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GENE THERAPY FOR PARKINSON'S DISEASE: STRATEGIES FOR THE LOCAL PRODUCTION OF DOPAMINE*

SHIN-ICHI MURAMATSU[†,§], SAYAKA ASARI[†], KEN-ICHI FUJIMOTO[†], KEIYA OZAWA[‡] and IMAHARU NAKANO[†]

[†] *Division of Neurology, Department of Medicine*
and
[‡] *Genetic Therapeutics, Center for Molecular Medicine*
Jichi Medical University, Tochigi, Japan
[§] *muramats@jichi.ac.jp*

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The cardinal motor symptoms of Parkinson's disease (PD) are associated with the profound depletion of dopamine in the striatum. The replacement of dopamine is the most straightforward strategy to improve motor performance in PD. Researchers have been developing gene therapy aimed at local production of dopamine via the introduction of dopamine-synthesizing enzyme genes into the putamen. Two phase I clinical studies have used recombinant adeno-associated virus (AAV) vectors to transfer the aromatic L-amino acid decarboxylase (AADC) gene into the putamen to restore efficient conversion of orally administered L-3,4-dihydroxyphenylalanine (L-dopa). The initial results of these studies have not only confirmed the safety of AAV vectors, but have also demonstrated the alleviation of motor symptoms associated with PD. Interestingly motor performance in the "off" medication state was improved after gene therapy, suggesting long-term modulation of dopaminergic signals in the striatal neurons was induced by gene transfer. Gene delivery of tyrosine hydroxylase (TH) and guanosine triphosphate cyclohydrolase I (GCH) in addition to AADC may help to avoid motor fluctuations associated with intermittent intake of L-dopa by continuously supplying dopamine in the putamen. A clinical study of such triple gene transfer is presently underway using equine infectious anemia virus (EIAV) vector.

Keywords: Adeno-associated virus; aromatic L-amino acid decarboxylase; L-dopa; guanosine triphosphate cyclohydrolase I; positron emission tomography.

Parkinson's Disease

Parkinson's disease (PD) is second only to Alzheimer disease as the most common neurodegenerative disorder among the elderly, with an estimated 1% of the population over 60 years old suffering from PD and a lifetime risk of 6.7% in men (Driver *et al.*, 2009). The pathological hallmarks of PD are the presence of Lewy bodies,

*Invited review article.
§Corresponding author.

cytoplasmic inclusions, in the substantia nigra pars compacta (SNc) neurons that project to the striatum and the loss of these neurons. The main protein component of Lewy bodies is α-synuclein, which accumulates in a phosphorylated and aggregated form (Dickson *et al.*, 2009).

The causes of PD remain largely unknown, although genetic causes have been elucidated in some familial cases including mutations in the gene encoding α-synuclein, leucine-rich repeat kinase 2 (LRRK2), or ubiquitin carboxy-terminal hydrolase-L1 (UCH-L1) in families with an autosomal dominant pattern of inheritance, and mutations in the genes encoding parkin, PTEN-induced putative kinase 1 (PINK1), or DJ-1 in families with an autosomal recessive pattern of inheritance (Nuytemans *et al.*, 2010). Duplication or triplication of the *SNCA* gene, which encodes α-synuclein, gives rise to late-onset and early-onset familial PD, thus suggesting the expression level of α-synuclein might be an important determinant of disease onset and severity. Although genome-wide association studies successfully revealed some susceptibility genes (Tsuji, 2010), purely genetic causes probably account for only a small number of PD patients and multiple factors including environmental factors may contribute to the development of sporadic PD. Postmortem investigations demonstrate that the rate of decrease of nigral neurons is fast in the initial stage of the disease, namely about 40–50% are lost in the first decade, with possibly a slower rate of degeneration occurring thereafter and finally approaching a normal age-related linear decline (Fearnley and Lees, 1991). Recent imaging studies using radiotracers for nigrostriatal nerve terminals support this progression pattern, thus suggesting the mechanisms underlying PD initiation and progression are probably different (Bruck *et al.*, 2009; Nandhagopal *et al.*, 2009). The reason for the selective susceptibility of nigral dopaminergic neurons and the temporal sequence of events leading to cell loss in PD, however, remain to be elucidated.

Dopamine Synthesis in the Striatum

Dopamine is synthesized almost exclusively in the terminals of nigrostriatal neurons in the normal striatum. Three enzymes are necessary for efficient dopamine synthesis (Fig. 1): tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), and guanosine triphosphate cyclohydrolase I (GCH). L-Tyrosine is converted to L-3,4-dihydroxyphenylalanine (L-dopa) by TH in the first rate-limiting step. AADC then converts L-dopa to dopamine. GCH is the rate-limiting enzyme for synthesis of the essential TH co-factor tetrahydrobiopterin (BH_4). Since low levels of endogenous BH_4 do not yield sufficient TH activity, GCH is considered to regulate TH activity via regulation of BH_4 biosynthesis, thus indirectly controlling dopamine production in TH-containing neurons (Nagatsu *et al.*, 1987). These three enzymes are transported from the SNc to the striatum in an anterograde manner.

The cardinal symptoms in PD including resting tremor, muscular rigidity, and bradykinesia become apparent after the 40–50% of the SNc neurons are lost and striatal dopamine is reduced to about 20% of normal levels. A severe loss of

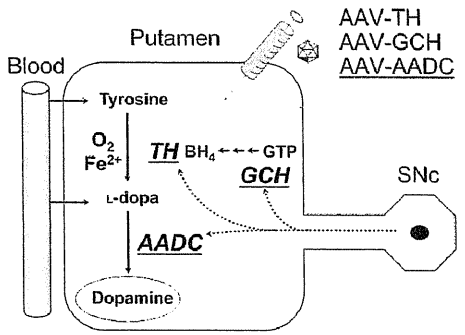

Fig. 1. Biosynthesis pathway of dopamine. Three enzymes are necessary for the efficient production of dopamine. Dopamine precursor, L-dopa is a standard drug for treating Parkinson disease. AADC, aromatic L-amino acid decarboxylase; BH_4, tetrahydrobiopterine; GCH, guanosine triphosphate cyclohydrolase I; TH, tyrosine hydroxylase; SNc, substantia nigra pars compacta.

dopaminergic nerve terminals in advanced PD is associated with an 80–95% depletion of the striatal TH and AADC activity (Zhong *et al.*, 1995; Nagatsu and Sawada, 2007) thus leading to a profound decrease of dopamine. There are several types of AADC-containing cells in the striatum, such as serotonin neurons, intrinsic dopamine neurons, AADC-containing "D" neurons, and glial cells. These cells may act as a local source of dopamine. However, endogenous AADC activity in the striatum is thought to be insufficient and the functional efficacy of dopamine produced from exogenous L-dopa in these cells may be limited at least in primates. The activity of GCH in the striatum has also been reported to decrease in PD (Nagatsu and Sawada, 2007). The restoration of the GCH activity is necessary to supply sufficient BH_4 in advanced PD, since the uptake of exogenous BH_4 from the blood is low (Hoshiga *et al.*, 1993) and the primary source of BH_4 in the brain is intracellular biosynthesis.

Complications of Long-Term L-dopa Therapy

The current accepted therapeutic strategy for PD is the replacement of dopamine in the striatum to alleviate motor dysfunction. Unlike dopamine, which does not cross the blood-brain barrier, the dopamine precursor, L-dopa can be transported into the brain and is the most effective drug in pharmacotherapy for PD. Virtually all patients experience a clinically meaningful benefit after receiving L-dopa treatment. However, as the disease progresses, the loss of AADC activity and the decreased capacity for dopamine storage in the synaptic vesicles lead to the failure of L-dopa therapy. Frequent systemic administration of high doses of L-dopa causes oscillations in motor performance with a variety of abnormal involuntary movements or dyskinesia (Fox and Lang, 2008). After 4–6 years of L-dopa treatment, 40 to 50% of patients are estimated to have motor complications (Ahlskog and Muenter, 2001). The diagnosis of idiopathic PD may be incorrect if a patient does

not show any fluctuations after several years of L-dopa therapy, since such fluctuations are almost invariable in PD patients. Many patients also start to experience some deleterious complications such as hallucinations and compulsive behaviors due to dopaminergic stimulation of the mesolimbic system (Aarsland *et al.*, 2009; Evans *et al.*, 2009; Voon *et al.*, 2009). Patients eventually become disabled. Novel therapeutic interventions in place of oral L-dopa administration are therefore required.

Preclinical Studies with Adeno-Associated Virus (AAV) Vectors

PD is a suitable candidate for gene therapy (Muramatsu *et al.*, 2005). PD is primarily confined to the well-defined nigrostriatal dopaminergic system and it is not necessary to deliver therapeutic genes to the entire brain, but only to a portion of the basal ganglia. Stereotactic surgical techniques to approach basal ganglia are established in clinical practice. In addition, well-characterized rodent and primate PD models are available for testing novel therapeutic interventions.

Viral vectors, in particular vectors derived from adeno-associated virus (AAV), are suitable for the transduction of neurons in the mammalian brain without significant toxicity. Recombinant AAV vectors have been applied in clinical trials for numerous disorders including hemophilia, cystic fibrosis and retinal degeneration (Daya and Berns, 2008). No adverse effects due to the administration of the vectors themselves have so far been found.

Researchers have been developing gene therapy method to restore local dopamine production in the striatum using AAV vectors. Gene transfer of TH, GCH and AADC, or AADC alone into the putamen has lead to behavioral recovery in primate models of PD (Fan *et al.*, 1998; Bankiewicz *et al.*, 2000; Shen *et al.*, 2000; Sanchez-Pernaute *et al.*, 2001; Muramatsu *et al.*, 2002; Bankiewicz *et al.*, 2006; Forsayeth *et al.*, 2006; Li *et al.*, 2006). In our study, cynomolgus monkeys (*Macaca fascicularis*) received the intravenous injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a selective toxin of dopaminergic neurons, once a week until a stable parkinsonian syndrome was achieved (Muramatsu *et al.*, 2002). Then mixtures of three AAV vectors that express TH, AADC, and GCH, respectively were stereotaxically injected into the unilateral putamen. Coexpression of the enzymes in the unilateral putamen resulted in remarkable improvement in manual dexterity on the contralateral to the vectors-injected side. Transduced cells were mainly medium spiny neurons and present in a large region of the putamen (>90% of the putamen). Microdialysis demonstrated that concentrations of dopamine in the vectors-injected putamen were increased in comparison to the control side. Moreover the level of dopamine was remarkably elevated after systemic administration of L-dopa with peripheral decarboxylase inhibitor. Monkeys showed no complications related to the AAV vector injection including dyskinesia.

Clinical Studies of AADC Gene Therapy

Two phase I clinical trials were conducted at the University of California San Francisco (UCSF) (Christine *et al.*, 2009) and Jichi Medical University (JMU)

(Muramatsu *et al.*, 2010) to evaluate the safety and potential efficacy of AAV vector-mediated gene delivery of AADC to the bilateral putamen in combination with the oral administration of L-dopa. Both trials confirmed the safety of the AAV vectors for clinical use in the human brain and the motor symptoms associated with PD were alleviated.

