCTCGCCTTTGC R: CACGATGGAGGGGAAGAC
BACE1	*B-Site Amyloid Precursor Protein-Cleaving Enzyme 1*	F: CGGGTGGAGATCAATGGACA R: CACACCAGCTGCTCTCCTAG

between age groups. The Ct values were inputted to obtain the Relative Quantity (RQ) through the formula $2^{-\Delta Ct}$. The RQ values were translated as the mRNA expression level in the form of fold change.

RESULTS AND DISCUSSION

The Ratio of BACE1 Gene Expression in the Long-Tailed Macaques Between Regions of the Brain

AD is a neurodegenerative disease caused by the gradual death of neural cells starting from the entorhinal cortex (EC) in the hippocampus region (van Hoesen et al., 1991). One of the synaptic effects in AD patients from the accumulation of Aβ is the long-term potential (LTP) decrease in the hippocampus (Chen et al., 2000). The research compared the gene expression of BACE1 in the hippocampus and cortex through the fold change obtained from the RQ values.

Figure 1 shows the insignificant difference in the gene expression level of the mRNA gene BACE1 in the Long-tailed macaque's hippocampus and cortex region of the brain. The insignificance is also proven by the paired t-test value shown in Table 2 with the p-value of 0,502 (p-value > 0,05). Regarding the expression level, the BACE1 expression appears to be higher in the hippocampus region.

The Ratio of BACE1 Gene Expression in the Long-tailed Macaques Between the Adults and the Elderly

The expression level of BACE1 in the Long-tailed macaques is also distinguished by the age group. The group consists of the adults whose age ranges from 10-15 years, and the elderly whose age is above 15 years. Figure 2 shows the BACE1 gene expression level in the two groups. The elderly group shows lower expression for only up to 0,2 folds.

The statistical calculation shown in Table 3 implies no significant difference in the BACE1 gene expression between the adults and the elderly group. The comparison resulted in the p-value of 0,066 (p-value > 0,05). Despite being insignificantly different, the average value of the expression level appears to be higher in the adult group compared to that in the elderly group.

The Ratio of BACE1 Gene Expression in the Long-tailed Macaques Between Regions of the Brain

The mRNA expression level of BACE1 appears to be different in each region of the brain, including the cortex and the hippocampus. The data were normalized using ACTB as the housekeeping gene. Park et al. (2015) Research showed that the BACE1 expression level in mice is higher in the cortex regions rather than the hippocampus. However, despite the BACE1 gene itself being distributed to most of the brain regions, the BACE1-specific immunoreactivities are localized in the hippocampus. The hippocampus region is responsible for learning and memory, making it a prone region in AD patients (Laird et al., 2005).

The Ratio of BACE1 Gene Expression in the Long-Tailed Macaques Between the Adults and the Elderly

Several studies show that the expression level of the BACE1 gene appears to increase along with the maturing brain in humans, primates, and mice,

Table 2. Statistical analysis of BACE1 gene expression based on the brain regions

Brain Region	Amount of Samples	Average of RQ ± SD
Cortex	6	0,2032 ± 0,2697[a]
Hippocampus	6	0,3336 ± 0,4193[a]

[a] The values in the same column followed by the same letter show no significant difference at the 5% test level (paired t-test).

Table 3. Statistical analysis of BACE1 gene expression based on the age groups

Brain Region	Age Group	Amount of Samples	Average of RQ ± SD
Cortex	Adults	3	0,3172 ±0,3742[a]
	Elderlies	3	0,0892 ±0,0527[a]
Hippocampus	Adults	3	0,5778 ±0,5007[b]
	Elderlies	3	0,0894 ±0,1002[b]

[a,b] The values in the same column followed by the same letter show no significant difference at the 5% test level (independent t-test).

