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LITERATURE REVIEW

CRISPR-Cas9 through AAV delivery system as a gene therapy in Parkinson's disease

Maulana Bagus Adi Cahyono[1], Ilham Rahmanto[1], Zahras Azimuth Doman[1], Galuh Senjani Yulfani Putri[1], Christopher Surya Lodianto[1], Pradana Zaky Romadhon[1,2,3]

1) Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia
2) Department of Internal Medicine, Dr. Soetomo Teaching Hospital, Surabaya, Indonesia
3) Department of Internal Medicine, Airlangga University Hospital, Surabaya, Indonesia

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Correspondence:

zaky.romadhon@fk.unair.ac.id

ABSTRACT

The global population living with Parkinson's disease is estimated to reach 9.4 million people, which has increased significantly since 2016, with a total of 6 million people. Parkinson's is a neurodegenerative disease of the substantia nigra that causes a decrease in dopamine production and is characterized by the appearance of cytoplasmic misfold proteins called Lewy bodies. The study found that abnormalities or mutations in the SNCA and LRRK2 genes correlated with the overproduction of the α-synuclein protein, which forms Lewy bodies that cause Parkinson's. Current Parkinson's medications only temporarily replace lost dopamine but do not treat the direct cause of Parkinson's; this research used qualitative literature study with content analysis, observation, and development; the use of CRISPR-Cas9 through AAV genetic engineering in repairing SNCA and LRRK2 mutant gene. This genetic therapy works by cutting the mutant DNA base sequences in the SNCA and LRRK2 genes and then replacing them with normal sequences through a homology-direct repair mechanism. As a result, the abnormalities or mutations that cause Parkinson's in these two genes can be corrected, so that dopaminergic levels in the brain can return to normal and excessive accumulation of α-synuclein protein can be suppressed.

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INTRODUCTION

Aging is a natural process that every human being must experience without exception. The brain is one of the organs most vulnerable to the aging process, characterized by various neurodegenerative disorders, one of which is Parkinson's disease. Parkinson's disease generally affects the elderly with an average age of ≥ 65 years and is dominated by men compared to women (Samaila *et al.*, 2019). In 2020, the global population with this disease is estimated to reach 9.4 million people, which has increased significantly since 2016, with a total of 6 million people. Meanwhile, in Indonesia, around ten people are estimated to suffer from this neurodegenerative disease each year, with the total number of sufferers currently ranging from 200,000 to 400,000 people (Kasandra, 2017). This number is predicted to increase over time in the future. This global health problem needs to be addressed seriously, considering that Parkinson's will greatly affect a person's quality of life.

Parkinson's is a neurodegenerative disease of the substantia nigra that causes a decrease in dopamine production and is characterized by the appearance of cytoplasmic misfold proteins called Lewy bodies (Gómez-Benito *et al.*, 2020). Dopamine is one of the neurotransmitters or chemicals in the brain that plays an important role in producing fine motor movements and non-motor functions. Thus, the clinical symptoms of Parkinson's disease consist of motor symptoms characterized by tremors, slowing of movements (bradykinesia), rigidity, and reduced postural reflexes, and can be followed by non-motor symptoms such as neuropsychiatric disorders (depression, anxiety, and dementia) (Fernandez, 2012). The cause of Parkinson's is believed to come from internal and external factors. The study found that abnormalities or mutations in the SNCA and LRRK2 genes correlated with the overproduction of the α-synuclein protein, which forms Lewy bodies that cause Parkinson's (Daher, 2017). In other words, an approach by molecularly repairing the SNCA and LRRK2 mutant genes has the potential to be the next step in Parkinson's treatment.

Until now, there is no definite treatment that can cure Parkinson's directly from the source of the cause. Current Parkinson's medications, including levodopa, dopamine agonists, and monoamine oxidase (MAO) type B inhibition, only temporarily replace lost dopamine levels in the central nervous system (Ellis *et al.*, 2017). These drugs only relieve motor and non-motor symptoms but do not treat the direct cause of Parkinson's, namely abnormalities in the SNCA and LRRK2 genes (Kearney *et al.*, 2017). In addition, the use of these drugs will cut costs a lot since Parkinson's patients will take these drugs in the long term and even for life to maintain their quality of life. On the other hand, these drugs have various side effects if consumed for a long time (DeMaagd *et al.*, 2015). Therefore, the current global health pain is finding the latest Parkinson's treatment that is potentially safe and relatively inexpensive.