A low dose cohort of five patients received 1×10^{11} vector genome (vg) and a high dose cohort of five patients received 3×10^{11} vg of AAV vector expressing AADC (AAV-AADC) in the UCSF study. The mean improvements of the ten patients in the total score of unified Parkinson's disease rating scale (UPDRS) were 31% (10.5 points) in the "off" medication state and 32% (10 points) in the "on" medication state. The mean improvements on the motor score of UPDRS were 36% (12 points) in the "off" state and 28% (4.3 points) in the "on" state. Positron emission tomography (PET) using [^{18}F]fluoro-L-m-tyrosine (FMT), a tracer for AADC, revealed a 30 and 75% increase in Ki^c values in the putamen of the low-dose and high-dose cohort, respectively, at 6 months after the gene delivery.

Six patients received 3×10^{11} vg of AAV-AADC in the JMU study. The motor function in the "off" state improved to a mean of 46% (11.6 points) based on the motor score of UPDRS at six months after surgery. PET revealed a 56% increase in FMT activity, which persisted for up to 96 weeks (Fig. 2).

It is worth noting that motor function in the "off" state improved in both studies. The anti-parkinsonian effects observed after L-dopa administration have generally been recognized as short- and long-duration responses. The short-duration response roughly parallels the plasma L-dopa concentrations, while the long-duration response builds up over weeks and improves trough (worst) motor performance in the "off" state (Nutt, 2008). The pattern of the short-duration response to L-dopa did not change after gene therapy in the JMU study and the beneficial effect on the "off" state appears to be attributed to augmentation of the long-term response to L-dopa. Although the mechanism underlying the long-duration response is not sufficiently understood, improving the trough or "off" state motor function by augmenting the long-term response would likely reduce motor fluctuation.

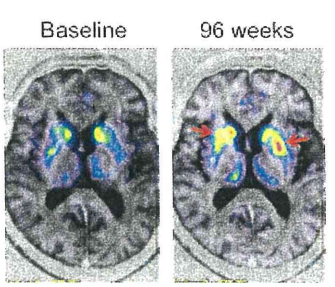

Fig. 2. FMT-PET images. Axial images at the level of the putamen are shown before and 96 weeks after gene therapy. The uptake of FMT was observed to increase after the AAV vector-mediated gene delivery of AADC (red arrows). FMT, 6-[^{18}F]fluoro-L-*m*-tyrosine, a tracer for AADC; PET, positron emission tomography.

Continuous Synthesis of Dopamine

Gene transfer of AADC alone in combination with oral L-dopa administration would be a safer strategy for initial clinical trials because excess production of dopamine could be avoided by reducing the dose of L-dopa. However, TH and GCH gene transfer into striatal cells may appear offer a more efficient method of supplying L-dopa continuously in comparison to the administration of exogenous L-dopa and avoid motor fluctuations associated with intermittent administration of L-dopa.

A phase I/II trial involving the triple gene transfer of TH, GCH and AADC into the bilateral putamen using a vector derived from the equine infectious anemia virus (EIAV) has been initiated at the Henri Mondor Hospital in France. Two dose levels have been evaluated in cohorts of three patients per dose (Oxford BioMedica, 2010). The first cohort of patients, treated at the lowest initial dose level, has now completed two-year assessments. The mean improvement from baseline in the motor score of UPDRS was 20%, in two of the three patients sustained effects of 30% improvement were observed without any increase in L-dopa dose. The third patient remained at levels of improvement similar to baseline. The maximum improvement in motor function at one year was 56% in the higher dose group and the mean was 28% for both doses relative to the patients' pre-treatment motor function.

Although it is difficult to package multiple genes in a single AAV vector because of the limited capacity on the size of the DNA (<5 Kb), one cell could be simultaneously transduced with different AAV vectors (Shen *et al.*, 2000; Muramatsu *et al.*, 2002). AAV vectors would therefore be the next choice for clinical trials for triple gene transfer of the TH, AADC, and GCH.

Other Strategies and Future Prospects

Gene therapy for PD has been tested in clinical trials due to the development of efficient viral vectors (Kaplitt, 2010). Two other strategies are developed in addition to dopamine replacement. One is designed to protect nigrostriatal projection by producing neurturin, a trophic factor for dopaminergic neurons, in the putamen. The other strategy is to modulate the neural activity along the output pathway of the basal ganglia by transducing the subthalamic nucleus with vectors expressing glutamic acid decarboxylase (GAD-65, GAD-67), a key enzyme for synthesis of the inhibitory transmitter γ-aminobutyric acid (GABA). Initial results of phase I studies indicate that these two strategies are also promising (Kaplitt *et al.*, 2007; Marks *et al.*, 2008). However, preliminary results of a phase II double-blind study of the neurturin strategy failed to show significant beneficial effects over the sham operation group, thus earlier intervention may be required for success of this kind of neuro-protective approach in future trials. Another phase I/II study is ongoing in which the patients receive AAV vectors expressing neurturin both in the putamen and the SNc.

There are several PD symptoms that do not respond well to L-dopa, such as cognitive dysfunction, depressive state, frozen gait, posture reflex disturbance and

sleep disturbance (Sethi, 2008). Effective therapeutic genes must be identified for the treatment of these symptoms, and then to be delivered into the appropriate areas of the brain. The development of vector constructs that avoid over-expression is also required for increasing safety (Li *et al.*, 2006). Gene therapy is therefore expected to become the therapy of choice for PD in the near future.

References

Aarsland, D., Marsh, L. and Schrag, A. (2009). Neuropsychiatric symptoms in Parkinson's disease. *Mov Disord*, **24**: 2175–2186.

Ahlskog, J. E. and Muenter, M. D. (2001). Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord*, **16**: 448–458.

Bankiewicz, K. S., Eberling, J. L., Kohutnicka, M., Jagust, W., Pivirotto, P., Bringas, J., Cunningham, J., Budinger, T. F. and Harvey-White, J. (2000). Convection-enhanced delivery of AAV vector in parkinsonian monkeys; *in vivo* detection of gene expression and restoration of dopaminergic function using pro-drug approach. *Exp Neurol*, **164**: 2–14.

Bankiewicz, K. S., Forsayeth, J., Eberling, J. L., Sanchez-Pernaute, R., Pivirotto, P., Bringas, J., Herscovitch, P., Carson, R. E., Eckelman, W., Reutter, B. and Cunningham, J. (2006). Long-term clinical improvement in MPTP-lesioned primates after gene therapy with AAV-hAADC. *Mol Ther*, **14**: 564–570.

Bruck, A., Aalto, S., Rauhala, E., Bergman, J., Marttila, R. and Rinne, J. O. (2009). A follow-up study on 6-[18F]fluoro-L-dopa uptake in early Parkinson's disease shows nonlinear progression in the putamen. *Mov Disord*, **24**: 1009–1015.

Christine, C. W., Starr, P. A., Larson, P. S., Eberling, J. L., Jagust, W. J., Hawkins, R. A., VanBrocklin, H. F., Wright, J. F., Bankiewicz, K. S. and Aminoff, M. J. (2009). Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology*, **73**: 1662–1669.

Daya, S. and Berns, K. I. (2008). Gene therapy using adeno-associated virus vectors. *Clin Microbiol Rev*, **21**: 583–593.

Dickson, D. W., Braak, H., Duda, J. E., Duyckaerts, C., Gasser, T., Halliday, G. M., Hardy, J., Leverenz, J. B., Del Tredici, K., Wszolek, Z. K. and Litvan, I. (2009). Neuropathological assessment of Parkinson's disease: refining the diagnostic criteria. *Lancet Neurol*, **8**: 1150–1157.

Driver, J. A., Logroscino, G., Gaziano, J. M. and Kurth, T. (2009). Incidence and remaining lifetime risk of Parkinson disease in advanced age. *Neurology*, **72**: 432–438.

Evans, A. H., Strafella, A. P., Weintraub, D. and Stacy, M. (2009). Impulsive and compulsive behaviors in Parkinson's disease. *Mov Disord*, **24**: 1561–1570.

Fan, D. S., Ogawa, M., Fujimoto, K. I., Ikeguchi, K., Ogasawara, Y., Urabe, M., Nishizawa, M., Nakano, I., Yoshida, M., Nagatsu, I., Ichinose, H., Nagatsu, T., Kurtzman, G. J. and Ozawa, K. (1998). Behavioral recovery in 6-hydroxydopamine-lesioned rats by cotransduction of striatum with tyrosine hydroxylase and aromatic L-amino acid decarboxylase genes using two separate adeno-associated virus vectors. *Hum Gene Ther*, **9**: 2527–2535.

Fearnley, J. M. and Lees, A. J. (1991). Ageing and Parkinson's disease: substantia nigra regional selectivity. *Brain*, **114 (Pt 5)**: 2283–2301.

Forsayeth, J. R., Eberling, J. L., Sanftner, L. M., Zhen, Z., Pivirotto, P., Bringas, J., Cunningham, J. and Bankiewicz, K. S. (2006). A dose-ranging study of AAV-hAADC therapy in Parkinsonian monkeys. *Mol Ther*, **14**: 571–577.

Fox, S. H. and Lang, A. E. (2008). Levodopa-related motor complications–phenomenology. *Mov Disord*, **23 Suppl 3**: S509–S514.

Hoshiga, M., Hatakeyama, K., Watanabe, M., Shimada, M. and Kagamiyama, H. (1993). Autoradiographic distribution of [^{14}C]tetrahydrobiopterin and its developmental change in mice. *J Pharmacol Exp Ther*, **267**: 971–978.

Kaplitt, M. G. (2010). Parkinson disease: Another player in gene therapy for Parkinson disease. *Nat Rev Neurol*, **6**: 7–8.

Kaplitt, M. G., Feigin, A., Tang, C., Fitzsimons, H. L., Mattis, P., Lawlor, P. A., Bland, R. J., Young, D., Strybing, K., Eidelberg, D. and During, M. J. (2007). Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet*, **369**: 2097–2105.

Li, X. G., Okada, T., Kodera, M., Nara, Y., Takino, N., Muramatsu, C., Ikeguchi, K., Urano, F., Ichinose, H., Metzger, D., Chambon, P., Nakano, I., Ozawa, K., Muramatsu, S. (2006). Viral-mediated temporally controlled dopamine production in a rat model of Parkinson disease. *Mol Ther*, **13**: 160–166.

Marks, W. J. Jr., Ostrem, J. L., Verhagen, L., Starr, P. A., Larson, P. S., Bakay, R. A., Taylor, R., Cahn-Weiner, D. A., Stoessl, A. J., Olanow, C. W. and Bartus, R. T. (2008). Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: An open-label, phase I trial. *Lancet Neurol*, **7**: 400–408.

