

# Gene therapy improves motor and mental function of aromatic L-amino acid decarboxylase deficiency

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In patients with aromatic L-amino acid decarboxylase (AADC) deficiency, a decrease in catecholamines and serotonin levels in the brain leads to developmental delay and movement disorders. The beneficial effects of gene therapy in patients from 1 to 8 years of age with homogeneous severity of disease have been reported from Taiwan. We conducted an open-label phase 1/2 study of population including adolescent patients with different degrees of severity. Six patients were enrolled: four males (ages 4, 10, 15 and 19 years) and one female (age 12 years) with a severe phenotype who were not capable of voluntary movement or speech, and one female (age 5 years) with a moderate phenotype who could walk with support. The patients received a total of  $2 \times 10^{11}$  vector genomes of adeno-associated virus vector harbouring *DDC* via bilateral intraputamenal infusions. At up to 2 years after gene therapy, the motor function was remarkably improved in all patients. Three patients with the severe phenotype were able to stand with support, and one patient could walk with a walker, while the patient with the moderate phenotype could run and ride a bicycle. This moderate-phenotype patient also showed improvement in her mental function, being able to converse fluently and perform simple arithmetic. Dystonia disappeared and oculogyric crisis was markedly decreased in all patients. The patients exhibited transient choreic dyskinesia for a couple of months, but no adverse events caused by vector were observed. PET with 6-[<sup>18</sup>F]fluoro-L-*m*-tyrosine, a specific tracer for AADC, showed a persistently increased uptake in the broad areas of the putamen. In our study, older patients (>8 years of age) also showed improvement, although treatment was more effective in younger patients. The genetic background of our patients was heterogeneous, and some patients suspected of having remnant enzyme activity showed better improvement than the Taiwanese patients. In addition to the alleviation of motor symptoms, the cognitive and verbal functions were improved in a patient with the moderate phenotype. The restoration of dopamine synthesis in the putamen via gene transfer provides transformative medical benefit across all patient ages, genotypes, and disease severities included in this study, with the most pronounced improvements noted in moderate patients.

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**Keywords:** aromatic L-amino acid decarboxylase (AADC) deficiency; adeno-associated virus (AAV) vector; dopamine; putamen; dystonia

**Abbreviations:** AADC = aromatic L-amino acid decarboxylase; AAV = adeno-associated virus; AIMS = Alberta Infant Motor Scale; FMT = 6-[<sup>18</sup>F] fluoro-L-*m*-tyrosine; HVA = homovanillic acid; OGC = oculogyric crisis

## Introduction

Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM #608643) is an autosomal recessive neurotransmitter disorder caused by defects in the dopa decarboxylase (*DDC*) gene, which encodes AADC. About 140 patients have been reported worldwide thus far (Hyland and Clayton, 1992; Pons *et al.*, 2004; Ito *et al.*, 2008; Manegold *et al.*, 2009; Brun *et al.*, 2010; Ide *et al.*, 2010; Chen *et al.*, 2014; Atwal *et al.*, 2015; Kojima *et al.*, 2016; Lee *et al.*, 2017; Wassenberg *et al.*, 2017). AADC catalyses the formation of neurotransmitters from L-DOPA and 5-hydroxytryptophan to dopamine and serotonin, respectively. Consequently, the depletion of dopamine and serotonin, as well as norepinephrine and epinephrine, which are synthesized from dopamine, is induced in patients with AADC deficiency. In the CSF and serum of patients with AADC deficiency, the concentrations of these biogenic amines and metabolites are reduced, while those of L-DOPA and 3-O-methyldopa are elevated (Hyland and Clayton, 1992; Wassenberg *et al.*, 2017). The main phenotype of AADC deficiency is movement disorder, including loss of voluntary movement, hypotonia, intermittent oculogyric crisis (OGC) and limb dystonia (Korenke *et al.*, 1997; Brun *et al.*, 2010). OGC presents as a sustained upward or lateral deviation of the eyes in combination with backward and lateral flexion of the neck. Patients also present with autonomic dysfunction characterized by impairment of the sympathetic regulation of heart rate and blood pressure, excessive sweating and hyper-salivation, intellectual disability, hypoglycaemia, epilepsy, emotional instability and sleep disturbance (Hyland and Clayton, 1992; Brun *et al.*, 2010; Arnoux *et al.*, 2013; Helman *et al.*, 2014; Lee *et al.*, 2014; Spitz *et al.*, 2017). The disease onset is in early infancy, and most patients with the severe phenotype are bedridden for life. However, some relatively mild phenotypes have also been reported (Hyland *et al.*, 1992; Swoboda *et al.*, 2003; Tay *et al.*, 2007; Leuzzi *et al.*, 2015). In the guideline for AADC deficiency, cases are classified as severe (80%: no or very limited achievement of developmental milestones, fully dependent), mild (5%: mild developmental delay and intellectual disability, ambulatory without assistance) and moderate (15%: between severe and mild) (Wassenberg *et al.*, 2017). Several drugs have been applied for treatment, including vitamin B<sub>6</sub>, which is a coenzyme of AADC, L-