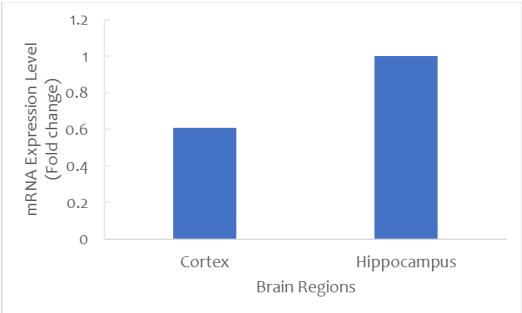

Figure 1. The BACE1 gene expression level based on the brain regions.

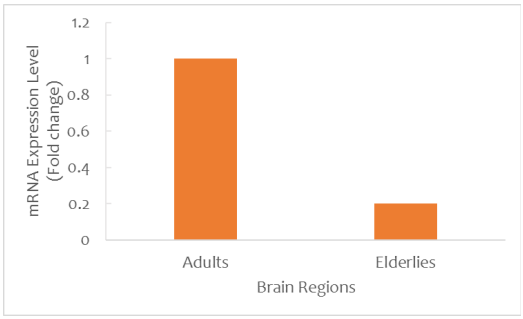

Figure 2. The BACE1 gene expression leel based on the age groups.

specifically in the area related to memory and higher cognitive function (Fukumoto et al., 2004; Hartmann et al., 2018).

Based on Duffy et al., (2019) research, the Aβ aggregation is found in the frontal and temporal cortex as well as the hippocampus in the elder Indochinese rhesus macaque (*Macaca mulatta*). The Aβ formation is induced by the BACE1. The BACE1 itself is significantly elevated in the adult Indochinese rhesus macaques, specifically in the cortex, before dropping along with the start of the elderly phase.

The Difference of the BACE1 Gene Expression in Humans and Long-tailed Macaque

The BACE1 gene naturally exists in the brain as it is responsible for sensorimotoric regulations as well as synaptic function. The lack of the BACE1 gene might lead to sensorimotor issues and seizures. The lack of BACE1 is also reported to be a cause of postnatal death in mice (Harrison et al., 2003; Kobayashi et al., 2008). One of the factors that leads to higher expression of BACE1 is the exposure to lead (Pb) pollution from the environment. The long-tailed macaques exposed to lead during the infant stage appear to show higher Aβ aggregation in the elderly stage (Wu et al., 2008).

BACE1 is responsible for accumulating Aβ. Aβ is the main component of the neuritic plaque, which becomes the molecular mark of AD. BACE1 is also found in the cerebrospinal fluid (CSF) in AD patients, which is often correlated with the high level of phospho-Tau (p-Tau).

The research shows that there is no significant difference in the BACE1 gene expression in both the cortex and the hippocampus regions. Despite being insignificantly different, the BACE1 expression level in the adult cortex is higher than that in the hippocampus. The overall gene expression of BACE1 is indeed higher in adults than in the elderly. However, this finding is reciprocal with that found in the human brain, based on Fukumoto et al. (2004), who stated that the BACE1 gene expression level in humans is found to be higher in the elderly stage. The factors affecting the significantly higher level of BACE1 gene expression in the adult stage cannot be overlooked and need further studies in terms of the correlation between the BACE1 expression level and the cognitive function affected by the gene activity.

CONCLUSION

Although no statistically significant differences were observed, the findings indicate a tendency toward variation in BACE1 expression across brain regions and age groups. Higher expression levels were descriptively noted in the hippocampus and in the adult group compared to the cortex and old group. These trends may provide preliminary insight for further studies investigating the molecular mechanisms of Alzheimer's disease in long-tailed macaques.

AUTHORS CONTRIBUTION

A.K.K.T. and N.A.J. were involved in conducting the experiments, collecting and processing samples, performing molecular analyses, and preparing the initial manuscript draft. F.N.A.D. assisted in laboratory work, data compilation, and preliminary data analysis. U.S. and L.R. contributed to methodological support,

data verification, and interpretation of the results. H.S.D. contributed to the study conception, overall research supervision, and critical revision of the manuscript. All authors have read and approved the final version of the manuscript.

"The authors declare that there is no conflict of interest with any parties involved in this research".

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