Muramatsu, S., Fujimoto, K., Ikeguchi, K., Shizuma, N., Kawasaki, K., Ono, F., Shen, Y., Wang, L., Mizukami, H., Kume, A., Matsumura, M., Nagatsu, I., Urano, F., Ichinose, H., Nagatsu, T., Terao, K., Nakano, I. and Ozawa, K. (2002). Behavioral recovery in a primate model of Parkinson's disease by triple transduction of striatal cells with adeno-associated viral vectors expressing dopamine-synthesizing enzymes. *Hum Gene Ther*, **13**: 345–354.

Muramatsu, S., Tsukada, H., Nakano, I. and Ozawa, K. (2005). Gene therapy for Parkinson's disease using recombinant adeno-associated viral vectors. *Expert Opin Biol Ther*, **5**: 663–671.

Muramatsu, S. I., Fujimoto, K. I., Kato, S., Mizukami, H., Asari, S., Ikeguchi, K., Kawakami, T., Urabe, M., Kume, A., Sato, T., Watanabe, E., Ozawa, K. and Nakano, I. (2010). A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease. *Mol Ther* (Epub 2010 July 6).

Nagatsu, T. and Sawada, M. (2007). Biochemistry of postmortem brains in Parkinson's disease: historical overview and future prospects. *J Neural Transm Suppl*, 113–120.

Nagatsu, T., Horikoshi, T., Sawada, M., Nagatsu, I., Kondo, T., Iizuka, R., Narabayashi, H. (1987). Biosynthesis of tetrahydrobiopterin in parkinsonian human brain. *Adv Neurol*, **45**: 223–226.

Nandhagopal, R., Kuramoto, L., Schulzer, M., Mak, E., Cragg, J., Lee, C. S., McKenzie, J., McCormick, S., Samii, A., Troiano, A., Ruth, T. J., Sossi, V., de la Fuente-Fernandez, R., Calne, D. B. and Stoessl, A. J. (2009). Longitudinal progression of sporadic Parkinson's disease: A multi-tracer positron emission tomography study. *Brain*, **132**: 2970–2979.

Nutt, J. G. (2008). Pharmacokinetics and pharmacodynamics of levodopa. *Mov Disord* **23 Suppl 3**: S580–S584.

Nuytemans, K., Theuns, J., Cruts, M. and Van Broeckhoven, C. (2010). Genetic etiology of Parkinson disease associated with mutations in the SNCA, PARK2, PINK1, PARK7, and LRRK2 genes: A mutation update. *Hum Mutat*, **31**: 763–780.

Oxford BioMedica (2010). Two-year phase I/II results of ProSavin in Parkinson's disease. http://www.oxfordbiomedica.co.uk.

Sanchez-Pernaute, R., Harvey-White, J., Cunningham, J. and Bankiewicz, K. S. (2001). Functional effect of adeno-associated virus mediated gene transfer of aromatic L-amino acid decarboxylase into the striatum of 6-OHDA-lesioned rats. *Mol Ther*, **4**: 324–330.

Sethi, K. (2008). Levodopa unresponsive symptoms in Parkinson disease. *Mov Disord*, **23 Suppl 3**: S521–S533.

Shen, Y., Muramatsu, S., Ikeguchi, K., Fujimoto, K., Fan, D. S., Ogawa, M., Mizukami, H., Urabe, M., Kume, A., Nagatsu, I., Urano, F., Suzuki, T., Ichinose, H., Nagatsu, T., Monahan, J., Nakano, I. and Ozawa, K. (2000). Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic-L-amino-acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. *Hum Gene Ther*, **11**: 1509–1519.

Tsuji, S. (2010). Genetics of neurodegenerative diseases: Insights from high-throughput resequencing. *Hum Mol Genet*, **19**: R65–R70.

Voon, V., Fernagut, P. O., Wickens, J., Baunez, C., Rodriguez, M., Pavon, N., Juncos, J. L., Obeso, J. A. and Bezard, E. (2009). Chronic dopaminergic stimulation in Parkinson's disease: from dyskinesias to impulse control disorders. *Lancet Neurol*, **8**: 1140–1149.

Zhong, X. H., Haycock, J. W., Shannak, K., Robitaille, Y., Fratkin, J., Koeppen, A. H., Hornykiewicz, O. and Kish, S. J. (1995). Striatal dihydroxyphenylalanine decarboxylase and tyrosine hydroxylase protein in idiopathic Parkinson's disease and dominantly inherited olivopontocerebellar atrophy. *Mov Disord*, **10**: 10–17.

The current status of gene therapy for Parkinson's disease

Shin-ichi Muramatsu, M.D, Ph.D

Division of Neurology, Department of Medicine, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi 329-0498, JAPAN

ABSTRACT

The recent development of viral vectors, especially vectors derived from adeno-associated virus (AAV), has translated gene therapy for Parkinson's disease (PD) from animal experiments into clinical trials. The current gene therapy protocols used are based on three major strategies. The first protocol involves local production of dopamine via the introduction of dopamine-synthesizing enzyme genes into the putamen. The aromatic L-amino acid decarboxylase (AADC) gene has been transferred in this manner with the aim of efficiently converting orally administered L-dopa. The delivery of triple genes including tyrosine hydroxylase (TH), guanosine triphosphate cyclohydrolase I (GCH) and AADC is also being undertaken, and is aimed at continuously supplying dopamine into the putamen. The second protocol involves the protection of nigrostriatal projections via the production of neurturin, a trophic factor for dopaminergic neurons in the putamen. The final method includes the modulation of neural activity along the output pathway of the basal ganglia by transducing the subthalamic nucleus with vectors expressing glutamic acid decarboxylase (GAD-65, GAD-67), a key enzyme required for the synthesis of the inhibitory transmitter γ-aminobutyric acid (GABA). The initial results of phase 1 studies using AAV vectors have not only confirmed the safety of these vectors, but have also revealed the alleviation of motor symptoms associated with PD.

KEY WORDS : Adeno-associated virus, Aromatic L-amino acid decarboxylase, Neurturin, Glutamic acid decarboxylase, Positron emission tomography
Corresponding Author : Shin-ichi Muramatsu, M.D, Ph.D, E-mail : muramats@jichi.ac.jp; Tel: +81-285-58-7352, Fax: +81-285-44-5118

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Introduction

Almost two decades have passed since the first gene therapy clinical trial was conducted for the treatment of adenosine deaminase deficiency at the National Institute of Health in the United States in 1990. Although gene therapy appeared to be a ground breaking form of medical treatment at the time, it has not proven to be as successful in treating disease as initially anticipated. A patient with ornithine transcarbamylase deficiency died of systemic inflammatory response syndrome (SIRS) following the administration of a large quantity of adenoviral vector into the hepatic artery.[1] In addition, some children with X-linked severe combined immunodeficiency (X-SCID) developed leukemia due to the activation of an oncogene after gene therapy using a retroviral vector.[2,3] These reports describing the severe, adverse effects of gene therapy dampened the excitement underlying the success of gene therapy treatment to some extent. However, encouraging results have been obtained more recently with clinical studies for Parkinson's disease (PD).[4-7]

Table. Gene therapy clinical trials for Parkinson's disease.

Gene	AADC		TH/GCH/AADC	Neurturin		GAD	
Function	Convert L-dopa to dopamine		Synthesis of dopamine from tyrosine	Neurotrophic factor for dopaminergic neurons		Synthesis of inhibitory neurotransmitter GABA	
Vector	AAV		EIAV	AAV		AAV	
Phase	I		I	I	II	I	II
Institute	UCSF[7]	JMU	Henri Mondor Hospital[22]	UCF[6]	Multi-center[26]	NYP Hospital[5]	Multi-center
Dose	$9x10^{10}$ $3x10^{11}$	$3x10^{11}$	1x 2x	$1.3x10^{11}$ $5.4x10^{11}$	$5.4x10^{11}$ Sham	$3.5x10^{9}$ $1x10^{10}$ $3.5x10^{10}$	$1x10^{11}$ Sham
Number of subjects	10	6	6	12	54	12	40
Target	Putamen (Bilateral)		Putament (Bilateral)	Putamen (Bilateral)		STN (Unilateral)	STN (Bilateral)
PET tracer	[^{18}F] fluro-m-tyrosine			[^{18}F] fluro-DOPA		[^{18}F] fluro-deoxyglucase	

AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated virus; EIAV, equine infectious anemia virus; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GCH, guanosine triphosphate cyclohydrolase I; TH, tyrosine hydroxylase; STN, subthalamic nucleus. Dose of AAV vectors are represented as vector genome/patient.

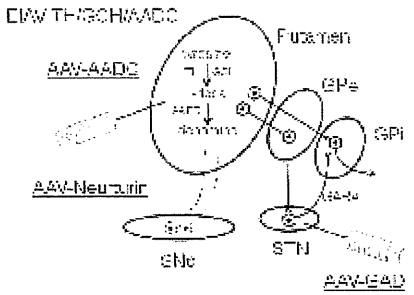

Figure. Current gene therapy strategies for the treatment of Parkinson's disease

Three gene therapy strategies are currently available for the treatment of (PD. 1) production of dopamine. AAV vectors expressing AADC or EIAV vector expressing TH, GCH and AADC are infused into the putamen. 2) Protection of the nigrostriatal pathway. The neurotrophic factor neurturin is produced continuously in the putamen. 3) Modification of neuronal activity in the STN by introducing the GAD gene.

AADC, aromatic L-amino acid decarboxylase; AAV, adeno-associated virus; EIAV, equine infectious anemia virus; GABA, γ-aminobutyric acid; GAD, glutamic acid decarboxylase; GCH, guanosine triphosphate cyclohydrolase I; GPe, globus pallidus external segment; GPi, globus pallidus internal segment; TH, tyrosine hydroxylase; SNc, substantia nigra pars compacta; STN, subthalamic nucleus.

Adeno-associated virus vectors

Technology that efficiently introduces a therapeutic gene into target cells is essential for successful gene therapy. Viral vectors, in particular vectors derived from adeno-associated virus (AAV), have been shown to be suitable for the transduction of neurons in the mammalian brain using stereotaxic surgery.[8] AAVs are small (25 nm), single stranded DNA viruses that belong to Parvoviridae.[9] No clear pathogenicity in humans has been reported, and many adults synthesize antibodies against AAVs following latent infection in childhood.[10,11] The AAV genome exists in episomes in the nucleus and is rarely incorporated in the chromosomes. To date, more than one hundred genotypes have been defined for primate AAVs. The vast majority of vectors used in clinical applications are derived from serotype 2 (AAV2).