The use of CRISPR-Cas9 genetic engineering in repairing SNCA and LRRK2 mutant gene abnormalities in brain neurons with Parkinson's disease can be an alternative therapy that is potentially safe, and relatively inexpensive. Gene therapy is a technology that makes it possible to edit the genome as desired, so it has the potential to treat diseases related to gene disorders, including Parkinson's disease (Artyukhova *et al.*, 2019). This innovation is inspired by a successful clinical trial on the use of CRISPR-Cas9 in improving the

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clinical condition of transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) in Germany (Frangoul *et al.*, 2021). Kantor et al. (2018) have done an in vitro study about the use of CRISPR-Cas9 to treat Parkinson's disease giving promising results. this is expected to be a renewable step to increase the cure rate for Parkinson's sufferers.

METHOD

This study uses a literature review method using a comprehensive strategy such as searching for articles in research journal databases. The databases used are Pubmed, Scopus, and Web of Science. The inclusion criteria in this study were journals in the last ten years that discussed Parkinson's disease, CRISPR-Cas9, drugs for Parkinson's, and Gene mutation in Parkinson's. The exclusion criteria in this study were international journals written in languages other than English.

LITERATURE REVIEW

Gene Mutation in Parkinson's Disease

Parkinson's disease is a neurodegenerative disorder characterized by a progressive decrease in dopaminergic levels in the substantia nigra pars compacta, causing motor and non-motor disturbances due to low dopamine levels (Balestrino *et al.*, 2020). Until now, the main cause of Parkinson's disease is still not known with certainty also it is thought that genetic and environmental factors may contribute to the course of this disease. Recent studies have shown that excessive accumulation of α-synuclein proteins that form Lewy bodies due to the SNCA and LRRK2 mutant genes is positively correlated with the manifestations of Parkinson's disease (Kearney *et al.*, 2017).

The α-synuclein protein is synthesized by the SNCA gene at normal levels and plays a role in regulating synaptic function, neurotransmission, and brain plasticity (Daher, 2017). SNCA genes are located in the gene locus 4q22.1 (Siddiqui *et al.*, 2016). However, if there is a point mutation, frameshift mutation, also duplication, it will cause an abnormal increase in the α-synuclein protein which shows a toxic effect on nerve cells, by destructing locus coeruleus and substansia nigra (Ding *et al.*, 2020). SNCA gene mutation is contributed to the early onset of Parkinson's disease (Chen *et al.*, 2020). On the other hand, the LRRK2 gene modulates aggregation and toxicity upstream of α-synuclein (Kearney *et al.*, 2017). It is located in the gene locus 12q12 (Labonne *et al.*, 2020) and is associated with late-onset disease (Trinh *et al.*, 2014). Mutations in this gene also lead to the accumulation of α-synuclein through diverse pathomechanisms that alter cellular function and signaling pathways, including the immune system, autophagy, vesicle trafficking, and retromer complex modulation (Daher, 2017). In other words, targeting that corrects abnormalities in these genes can be one of the solutions for Parkinson's treatment. An illustration of differences in brain dopaminergic mechanisms between normal individuals and Parkinson's can be seen in Figure 1.