AAV2 has a 4.7-kb genome, both ends of which contain hairpin structures called inverted terminal repeats (ITR). A region encoding the non-structural protein Rep and capsid protein VP are also present. These regions for Rep and VP can be substituted with a therapeutic gene when constructing vectors. AAV vectors express an exogenous gene in the brain for long term use (more than seven years) and do not produce any significant inflammatory or immuno-logical reactions.[12] Although the size of the genes that can be inserted into the AAV2 genome is limited to less than 4.5 kb, most of the therapeutic genes fit into this range. It has also been shown that several AAV vectors are able to infect one cell at the same time and can express plural genes.[13] AAV vectors have been applied in clinical trials for numerous disorders including hemophilia,[14] cystic fibrosis[15] and retinal degeneration.[16] To date, adverse effects due to the administration of the vectors themselves have not been found. In addition to gene therapy, AAV vectors have also been recently used as a genetic tool in the neurosciences.[17,18]

Gene transfer of dopamine-synthesizing enzymes

Dopamine is synthesized in the brain from diet-derived L-tyrosine. Three enzymes are essential in this production process.[19] Tyrosine hydroxylase (TH) is the rate-limiting enzyme that converts L-tyrosine to L-3, 4-dihydroxy-phenylalanine (L-dopa). Guanosine triphosphate cyclohydrolase I (GCH) is the rate-limiting enzyme that synthesizes the essential TH co-factor tetrahydrobiopterine (BH_4), while aromatic L-amino acid decarboxylase (AADC) converts L-dopa to dopamine. These three enzymes are transported from the substantia nigra in an anterograde manner to the striatum. A severe loss of the nerve terminals in advanced Parkinson's disease (PD) is associated with an 80-95% depletion of striatal enzyme activity. Gene transfer of TH, GCH and AADC,[13,20] or AADC alone,[12,21] into the striatal neurons has led to behavioral recovery in primate models of PD.

Two phase I clinical trials were conducted at the University of California San Francisco (UCSF) and Jichi Medical University (JMU) to evaluate the safety and potential efficacy of AAV vector-mediated gene delivery of AADC to the bilateral putamen. Alleviation of motor symptoms associated with PD was observed in both trials. In the UCSF study,[7] a low dose cohort of five patients received 110^{11} vector genome (vg) and a high dose cohort of five patients received 310^{11} vg of AAV-AADC. The mean improvements of the ten patients in the total score of unified Parkinson's disease rating scale (UPDRS) were 31% (10.5 points) in the off-state and 32% (10 points) in the on-state. The mean improvements in the motor score of UPDRS were 36% (12 points) in the off-state and 28% (4.3 points) in the on-state. Positron emission tomography (PET) using [^{18}F]fluoro-m-tyrosine (FMT), a tracer for AADC, revealed a 30 and 75% increase in K^{ic} values in the putamen of the low-dose and high-dose cohort, respectively, at 6 months after the gene delivery. In the JMU study, six patients received 310^{11} vg of AAV-AADC. Motor function in the off-state improved to a mean of 46% (11.6 points) based on the UPDRS scores at six months after surgery, without any apparent changes in the short-duration response to levodopa. PET revealed a 56% increase in FMT activity, which persisted for up to 96 weeks (manuscript in preparation). Phase 2 trials of AAV-AADC are currently in the planning stages.

Using a vector derived from the equine infectious anemia virus (EIAV), a type of lentivirus, a phase I/II trial involving the triple gene transfer of TH, GCH and AADC into the bilateral putamen has been initiated at the Henri Mondor Hospital in France.[22] Two dose levels have been evaluated in cohorts of three patients per dose. All patients treated at the second dose level have completed their six-month assessments and have shown improvement in motor function in the off-state when evaluated on the motor score of UPDRS. The mean improvement was 34% relative to the patients' pre-

treatment motor function.[22] Studies into the high dose (5 times the lower dose) cohort are planned. Using this triple gene transfer, dopamine will be continuously produced in the putamen and may reduce "wearing-off" effects by avoiding pulsatile stimulation of dopaminergic receptors.

Gene transfer of neurturin

An alternative approach to gene therapy for PD is the protection of the nigrostriatal pathways from progressive degeneration by providing genes encoding for neurotrophic factors. Neurturin is a natural analog of glial cell line-derived neurotrophic factor (GDNF).[23] GDNF is a small glycoprotein that provides strong trophic support for the dopaminergic neurons. However, GDNF protein has limited usefulness as a therapeutic agent due to its short duration of activity and poor ability to cross the blood-brain barrier. In animal models of neurotoxin induced PD, viral vector-mediated gene delivery halts ongoing degeneration of the nigrostriatal pathway, resulting in functional recovery, even after substantial numbers of dopaminergic cells have been depleted.[24,25]

A phase I gene therapy trial that introduced the neurturin gene into the bilateral putamen was conducted at the UCSF[6]. The first six patients entered into the study receiving a dose of 310^{11} vg, while the next six patients received a dose of 5.410^{11} vg of AAV vector. A mean improvement of 36% (14 points) in the UPDRS motor score in the off-state and a mean increase of 25% (2.3 h) in on time without troublesome dyskinesia were observed one year after surgery. Subsequently, a phase II double–blind control study was undertaken at nine academic institutions in the United States, and in which one-third of a total of 54 patients received sham surgery (partial burr hole without infusion of vectors). Significant differences were not obtained in terms of the degree of motor performance between the gene transfer group and the control group at 12 months, although some treatment effects have since been observed in 30 subjects followed for 18 months under blind conditions.[26] Analysis of post-mortem brain tissue from two patients treated with AAV vectors demonstrated that neurturin was expressed in the putamen, but not in the substantia nigra. Earlier intervention may be required for success in future trials of this kind of neuro-protective approach, as most of the nigrostriatal fibers have already been lost when the PD symptoms appear.

Gene transfer of glutamic acid decarboxylase

In PD, depletion of dopamine in the striatum leads to an increase in the activity of the subthalamic nucleus (STN).[27] The increased excitatory drive of the STN to the internal portion of the globus pallidus (GPi) and to the substantia nigra pars reticulata (SNr) then exerts inhibitory effects on the thalamo-cortical projection and brainstem nucleus, resulting in motor symptoms such as bradykinesia and rigidity. During the last two decades, deep brain stimulation of the STN, which is thought to modify STN output by high-frequency stimulation, has become routine treatment for advanced PD patients and has resulted in the improvement of motor function. Gene transfer of glutamic acid decarboxylase (GAD-65 and GAD-67), a rate-limiting enzyme required for the synthesis of inhibitory transmitter γ-aminobutyric acid (GABA), into the STN is aimed at converting excitatory output to inhibitory output, thus, obtaining a similar effect to electrical stimulation.[28]

An open-label phase I clinical trial has been conducted at the New York Presbyterian Hospital.[5] A total of 12 patients in three dose-escalation cohorts received AAV-GAD into the unilateral STN contra-lateral to more severe motor symptoms. At 12 months after the vector infusion, the mean improvement on motor score of UPDRS was 27% in the off-state and 24% in the on-state.[5] A PET scan using [^{18}F]fluorodeoxyglucose (FDG) as a tracer revealed a decrease in uptake into the ventrolateral nucleus (VL) and dorsomedial nucleus (MD) of the thalamus on the operated side, and an increased uptake in the ipsilateral premotor and motor cortical regions.[29]

The underlining physiological changes in PD include, in addition to increases in the firing rate of the STN, the tendency of pallidal neurons to fire in more irregular patterns, as well as abnormal oscillatory synchronization in the basal ganglia.[30] Thus, mechanisms underlying how DBS and AAV-GAD gene therapy is effective remain to be defined.[31] A double-blinded phase II study of AAV-GAD infusion into the bilateral STN is currently underway for 40 subjects, including 20 subjects that received sham surgery.

Future prospects

Owing to the development of efficient viral vectors, gene therapy for PD has been tested in clinical trials, with the initial results of phase I studies proving encouraging. In contrast to cell transplantation, immunosuppressive drugs are not necessary for the current gene therapy strategies. If the primary purpose of treatment is the supplementation of dopamine into the striatum for improving motor performance, then gene therapy appears to be simpler than cell transplants. Cell therapy may prove useful in treating Parkinsonism including cerebral ischemia, striato-nigral degeneration and cortico-basal degeneration, where neurons in the striatum are damaged. However, several PD symptoms that L-dopa is not effective at rescuing including cognitive dysfunction, depressive state, frozen gait, posture reflex disturbance and sleep disturbance have also been reported.[32] For the treatment of these symptoms, effective therapeutic genes must be identified and delivered into the appropriate areas of the brain. Development of vector constructs that avoid over-expression is also required for increasing safety.[33] Although AAV and EIAV are reported to be non-pathogenic for humans, the long-term safety must be confirmed. It is expected that in the near future gene therapy will become the general choice for the treatment of PD.

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References

1. Wilson JM, Lessons learned from the gene

therapy trial for ornithine transcarbamylase deficiency. Mol Genet Metab 2009:96:151-7.

2. Hacein-Bey-Abina S, Garrigue A, Wang GP, *et al.* Insertional oncogenesis in 4 patients after retrovirus-mediated gene therapy of SCID-X1. J Clin Invest 2008:118:3132-42.
3. Howe SJ, Mansour MR, Schwarzwaelder K, *et al.* Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients. J Clin Invest 2008:118:3143-50.
4. Kaplitt MG, Parkinson disease: Another player in gene therapy for Parkinson disease. Nat Rev Neurol 2010; 6:7-8.
5. Kaplitt MG, Feigin A, Tang C *et al.* Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. Lancet 2007; 369:2097-105.
6. Marks WJ, Jr., Ostrem JL, Verhagen L *et al.* Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. Lancet Neurol 2008; 7: 400-8.
7. Christine CW, Starr PA, Larson PS *et al.* Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. Neurology 2009; 73:1662-9.
8. Muramatsu S, Fujimoto F, Katoa S *et al.* A phase 1 study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. Mol Ther 2010: in press.
9. Shen Y, Post L, Viral vectors and their applications. Knipe DM, Howley PM, ed. Fields' virology. Philadelphia. Lippincott Williams & Wilkins, 2006; 1: 547-50.
10. Boutin S, Monteilhet V, Veron P, *et al.* Prevalence of serum IgG and neutralizing factors against adeno-associated virus types 1, 2, 5, 6, 8 and 9 in the healthy population: implications for gene therapy using AAV vectors. Hum Gene Ther 2010: in press.
11. Ito T, Yamamoto S, Hayashi T, *et al.* A convenient enzyme-linked immunosorbent assay for rapid screening of anti-adeno-associated virus neutralizing antibodies. Ann Clin Biochem 2009; 46:508-10.
12. Bankiewicz KS, Forsayeth J, Eberling JL *et al.* Long-term clinical improvement in MPTP-lesioned primates after gene therapy with AAV-hAADC. Mol Ther 2006; 14:564-570.
13. Shen Y, Muramatsu S, Ikeguchi K, *et al.* Triple transduction with adeno-associated virus vectors expressing tyrosine hydroxylase, aromatic L-amino acid decarboxylase, and GTP cyclohydrolase I for gene therapy of Parkinson's disease. Hum Gene Ther 2000; 11: 1509-19.
14. Hasbrouck NC, High KA. AAV-mediated gene transfer for the treatment of hemophilia B: problems and prospects. Gene Ther 2008; 15:870-5.
15. Mueller C, Flotte TR. Gene therapy for cystic fibrosis. Clin Rev Allergy Immunol 2008;35:164-78.
16. Maguire AM, High KA, Auricchio A, *et al.* Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. Lancet 2009;374:1597-605.
17. Fukushima F, Nakao K, Shinoe T, *et al.* Ablation of NMDA receptors enhances the excitability of hippocampal CA3 neurons. PLoS One 2009; 4 : e3993.
18. Kadkhodaei B, Ito T, Joodmardi E, *et al.* Nurr1 is required for maintenance of maturing and adult midbrain dopamine neurons. J Neurosci 2009; 29:15923-32.
19. Vinish M, Milstein J. Non-Motor aspects of Parkinso's disease. Annals of Neurosciences 2009;16 (4): 176-179.
20. Muramatsu S, Fujimoto K, Ikeguchi K *et al.* Behavioral recovery in a primate model of Parkinson's disease by triple transduction of striatal cells with adeno-associated viral vectors expressing dopamine-synthesizing enzymes. Hum Gene Ther 2002; 13:345-54.
21. Bankiewicz KS, Eberling JL, Kohutnicka M *et al.* Convection-enhanced delivery of AAV vector in parkinsonian monkeys; in vivo detection of gene expression and restoration of dopaminergic function using pro-drug approach. Exp Neurol 2000; 164:2-14.
22. Oxford Biomedica. Oxford Biomedica announces update on phase I/II study of ProSavin in Parkinson's disease and publication of preclinical results. Available from: http://www.oxfordbiomedica.co.uk (15 October 2009).
23. Horger BA, Nishimura MC, Armanini MP *et al.* Neurturin exerts potent actions on survival and function of midbrain dopaminergic neurons. J Neurosci 1998; 18:4929-37.
24. Wang L, Muramatsu S, Lu Y *et al.* Delayed delivery of AAV-GDNF prevents nigral neurodegeneration and promotes functional recovery in a rat model of Parkinson's disease. Gene Ther 2002; 9:381-9.
25. McGrath J, Lintz E, Hoffer BJ *et al.* Adeno-associated viral delivery of GDNF promotes recovery of dopaminergic phenotype following a unilateral 6-hydroxydopamine lesion. Cell Transplant 2002;11:215-27.
26. Ceregene. Ceregene presents additional clinical data from phase 2 trial of CERE-120 for Parkinson's disease. Available from: http://www.ceregene.com/ (May 27, 2009).
27. Obeso JA, Rodriguez-Oroz MC, Benitez-Temino B, *et al.* Functional organization of the basal ganglia: therapeutic implications for Parkinson's disease. Mov Disord 2008; 23 Suppl 3: S548-59.
28. During MJ, Kaplitt MG, Stern MB, *et al.* Subthalamic GAD gene transfer in Parkinson disease patients who are candidates for deep brain stimulation. Hum Gene Ther 2001;12:1589-91.
29. Feigin A, Kaplitt MG, Tang C, *et al.* Modulation of metabolic brain networks after subthalamic gene therapy for Parkinson's disease. Proc Natl Acad Sci USA 2007;104:19559-64.
30. Montgomery EB, Jr. Basal ganglia pathophysiology in Parkinson's disease. Ann Neurol 2009; 65:618; author reply 618-9.
31. Gradinaru V, Mogri M, Thompson KR, *et al.* Optical Deconstruction of Parkinsonian Neural Circuitry. Science 2009; 324:354-9.
32. Sethi K. Levodopa unresponsive symptoms in Parkinson disease. Mov Disord 2008; 23 Suppl 3: S521-33.
33. Li XG, Okada T, Kodera M *et al.* Viral-mediated temporally controlled dopamine production in a rat model of Parkinson disease. Mol Ther 2006; 13:160-6.

ALSにおけるRNA editing異常

日出山 拓人（ひでやま たくと） 東京大学大学院医学系研究科脳神経医学専攻神経内科学
東京大学保健・健康推進本部
郭 伸（かく しん） 東京大学大学院准教授
医学系研究科脳神経医学専攻神経内科学

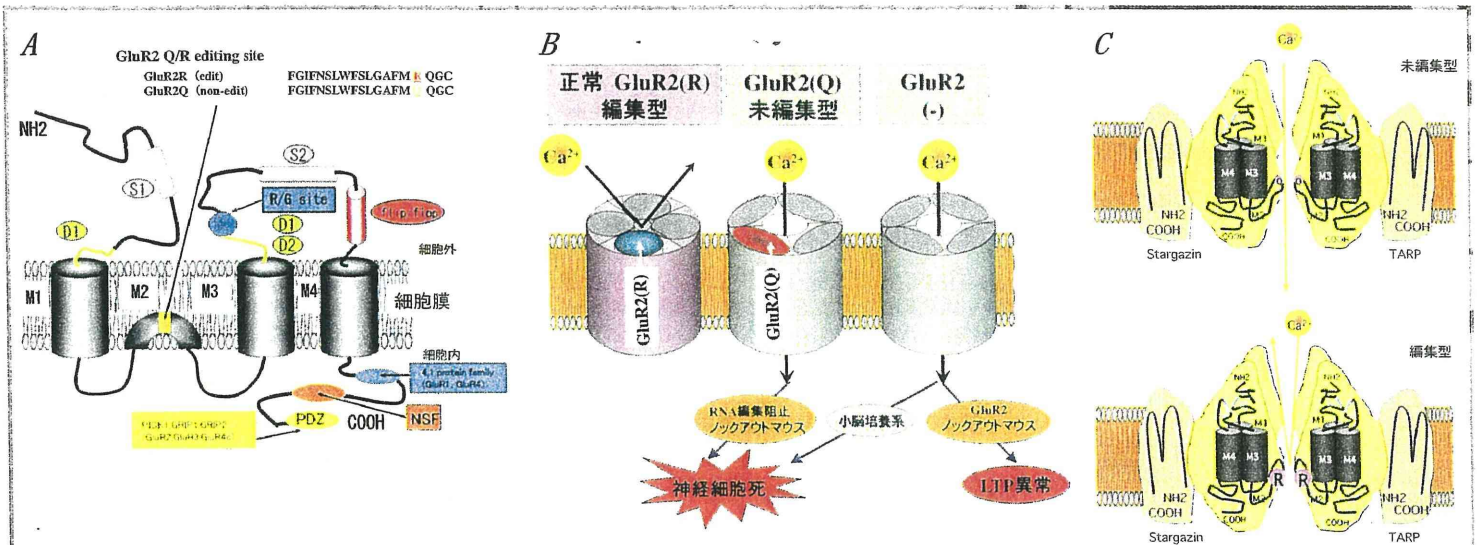

図 1　GluR2 サブユニットと Ca^{2+} 透過性による興奮性神経細胞死

A）AMPA 受容体の各サブユニットの M 2 には Q/R 部位と呼ばれる部位があり，同部位は GluR2 以外ではグルタミン(Q)であるのに対して，GluR2 だけはアルギニン(R)である．しかしゲノムレベルでは，GluR2 も Q をコードしている CAG という配列である．R となる理由は RNA へ転写後，mRNA になる前にアデノシン(A)がイノシン(I)へと塩基置換され，CAG(Q)から CIG(R)へのアミノ酸置換が生じるためである．リボソームで I はグアノシン(G)と同等であると見なされるため，CIG は CGG と見なされ R として翻訳される．この現象は RNA 編集と呼ばれる．B）サブユニットの中で，チャネルの Ca^{2+} 透過性決定に重要な役割を果たしているのは GluR2 である．AMPA 受容体の Ca^{2+} 透過性は GluR2 の有無によって決定されている．AMPA 受容体を構成する 4 つのサブユニットのうち GluR2 を 1 つ以上含む受容体は，Ca^{2+} 透過性が低いのに対し，Glu R1，3，4 のサブユニットだけで構成された受容体は，高い Ca^{2+} 透過性を示す．C）Q/R 部位が Ca^{2+} 透過性決定に重要なのはチャネルポアに面しており，陽電化の R が Ca^{2+} を弾くのに対して電気的に中性の Q ではこの作用が弱いためであると考えられている．

はじめに

筋萎縮性側索硬化症(amyotrophic lateral sclerosis：ALS)は，90％が家族歴を認めない孤発性で，原因不明かつ治療方法のない神経変性疾患である．著者らは，孤発性 ALS 脊髄運動ニューロンでは，グルタミン酸受容体である AMPA(α-3-hydroxy-5-methyl-4-isoxazole propionic acid)受容体チャネルの Ca^{2+} 透過性を亢進させる分子変化が疾患特異的，細胞選択的に起こっていることを見出し，この分子変化が神経細胞死の直接原因になることから，ALS の病因と考えられることを明らかにした[1]．この分子変化を生じる上流の現象が RNA 編集(RNA editing)である．

孤発性 ALS における興奮性神経細胞死と AMPA 受容体

錐体路の神経伝達物質はグルタミン酸で，脊髄運動ニューロンもグルタミン酸受容体が豊富に発現する．グルタミン酸による興奮が過剰になるとイオン透過性亢進により内部恒常性の破綻を来し，細胞死のカスケードが働く，というのが興奮性神経細胞死のメカニズムである．主に興奮性神経細胞死は，虚血や低血糖，外傷，てんかん重積などの急性の神経細胞死に働くと考えられていた．一方で，培養細胞系，*in vivo* 動物実験系で急性には神経細胞死を引き起こさない薄い濃度でも，受容体が長期間持続的に興奮することで遅発性の神経細胞死が起こることが明らかにされた．このことから，慢性に進行する神経変性疾患である ALS において遅発性の神経細胞死の関与が注目されるようになった．さらに，運動ニューロンは興奮性細胞死に脆弱であり，過剰な Ca^{2+} 流入による細胞内 Ca^{2+} 濃度の持続的上昇が細胞死の原因と考えられている．神経細胞内 Ca^{2+} 濃度上昇の機構には AMPA 受容体の Ca^{2+} 透過性の関与が大きい．

AMPA 受容体と RNA 編集

AMPA 受容体は 4 種のサブユニット(GluR1-4)の単独，または様々な組み合わせからなる四量体である．AMPA 受容体の Ca^{2+} 透過性を決定する因子には，GluR2 サブユニット，GluR2 サブユニットの RNA 編集(特に Q/R 部位)，flip/flop splicing varient，AMPA 受容体密度が挙げられる．しかし，Ca^{2+} 透過性を規定する因子の全てが細胞死に直接関連するわけではない．GluR2 のノックアウトにより小脳培養系では細胞死が生じるが[2]，GluR2 ノックアウトマウスでは LTP 異常のみで細胞死は生じない[3]（図 1 B）．Flip/flop isoform は細胞死を生じないが，AMPA 受容体毒性を強める[4]．AMPA 受容体密度の変化のみでは神経細胞死を生じない[5]．これに対し，RNA 編集を阻止した変異マウスでは生後 20 日以内に痙攣による個体死が認められる[6]（図 1 B）．