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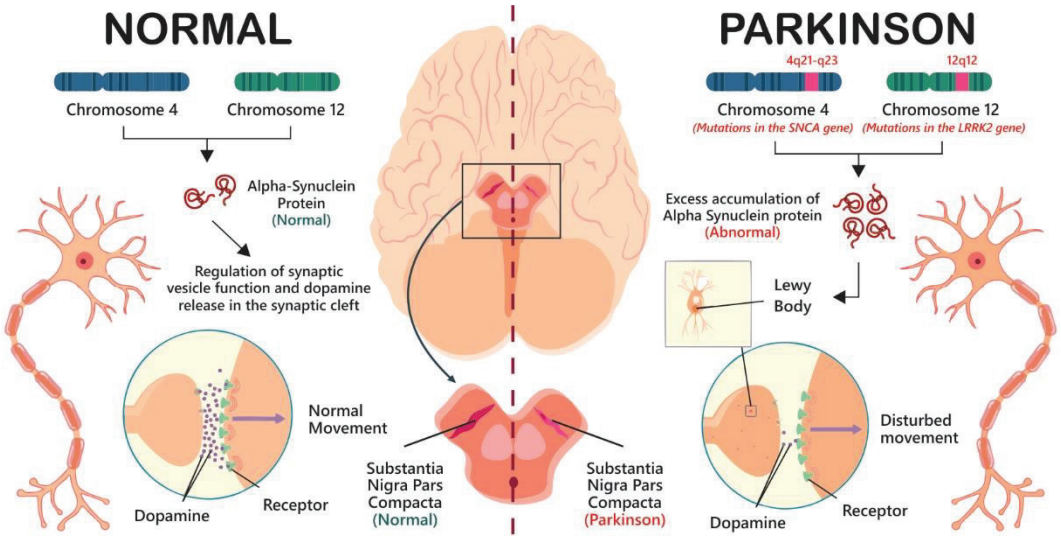

Figure 1. The production of dopamine between normal and parkinson's individual (source : original picture)

Drug Used in Parkinson's Disease

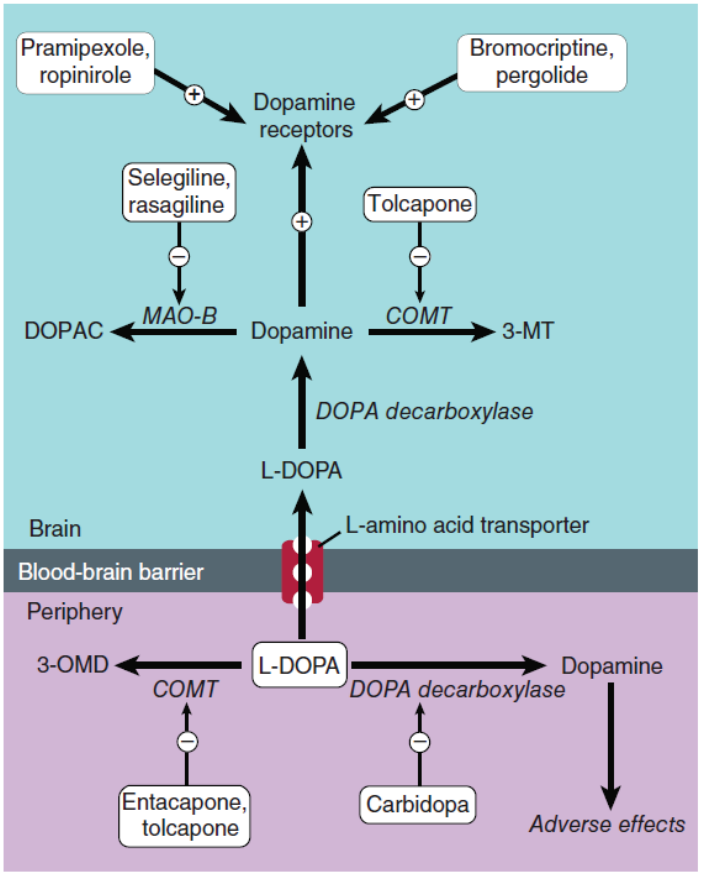

Figure 1. The drug's mechanism of action is used in Parkinson's disease (source: Katzung, Bertram G.; Trevor, 2019).

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A. Levodopa

Levodopa (L-dopa) is the precursor of dopamine with a higher bioavailability and readily crosses the blood-brain barrier. L-dopa enters the brain via the l-amino acid transporter. Levodopa is a drug of choice for the general condition of Parkinson's disease. However, the drug does not cure parkinsonism, and responsiveness fluctuates and gradually decreases with time, which may reflect the progression of the disease, so catechol-*O*-methyltransferase (COMT) inhibitors are often used adjunctively to reduce fluctuations in levodopa responses. Adverse effects are dose-dependent. In the early stage of treatment, the patients cannot tolerate postural hypotension and gastrointestinal disturbance. Dyskinesias occur in up to 80% of patients, with choreoathetosis of the face and distal extremities occurring most often. L-dopa also causes behavioral abnormalities such as anxiety, agitation, confusion, delusions, hallucinations, and depression. Some patients also reported movement disorders like tics, chorea, ballismus, myoclonus, and tremor (Katzung, Bertram G.; Trevor, 2019).