AMPA 受容体サブユニット発現と ALS の運動ニューロン死

これらの結果をふまえ，神経細胞死に関連する分子変化である GluR2 の減少ないし GluR2 Q/R 部位の編集率低下の有無を著者らのグループは検討した．Kwak らは laser microdissector を用いて凍結剖検脊髄組織から単一運動神経細胞を切り出し，RT-PCR 法により GluR2 mRNA 由来の cDNA を増幅し，AMPA 受容体各サブユニット mRNA を定量する方法および GluR2 Q/R 部位 RNA 編集率を定量する方法を確立した．そして，孤発性 ALS 脊髄運動

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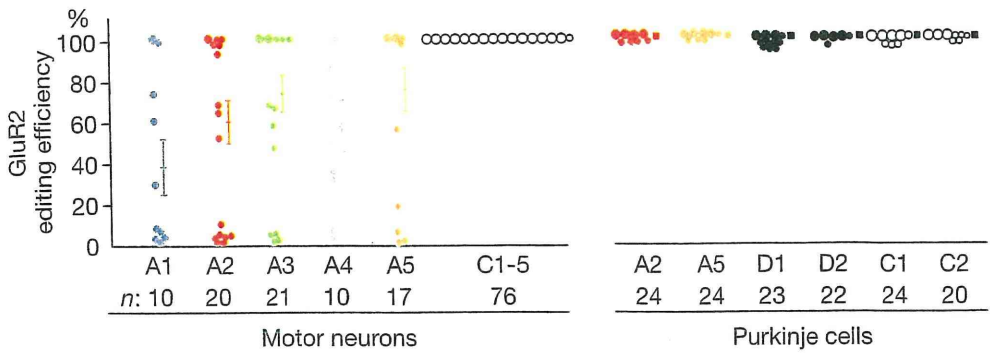


図 2　単一神経細胞における GluR2 Q/R 部位 RNA 編集率
(Kawahara ら[1]より改変)

各点(大きな点は 5 細胞，小さな点は 1 細胞)は，ALS 群 5 例(A1-A 5)，コントロール群 5 例(C 1-C 5)の単一脊髄運動ニューロンにおける GluR2 Q/R 部位の RNA 編集率と，ALS 群 2 例(A 2, A 5)，Dentatorubral-pallidoluysian atrophy(DRPLA)群 2 例(D 1, D 2)，コントロール群 2 例(C 1，C 2)の単一小脳プルキンエ細胞の編集率を表している．平均値±標準誤差と解析した細胞数(n)も示した．運動ニューロンにおける正常コントロール 76 個の内訳は，C 1：28，C 2：12，C 3：13，C 4：12，C 5：11 である．運動ニューロンでは正常コントロール群のすべての細胞において，例外なく編集率は 100%であった．これに対して，ALS 群では解析した 5 例すべてにおいて，編集率は 0〜100%まで大きくばらつき，正常コントロール群と比較し有意に低下していた(Mann-Whitney U test，$p<0.001$)．一方，小脳プルキンエ細胞における編集率については，ALS 群，DRPLA 群とコントロール群の間には有意差はない(Mann-Whitney U test，$p>0.05$)．また，他の変性疾患の病変組織や他の運動ニューロン病(SBMA)では，編集率の低下は認めない．このような選択特異性を生む機序としては，脊髄運動ニューロンの AMPA 受容体総 mRNA 発現量および GluR2 サブユニット比率が，他のニューロンに比べて低く，もともと Ca^{2+} 透過性 AMPA 受容体の割合が多いこと，したがって RNA 編集低下の影響を受けやすいことが挙げられる．

表；既知の A→I RNA 編集部位および神経・精神疾患

	基質名	編集部位	編集により変化する性質	マウス全脳由来 mRNA 編集効率	担当酵素	疾患との関連
AMPA 型グルタミン酸受容体	GluR2	Q/R 部位	受容体チャネルの Ca^{2+} 透過性	100%	ADAR2	ALS(編集効率低下) 悪性膠芽腫(編集効率低下†)
	GluR2, GluR3, GluR4	R/G 部位	受容体チャネルの脱感作時間	GluR2：75%，GluR3：90%，GluR4：45%	ADAR1，ADAR2	
カイニン酸型グルタミン酸受容体	GluR5	Q/R 部位	受容体チャネルの Ca^{2+} 透過性	64%	ADAR1，ADAR2	
	GluR6	Q/R 部位，I/V 部位，Y/C 部位	受容体チャネルの Ca^{2+} 透過性	Q/R 部位：86%，I/V 部位：87%，Y/C 部位：90%	ADAR1，ADAR2	側頭葉てんかん (Glu R6 Q/R 部位編集効率上昇)
セロトニン受容体	5-HT_{2C}R	A〜E 部位	G タンパク結合効率	A 部位：75%，B 部位：80%，C 部位：15%，D 部位：70%，E(C′)部位	A，B 部位：ADAR1，C，D 部位：ADAR2	うつ病 (5-HT_{2C}受容体 D，E 部位編集効率上昇)
膜電位依存型カリウムチャネル	Kv1.1	S6 ドメイン内	チャネルの不活化	48%	ADAR2	Episodic ataxia type I (編集異常は不明)††
RNA 編集酵素	ADAR2	自己編集部位	酵素活性	15%	ADAR2	

*正常の白質組織と比較すると低下していない．**本態は point mutation であり，RNA 編集異常の関与が考えられている．

RNA 編集の意義としては，RNA 編集により特定のアミノ酸が置換され，翻訳産物の大きさや分子配列が変化することで翻訳産物が別の機能を獲得する場合がある．RNA 編集は，シトシン(C)→ウラシル(U)とアデノシン(A)→イノシン(I)の 2 種類が知られている．C→U の RNA 編集は植物から哺乳類まで保存され，植物ミトコンドリアのある遺伝子には開始コドンがないが，ACG→AUG と編集されることにより開始コドンが出来，翻訳可能な配列になる correctional editing などが知られている．哺乳類では C→U 編集は活発ではない．A→I 編集は中枢神経系で活発に起こっており，ヒト D 型肝炎ウイルス，線虫，ショウジョウバエ，イカ，哺乳類で確認されており，太古から存在している現象と考えられる．GluR2 Q/R 部位が哺乳類で最初に見つかった編集部位であり，この他に，表のように編集部位は受容体やイオンチャネルに多く見られ，R/G 部位は胎児発生の過程で徐々に増加し，イオンチャネルの脱感作を調節し，GluR6 の I/V，Y/C 部位はイオンチャネルの電気抵抗性に関与している．神経変性疾患の他，てんかん，精神疾患(統合失調症，うつ病，自殺)，遺伝性皮膚疾患(遺伝性対側性色素異常症)での検討が行われているが，現時点でヒトの疾患との関連が明らかにされたものは，著者らのグループで発見した ALS の運動ニューロンのみである．

ニューロンの単一神経細胞レベルで，GluR2 mRNA 発現量に有意な減少がないこと[7]，および ALS の単一運動ニューロンに GluR2 Q/R 部位 RNA 編集率低下という RNA 編集異常が細胞選択的かつ疾患特異的に見られることを報告した[1,8](図 2)．

RNA 編集と RNA 編集酵素 ADAR2

この GluR2 Q/R 部位の RNA 編集を特異的に制御しているのが RNA 編集酵素 adenosine deaminase acting on RNA type 2(以下 ADAR2)である[9]．一般に，あるタンパクへと翻訳される mRNA は遺伝子から転写された後，スプライシングを受け，イントロン部分が切り出されて形成される．遺伝情報は正確に転写されなければ生死に関わるような重大な障害を来す可能性があるが，遺伝子から mRNA が形成される過程で遺伝情報が書き換えられることがある．これを RNA 編集(RNA editing)と呼ぶ[10]．RNA 編集のうち特に転写後のコドン置換を伴う場合には，タンパクの機能を変える場合があり，生物学的にも重要な反応である．孤発性 ALS で認められるような A→I 編集は中枢神経系で最も活発に生じている(表参照)．中でも GluR2 の Q/R 部位は，胎生期から成熟期に至るまでほぼ 100%編集されているという点で特異的であり，これほど高い編集率を示す部位は他には見いだされていない．

著者らのグループは ADAR2 コンディショナルノックアウトマウスを作成し，神経細胞死を生じるかどうか検討中であり，ADAR2 の機能解明が孤発性 ALS の治療方法開発につながると期待している．

文　献

1) Kawahara Y, et al. Nature. 2004；427：801.
2) Iihara K, et al. J Neurosci. 2001；21：2224-39.
3) Jia Z, et al. Neuron. 1996；17：945-56.
4) Sun H, et al. J Neurochem. 2006；98：782-91.
5) Kwak S, et al. J Mol Med. 2005；83：110-20.
6) Brusa R, et al. Science. 1995；270：1677-80.
7) Kawahara Y, et al. J Neurochem. 2003；85：680-9.
8) Takuma H, et al. Ann Neurol. 1999；46：806-15.
9) Higuchi M, et al. Nature. 2000；406：78-81.
10) Keegan LP, et al. Nat Rev Genet. 2001；2：869-78.

グルタミン酸受容体と孤発性筋萎縮性側索硬化症

日出山拓人，山下雄也，郭　伸

孤発性筋萎縮性側索硬化症（amyotrophic lateral sclerosis，以下ALS）は，原因不明の神経変性疾患である．近年，私たちのグループではグルタミン酸受容体サブユニットであるGluR2のRNA編集異常が疾患特異的に生じていることを，孤発性ALS例と疾患対照，正常対照例の検討から明らかにした．これは原因解明とともに疾患特異的な治療方法を開発できる可能性を示唆するものである．本稿では，これまでの歴史的な経緯と最近の知見について述べてみたい．

はじめに：筋萎縮性側索硬化症とは

筋萎縮性側索硬化症（amyotrophic lateral sclerosis：以下，ALS）は，運動ニューロンがある時期から変性に陥る，原因不明で治療法のない神経変性疾患である．ALSの発症率は人口10万人あたり0.8～7.3人/年程度，有病率は2～8人程度であり頻度や人種差は認められていないため，共通の発生機序の存在が示唆される．臨床的に典型例では一側上肢の遠位部小手筋から筋力低下・筋萎縮が始まる．やがて嚥下障害，呼吸筋麻痺などによって，多くは2～5年（平均3年）程度で死に至る難病である．

病理学的には，脊髄前角にも側索にもグリオーシスを認め，脊髄前角の大型運動ニューロンの脱落が著明である．残存ニューロン内には，Bunina小体と呼ばれるエオジン好性のシスタチン抗体陽性，トランスフェリン抗体陽性の細胞内封入体やユビキチン陽性，TDP-43陽性封入体であるskein-like inclusionやLewy body-like hyaline inclusionなどの封入体がみられる．

孤発性ALSは全患者の90％以上を占め，既知の家族性ALSの責任遺伝子異常は大多数の症例で見出されていないため，家族性ALSとは発症メカニズムが異な

[キーワード＆略語]
RNA編集，AMPA受容体，ALS，GluR2，Q/R部位

ALS：amyotrophic lateral sclerosis（筋萎縮性側索硬化症）
AMPA：α-3-hydroxy-5-methyl-4-isoxazole propionic acid
DRPLA：dentatorubropallidoluysian atrophy（歯状核赤核淡蒼球ルイ体萎縮症）
MSA：multiple system atrophy（多系統萎縮症）
ADAR：adenosine deaminase acting on RNA
ECS：exon complementary sequence
CYFIP：cytoplasmic fragile X mental retardation protein interacting protein