B. Dopamine Agonists

Pramipexole

As a dopamine agonist, pramipexole has a high affinity for the D_3 receptor. It can be used as monotherapy in mild disease or in combination with levodopa in more advanced diseases. It is contraindicated in psychotic patients, myocardial infarction, and peptic ulcer disease. The common side effects are nausea, vomiting, anorexia, postural hypotension, and dyskinesia. In rare cases, narcolepsy may occur (Perez-Lloret *et al.*, 2011).

Bromocriptine

Bromocriptine is an ergot alkaloid and binds to the D2 receptor in the brain as a partial agonist. Bromocriptine can be used in patients who are refractory to levodopa. Anorexia, nausea, vomiting, dyskinesias, and postural hypotension are reported as common side effects. Behavioral effects, which occur more commonly with bromocriptine than with newer dopamine agonists, include confusion, hallucinations, and delusions. Ergot-related effects include erythromelalgia and pulmonary infiltrates. Bromocriptine is now considered obsolete in patients with Parkinson's disease (Cacabelos, 2017).

C. Monoamine Oxidase Inhibitors

Monoamine oxidase is an enzyme that breaks down several neurotransmitters in the brain, including dopamine. Selegiline and rasagiline are the prototypes of monoamine oxidase type B (MAO-B) inhibitors. These drugs have relatively small efficacy for parkinsonism so they usually use adjunctively with levodopa. Adverse effects and interactions of monoamine oxidase inhibitors include insomnia, mood changes, dyskinesias, gastrointestinal distress, and hypotension (Ryan *et al.*, 2019).

D. Catechol-*O*-methyltransferase (COMT) Inhibitors

COMT will converts levodopa to 3-*O*-methyldopa (3-OMD). Increased plasma levels of 3-OMD are associated with poor response to levodopa partly because the compound competes with levodopa for active transport into the CNS. Levodopa dose reductions may be needed for the first few days of consuming COMT inhibitors. Entacapone and tolcapone are inhibitors of COMT and are used as adjuncts to levodopa-carbidopa, decreasing fluctuations, improving response, and prolonging on time. Gastrointestinal distress, postural hypotension, and dyskinesia are the side effect of these drugs. Sleep disturbance and increasing alt and ast are also reported (Cacabelos, 2017).

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There is currently no cure for Parkinson's disease, but treatments are available to help relieve the symptoms and maintain the quality of life by replacing temporarily lost dopamine. Commonly used drugs to relieve Parkinson's symptoms include levodopa, dopamine agonists, and type B monoamine oxidase (MAO) inhibitors (Ellis ***et al.***, 2017). Even though they are quite effective, the use of these drugs in the long term, such as levodopa, tends to lose their efficacy over time, thereby increasing the chance of unwanted side effects (Rizek ***et al.***, 2016). As a result, the costs required will be higher because the patients need to consume these drugs for a long time, even for a whole life. Therefore, researchers are currently looking for the best candidates for Parkinson's treatment methods that directly target the cause, namely abnormalities in the SNCA and LRRK2 genes which are autosomal dominant genes that cause this neurodegenerative disease.