AMPA receptor and sporadic amyotrophic lateral sclerosis
Takuto Hideyama[1)2)]/Takenari Yamashita[1)]/Shin Kwak[1)]：Department of Neurology, University of Tokyo, Graduate School of Medicine[1)]/Division for Health Service Promotion, University of Tokyo[2)]（東京大学大学院医学系研究科脳神経医学専攻神経内科学[1)]/東京大学保健・健康推進本部[2)]）

ると考えられる．中毒説，神経栄養因子欠乏説，細胞骨格タンパク異常説，逆行性軸索流異常説などが検討されてきたが，いずれも証明されていない．このような状況の中，Kwakらによって孤発性ALS脊髄運動ニューロンでは，グルタミン酸受容体であるAMPA（α-3-hydroxy-5-methyl-4-isoxazole propionic acid）受容体サブユニットの1つであり，Ca^{2+}透過性AMPA受容体を構成するGluR2のQ/R部位にRNA編集が起こらない未編集型のGluR2増加が，疾患特異的，細胞選択的に起こっていることが発見された[1]．

AMPA受容体と興奮性神経細胞死

1）グルタミン酸による遅発性興奮性細胞死

錐体路はグルタミン酸が神経伝達物質であり，脊髄運動ニューロンもこの興奮性入力を豊富に受けている．そのため運動ニューロンにおいてもグルタミン酸受容体が高密度で発現している．興奮性神経細胞死のメカニズムは，グルタミン酸による興奮が過剰になるとCa^{2+}などのイオン透過性亢進が引き起こされ，内部恒常性が破綻し，細胞死のカスケードが働くというものである．興奮性神経細胞死は，主に虚血や低血糖，外傷，てんかん重積などの急性の神経細胞死に働くと考えられていた．一方で，培養細胞系，*in vivo*動物実験系で急性には神経細胞死を引き起こさない低い濃度でもAMPA受容体が長期間持続的に興奮することで遅発性の神経細胞死が起こることが次々と明らかにされ，特にALSでAMPA受容体を介する神経細胞死がALSの神経細胞死に働いていることを支持する結果が得られている[2,3]．次に，AMPA受容体の特性と神経細胞死について論じる．

2）神経細胞死とAMPA受容体

グルタミン酸受容体は大きくイオンチャネル型と代謝調節型に分類される．そしてイオンチャネル型はさらにNMDA受容体，カイニン酸受容体，AMPA受容体に分けられる．NMDA受容体が急性の神経細胞死に関与するのに対して，AMPA受容体は速いシナプス伝達に関わるニューロンの遅発性細胞死に関与し，運動ニューロンは，特に後者の興奮性細胞死に脆弱であることが知られている．その分子メカニズムとして細胞死のトリガーとなるのは，過剰なCa^{2+}流入による細胞内Ca^{2+}濃度の持続的上昇である．神経細胞内Ca^{2+}濃度上昇の機構には，①NMDA受容体の活性化によるチャネルからのCa^{2+}流入，②Ca^{2+}透過性AMPA受容体の活性化，③代謝型グルタミン酸受容体などの興奮によるIP3産生を介する小胞体からのCa^{2+}動員，④膜の脱分極による膜電位依存性Ca^{2+}チャネルの開口などのメカニズム，⑤電位非依存性カチオン透過性チャネルの関与（transient receptor potential）が知られている．特にラット培養脊髄神経細胞の検討などから②のAMPA受容体を介した経路が重要であることがわかった．オートラジオグラフや免疫組織学的検討から，ヒトや動物の脊髄運動ニューロンには，カイニン酸受容体の発現が乏しいのに対して，AMPA受容体は豊富に発現しており，膜電位決定は主にAMPA受容体が担っている．また，ALS患者髄液を培養ラット皮質神経細胞に投与すると神経細胞死が生じ，AMPA受容体アンタゴニストによってレスキューできるがNMDA受容体アンタゴニストによっては防げないなど，NMDA受容体よりAMPA受容体を介した細胞死に脆弱であることが示されている[4]．

3）AMPA型受容体のCa^{2+}透過性決定因子

AMPA受容体は，電気生理，化学量論，超微形態など，多方面からの検討により4種のサブユニット（GluR1-GluR4）の単独またはさまざまな組み合わせからなる四量体と考えられている．各サブユニットは共通構造を持っており，相互に約70％のアミノ酸配列の相同性を持つ（**図1 A**）．

AMPA受容体のCa^{2+}透過性を決定する因子には，①GluR2サブユニット，②GluR2サブユニットのRNA編集（特にQ/R部位），③flip/flop splicing variantやR/G部位の編集率などチャネルの開口を編集するドメインがあり，細胞全体としては④AMPA受容体密度もCa^{2+}流入量を決定する大きな因子となる．しかし，Ca^{2+}透過性を規定する因子のすべてが細胞死に直接関連するわけではない．GluR2のノックアウトにより小脳培養系では細胞死が生じるが[5]，GluR2ノックアウトマウスではLTP異常のみで細胞死は生じない[6]．flip/flop isoformは，細胞死を生じないが，AMPA受容体毒性を強める[7]．AMPA受容体密度の変化のみでは神経細胞死を生じない[8]．これに対し，RNA編集を阻止した変異マウスでは生後20日以内に痙攣により死亡する[9]（**図1 B**）．

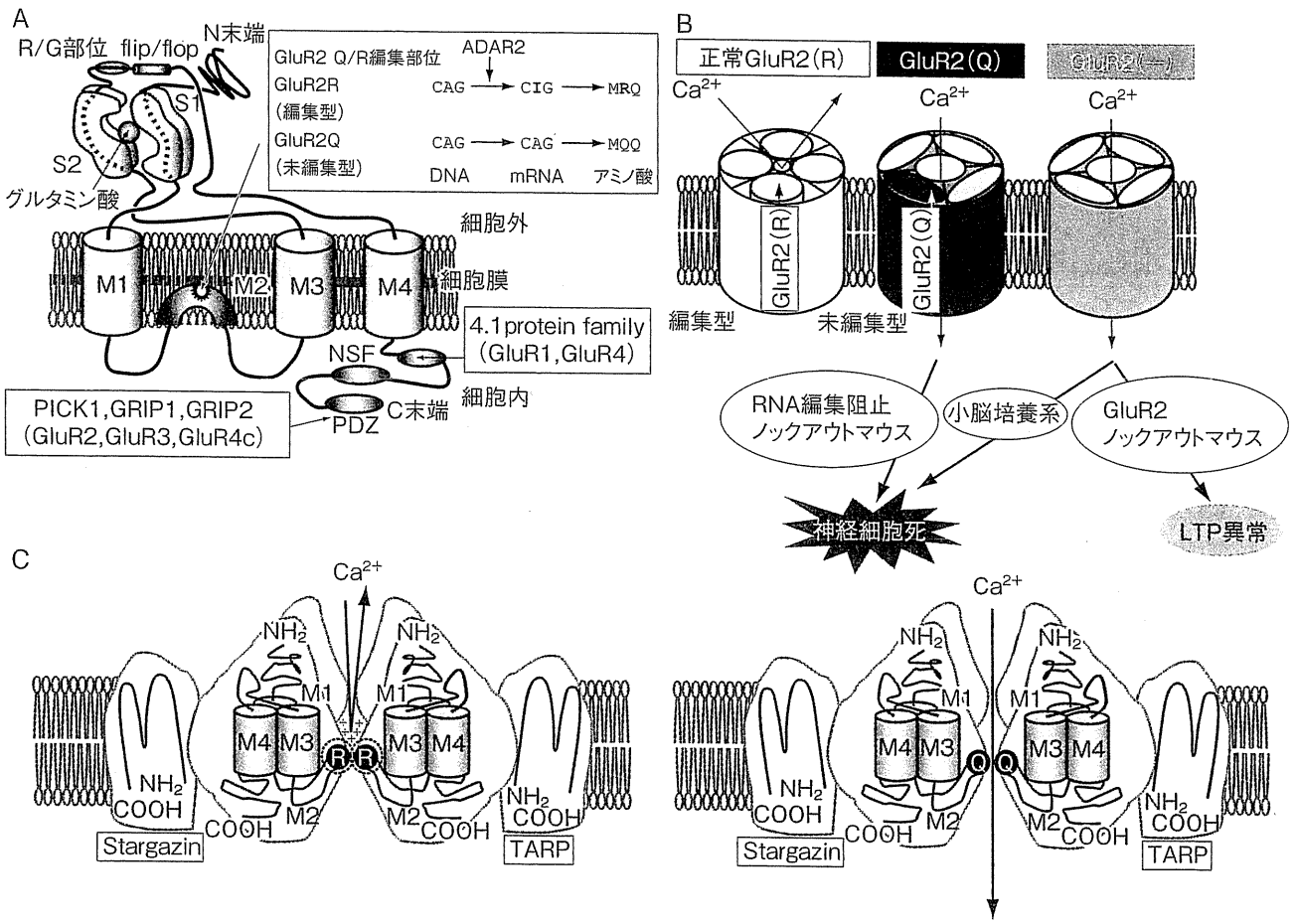


図1　AMPA受容体とCa^{2+}透過性による興奮性神経細胞死

A）AMPA受容体を構成するGluR1-4の各サブユニットは，細胞外のN末端，膜ドメイン（M1～M4），細胞内のC末端からなる．各サブユニットのM2にはQ/R部位と呼ばれる部位があり，同部位はGluR2以外ではグルタミン（Q）であるのに対して，GluR2だけはアルギニン（R）である．しかしゲノムレベルでは，GluR2もQをコードしているCAGという配列である．Rとなる理由は，RNAへ転写後，mRNAになる前にアデノシン（A）がイノシン（I）へと塩基置換され，CAG（Q）からCIG（R）へのアミノ酸置換が生じるためである．この現象は，RNA編集と呼ばれる．B）ラット小脳プルキンエ細胞や海馬錐体細胞などでは，他のサブユニットに比べGluR2が多く発現しており，AMPA型受容体のCa^{2+}透過性は低く，海馬のバスケット細胞，新皮質の非錐体細胞，小脳のBergmannグリア細胞のようにGluR2サブユニットがほとんど発現していないような細胞では，Ca^{2+}透過性は高い．つまりAMPA受容体のCa^{2+}透過性は，GluR2の有無によって規定されている．GluR2のノックアウトにより小脳培養系では細胞死が生じるが，GlluR2ノックアウトマウスではLTP異常のみで細胞死は生じない．RNA編集を阻止した変異マウスでは生後20日以内に痙攣による個体死が認められる．C）Q/R部位がCa^{2+}透過性決定に重要な理由はチャネル・ポアに面しており，編集型では陽電荷のRがCa^{2+}を弾くのに対して，未編集型では，電気的に中性のQではこの作用が弱いためと考えられている