CRISPR-Cas9 as a Gene Therapy for Parkinson's Disease

Today medical industry requires a breakthrough in treating Parkinson's directly at the level of the genome to treat abnormalities or mutations in the SNCA and LRRK2 genes. CRISPR-Cas9 genetic engineering is the most potential and up-to-date gene editing system that can treat various diseases caused by gene abnormalities, one of which is Parkinson's disease. CRISPR or Clustered Regularly Interspaced Short Palindromic Repeats is a defense mechanism bacteria have against harmful genetic material by creating single-guide RNA (sgRNA) that targets this genetic material. Meanwhile, CRISPR-associated 9 (Cas9) plays an important role in cutting the genes that have been targeted by sgRNA (Artyukhova et al., 2019). This genetic therapy works by cutting the mutant DNA base sequences in the SNCA and LRRK2 genes and then replacing them with normal sequences through a homology-direct repair mechanism. As a result, the abnormalities or mutations that cause Parkinson's in these two genes can be corrected, so that dopaminergic levels in the brain can return to normal and excessive accumulation of α-synuclein protein can be suppressed. The mechanism of CRISPR-Cas9 as a Parkinson's genetic therapy can be seen in Figure 3.

Recently a successful clinical trial has been carried out on the use of CRISPR-Cas9 through an Adeno-associated virus (AAV) delivery system, in improving the clinical condition of transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) in Germany (Frangoul *et al.*, 2021). Therapeutic strategies aimed at increasing fetal hemoglobin production in diseases such as beta-thalassemia and sickle cell anemia by inhibiting BCL11A. BCL11A is a zinc finger–containing transcription factor that represses γ-globin expression and fetal hemoglobin in erythroid cells. CRISPR-Cas9 nuclease system enables programmable targeting of insertions or deletions (indels) at a specific genomic DNA site, which is BCL11A (Fu *et al.*, 2022). In TDT patients, levels of fetal hemoglobin increased rapidly from 0.3 g per deciliter at baseline to 13.1 g at month 18. After the procedure, the hemoglobin level reached 12.1 g per deciliter without transfusion at month 4 and remained normal through month 18. The parallel result is reported in SCD patients. In SCD, hemoglobin levels also increase from 7.2 g per deciliter to 12 g per deciliter at month 12 without transfusion, and vaso-occlusive episodes are not reported again since the patient had seven serious episodes before the procedure. However, these procedures are not completely safe. Serious adverse events are reported such as pneumonia related to neutropenia, cholelithiasis, and sepsis, but all of these adverse events are resolved with treatment (Frangoul *et al.*, 2021). This result raised new hopes to be applied to Parkinson's disease.

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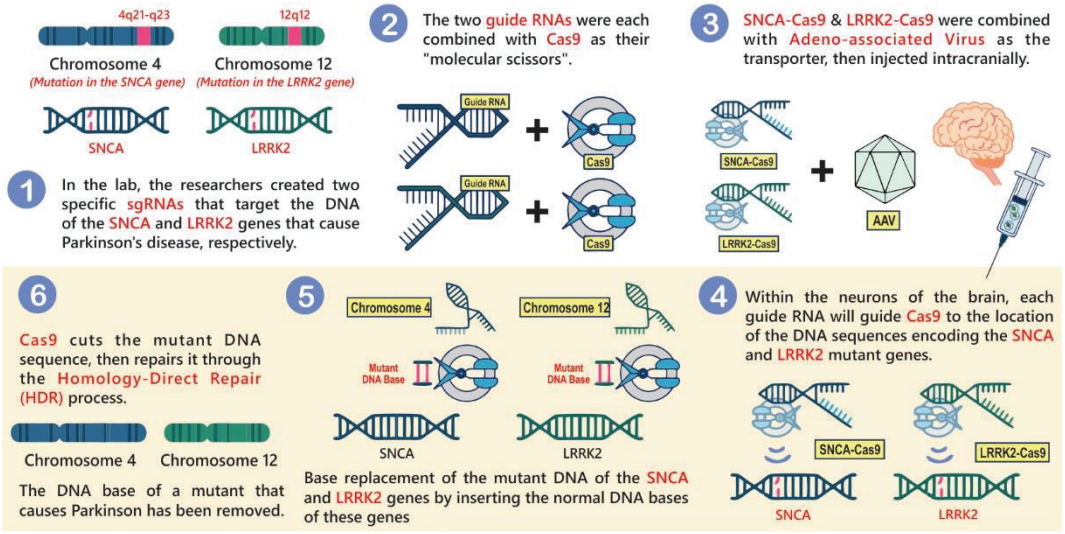

Figure 3. Mechanism of CRISPR-Cas9 in repairing the SNCA and LRRK2 mutant genes that cause Parkinson's (source: original picture).

In vitro, research conducted by Kantor et al. (2018) using CRISPR-Cas9 targeting the SNCA mutant gene on human induced pluripotent stem cell (hiPSC) dopaminergic neurons showed satisfactory results. CRISPR successfully corrected this gene, resulting in a downregulation of messenger-RNA (mRNA) of the SNCA gene and an over-accumulation of α-synuclein protein by almost 25% compared to controls. In addition, the results of this study also showed an increase in cellular viability and mitochondrial function that is more immune to oxidative stress. On the other hand, the research initiated by Qing et al. (2017) proved the potential of CRISPR-Cas9 in knowing the role of the LRRK2 mutant gene on the pathogenesis of parkinsonism in the hiPSC model. Based on these observations, it can be said that repairing the LRRK2 gene has the potential as a therapeutic option to improve α-synuclein induced neurodegeneration (Daher, 2017).

Gene therapy using CRISPR-Cas9 has promising potential in treating Parkinson's disease due to mutations in the SNCA and LRRK2 genes. Compared to other gene therapies such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), CRISPR offers results with high precision, ease, and time efficiency in terms of design and use, as well as its versatility (Artyukhova et al., 2019). Meanwhile, the Adeno-associated virus (AAV) was chosen as the 'vehicle' for CRISPR. This virus is the only current vector that is safe, and effective and has been approved as an intermediary for CRISPR into the human body (Wang *et al.*, 2020). AAV is non-pathogenic viral so it has minimum human immunogenicity (Hastie *et al.*, 2015). AAV will be delivered in the cytoplasm and then integrated with the human gene through DNA replication (Li *et al.*, 2020). The construction design of CRISPR-Cas9 as Parkinson's therapy can be seen in Figure 4.

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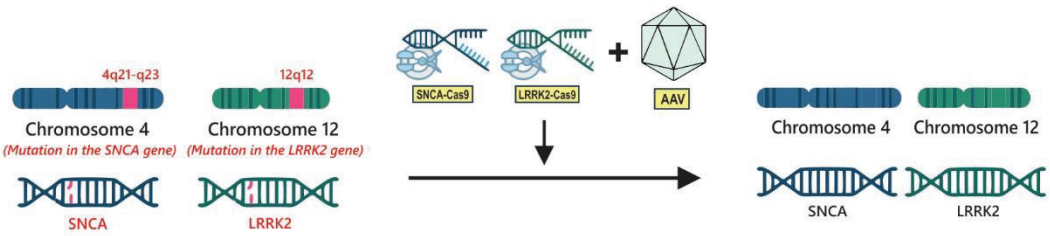

Figure 4. The construction design of CRISPR-Cas9 as Parkinson's therapy (source : original picture).

The AAV-encapsulated CRISPR-Cas9 complex is not recommended when given by injection through blood circulation because it will be difficult to penetrate the selective blood-brain barrier. However, this complex can be transported via intracranial injection that directly targets brain regions, so that maximum results will be obtained (Cota-Coronado *et al.*, 2019). On the other hand, AAV as a transporter has advantages over other viral vectors because it has a high level of precision with low cytotoxicity and provides stable gene expression results in the long term (Xu *et al.*, 2019). This step can be an alternative to levodopa replacement Parkinson's treatment which only relieves symptoms by replacing lost striatal dopamine (Kearney et al., 2017).

CONCLUSION

Genetic engineering CRISPR-Cas9 has the potential to be the latest therapy for Parkinson's disease caused by mutant genes SNCA and LRRK2. This gene therapy replaces mutant DNA base sequences with normal ones through a homology-direct repair (HDR) mechanism. Hence abnormal accumulation of α-synuclein protein can be suppressed and dopaminergic processes can function normally. Compared to current pharmacological treatments that only relieve Parkinson's symptoms, CRISPR-Cas9 offers a potential therapy with minimal side effects. In addition, the use of AAV as a transporter has high specificity and safety. This therapy is expected to be an alternative or combination therapy to increase the cure rate and quality of life for Parkinson's patients in Indonesia.

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