2 GluR2 Q/R部位と孤発性ALS

1）神経細胞死とGluR2サブユニット

上述したように神経細胞死に関連するCa^{2+}透過性を決定する因子は，GluR2である．AMPA受容体を構成する4つのサブユニットのうちGluR1，3，4のサブユニットだけで構成された受容体は高いCa^{2+}透過性を示すが，GluR2を1つ以上含む受容体はCa^{2+}透過性が低い（**図1B**）．

第二にAMPA受容体の各サブユニットのM2ドメインにあるQ/R部位がCa^{2+}透過性を制御している．同部位はGluR2以外のサブユニットGluR1，3，4ではグルタミン（Q）であるのに対して，GluR2だけはアルギニン（R）である．しかしゲノムレベルでは，GluR2

も他のサブユニット同様にQをコードしている．どうしてRになるのかというと，後述するRNA編集という現象が起こるためである．未編集型GluR2（Q）は他のサブユニット同様AMPA受容体のCa^{2+}透過性を制御できないので，編集型GluR2（R）を含んだAMPA受容体の割合が減少する，あるいは未編集型GluR2（Q）を含んだAMPA受容体の割合が増加すると細胞内へのCa^{2+}流入が高まる（**図1C**）．

2）GluR2 Q/R部位編集率低下は孤発性ALSの脊髄ニューロン特異的にみられる

私たちのグループは，孤発性ALSの神経細胞死に関連する分子変化が，GluR2サブユニットの減少（Ca^{2+}透過性AMPA受容体の割合の増加）なのか，それともGluR2 Q/R部位の編集率低下（Ca^{2+}透過性AMPA受容体の実質的増加）によるものかを検討した．Kwakらはレーザーマイクロダイセクターを用いて凍結剖検脊髄組織から単一運動ニューロンを切り出し，孤発性ALS脊髄運動ニューロンの単一神経細胞レベルの検討において，GluR2 mRNA発現量に有意な減少がないこと[10]，および脊髄前角組織レベル[11]で部位選択的・疾患特異的なGluR2 Q/R部位の編集率低下を確認した[1]．**図2A**に示すように，正常対照群の運動ニューロンでは，全例GluR2 Q/R部位は100％RNA編集されていたが，ALS群では検索したすべての症例で0～100％とばらつき，平均値は38～75％と低下していた．一方，ALSでは障害を受けない小脳プルキンエ細胞の編集率は，正常対照群と同様にほぼ100％に保たれていた[1]．また，小脳を侵す神経変性疾患である歯状核赤核淡蒼球ルイ体萎縮症（dentatorubropallidoluysian atrophy，以下DRPLA），多系統萎縮症（multiple system atrophy，以下MSA）の同細胞を検索したが，編集率は正常対照と同様のレベルによく保たれており[1)7)]，さらに他の脊髄小脳変性症の小脳，ハンチントン病の線条体[12]やアルツハイマー病の大脳皮質[13]など，さまざまな神経変性疾患で選択的に障害を受ける部位でも編集率は低下していないこと[14]も，この分子変化が細胞死に伴う非特異的なものではなく，孤発性ALSに疾患特異的な分子変化であることを支持する．

さらに，原因の違いにかかわらず運動ニューロンが変性する場合に共通して起こる非特異的な分子変化である可能性について検討した．運動ニューロンに変性を生じるトリプレット病であるSBMA（球脊髄性筋萎縮症）の運動ニューロン（**図2B**）と運動ニューロンに病変を生じる家族性ALSで最も頻度が高いALS1（**図2C**）とについても同様のメカニズムが生じているのか否か，について調べた．その結果，SBMAおよびSOD1関連性家族性ALS（ALS1）のモデルラット2系統の発症した個体とでは，単一運動ニューロンの編集率は正常コントロールと同様100％に保たれており[15]，GluR2 Q/R部位のRNA編集異常は運動ニューロンが細胞死に陥る際に生ずる非特異的な変化である可能性は除外された．さらに，運動ニューロン死の分子メカニズムは多様であり，SBMAやALS1の運動ニューロンでは孤発性ALSとは異なる細胞死のメカニズムが働いていると考えられた（**図3**）[16]．

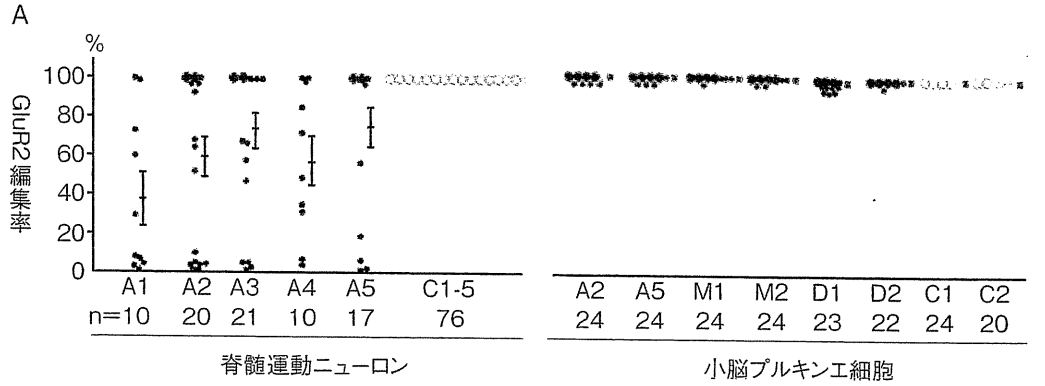
この分子異常が神経細胞死と関連するものであることは，GluR2 Q/R部位のRNA編集が起こらないように遺伝子改変したマウスが生後20日以内に痙攣重積によって死に至ること[17]からも示される．

3 RNA編集

1）GluR2 Q/R部位とRNA編集

一般に，転写後にmRNAレベルで，塩基の挿入・欠失・置換により遺伝情報が書き換えられることがある．これをRNA editing（RNA編集）と呼ぶ（**図4A**）[18]．RNA編集により翻訳領域の塩基置換を伴うとタンパク機能を変える場合があり，生物学的にも重要な反応である．一塩基置換を伴うRNA編集はアデノシン（A）からイノシン（I）（A→I編集）とシトシン（C）からウラシル（U）（C→U編集）とがあるが，哺乳類ではほとんどが前者であり，GluR2 Q/R部位は，DNAからRNAへ転写後，A→I編集が起こることでmRNAの塩基が置換（CAG→CIG）される．そして，リボソームでIはグアノシン（G）と同等であると見なされるため，CIGコドンはCGGと見なされアルギニン（R）として翻訳される（**図4B**）．これにより遺伝子上のグルタミン（Q）コドンはRにアミノ酸置換される．

GluR2 Q/R部位が，哺乳類で最初に見つかった編集部位であり，この他に，**表**のように編集部位は受容体やイオンチャネルに多くみられており，RNA編集の有無によりチャネル特性が変化すると考えられている．RNA編集はこのように生物界に広く行われている現象



B

年齢 性別	CAG リピート数	GluR2 Q/R部位 編集率100%
71 男	48	12/12
78 男	42	16/16
60 男	44	16/16

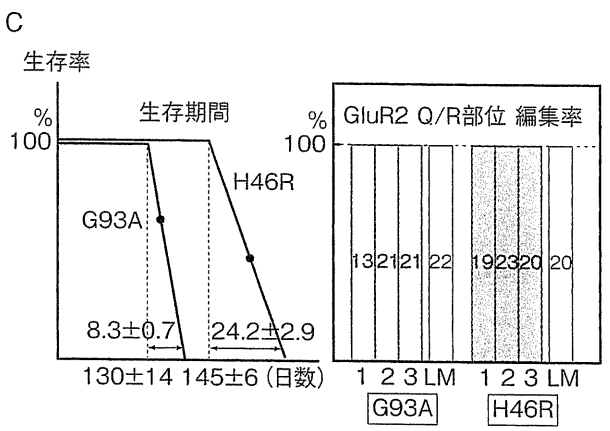


図2　単一神経細胞におけるGluR2 Q/R部位RNA編集率（文献1より改変引用）

A）各点（大きな点は5細胞，小さな点は1細胞）は，ALS群5例（A1-A5），コントロール群5例（C1-C5）の単一脊髄運動ニューロンにおけるGluR2 Q/R部位のRNA編集率と，ALS群2例（A2, A5），多系統萎縮症（MSA）群2例（M1, M2），歯状核赤核淡蒼球ルイ体萎縮症（DRPLA）群2例（D1, D2），コントロール群2例（C1, C2）の単一小脳プルキンエ細胞の編集率を表している．平均値±標準誤差と解析した細胞数（n）も示した．運動ニューロンにおける正常コントロール76個の内訳は，C1：28, C2：12, C3：13, C4：12, C5：11である．運動ニューロンでは，正常コントロール群のすべての細胞において，例外なく編集率は100％であった．これに対して，ALS群では，解析した5ケースすべてにおいて編集率は0％から100％まで大きくばらつき，正常コントロール群と比較し有意に低下していた（Mann-Whitney U test, $p < 0.001$）．一方，小脳プルキンエ細胞における編集率については，ALS群，MSA群，DRPLA群とコントロール群の間には有意差はない（Mann-Whitney U test, $p > 0.05$）．B）他の運動ニューロン病（SBMA）では，編集率の低下は認めない．表では検討した3例の年齢，性別，CAGリピート数，検討した運動ニューロン数を示した．一番上の行は71歳男性のSBMA患者でCAGリピート数は48，調べた運動ニューロン12個中12個すべてがGluR2 Q/R部位の編集率は100％である．検索した全例の運動ニューロンの編集率は完全に保たれていた．このような選択特異性を生む機序としては，脊髄運動ニューロンのAMPA受容体総mRNA発現量およびGluR2サブユニット比率が他のニューロンに比べて低く，もともとCa^{2+}透過性AMPA受容体の割合が多いことからRNA編集低下の影響を受けやすいことが挙げられる．C）家族性ALSで最も多いALS1のモデルラットの2系統（G93A, H46R）の運動ニューロンを調べた．左のグラフがそれぞれのラットの生存曲線で，それぞれ発症後の●で示した時点での運動ニューロンの編集率を各3匹ずつ，対照としてLM（littermate：同胞）を調べた．棒グラフ内の数字がそれぞれの個体において検索した運動ニューロン数で，検索したすべての個体の運動ニューロンは100％に完全に編集されていた

であり，その異常は個体にとり不利になる場合もありうるため，神経変性疾患のほか，脳腫瘍，てんかん，精神疾患（統合失調症，うつ病，自殺），遺伝性対側性色素異常症，ループスなどの膠原病で検討が行われているが，ヒト疾患との直接の関連が明らかにされたものは孤発性ALSのみである（**表**）．

2）ADAR2はどのようにしてRNA編集を制御しているのか？

GluR2 Q/R部位のRNA編集を特異的に制御しているのがRNA編集酵素adenosine deaminase acting on RNA type 2（以下ADAR2）である．ADAR2のほか，RNA編集活性を持つADARとしてはADAR1が