DOPA, dopamine agonists, and monoamine oxidase B (MAOB) inhibitors. However, only patients with the moderate or mild phenotype respond to drugs—mainly dopamine agonists and MAOB inhibitors (Fiumara *et al.*, 2002; Mastrangelo *et al.*, 2013; Leuzzi *et al.*, 2015)—with drug therapy providing little or no benefit for most severe patients (Hwu *et al.*, 2018).

The successful application of gene therapy in the treatment of four patients with AADC deficiency (age 4–6 years) was reported from Taiwan in 2012 (Hwu *et al.*, 2012), and 10 additional patients (age 1.67–8.42 years) were reported in 2017 (Chien *et al.*, 2017). The prevalence of AADC deficiency in the Taiwanese population is high because of the founder mutation IVS6+4A>T, and most Taiwanese patients present with the severe phenotype (Lee *et al.*, 2009; Hwu *et al.*, 2018). After gene therapy, some previously bedridden patients were able to sit or stand with support. The treatment vector (AAV-hAADC-2) was made by inserting the human AADC gene with cytomegalovirus (CMV) promoter into the type 2 adeno-associated virus (AAV) vector. The vector was then injected into the putamen bilaterally by a stereotactic operation (Hwu *et al.*, 2012). AAV-hAADC-2 was initially developed for clinical studies of gene therapy for Parkinson's disease (Muramatsu *et al.*, 2010).

In the present study, we performed gene therapy for six patients (4–19 years of age) with AADC deficiency using an AADC-expressing AAV vector similar to that reported in the Taiwanese study (Hwu *et al.*, 2012; Chien *et al.*, 2017). Our patients differed from the Taiwanese patients in that our patients had a variable genetic background, one had the moderate phenotype, and four were older than the Taiwanese patients.

## Materials and methods

### Study design

This study was planned as an open-label, phase 1/2 trial at Jichi Medical University Hospital (Tochigi, Japan) to investigate the effects of gene therapy in patients with AADC deficiency. The primary objective of this study was to verify the safety of the therapy; the secondary objective was to obtain preliminary data on the clinical response to the gene therapy by assessing the improvement in the motor function, cognitive function, autonomic function and other factors. We recorded

adverse events, as well as neurological findings, physical findings, laboratory findings (including the results of CSF catecholamine metabolites analysis) and brain MRI, CT and EEG findings. The study population included patients with AADC deficiency who were unable to stand, who presented motor disturbance and dystonia and in whom the diagnosis of AADC deficiency had been confirmed based on a CSF analysis, AADC enzyme activity or a genetic analysis. We excluded patients with mild-phenotype AADC deficiency who were able to stand and walk independently as well as seriously ill patients. We also excluded patients with normal findings on PET using the non-catecholic tracer 6-[<sup>18</sup>F] fluoro-L-*m*-tyrosine (FMT) (FMT-PET), a specific tracer for AADC (Muramatsu *et al.*, 2010), as well as patients in whom brain MRI could not be performed.

## Patients

Six patients with characteristic manifestations of AADC deficiency were enrolled from 1 May 2015, to 31 July 2017. The profiles of the patients are listed in Tables 1 and 2. Patients 1, 2 and 4–6, who showed the severe phenotype (Ito *et al.*, 2008; Ide *et al.*, 2010; Kojima *et al.*, 2016), were bedridden without voluntary movement, with OGCs and generalized dystonic attacks. Their mental status was relatively preserved, to the extent that they could visually follow objects, smile and distinguish people and situations. They understood simple words but could not speak. Patient 4 had refractory tonic convulsions. Patient 3 showed the moderate phenotype (Kojima *et al.*, 2016). The patient had been bedridden until 3 years of age before receiving a diagnosis of AADC deficiency. After MAOB inhibitor treatment was started, the patient gained the ability to walk unsteadily with support and to speak several words unclearly (Kojima *et al.*, 2016). However, this improvement stopped (Kojima *et al.*, 2016), so we decided to perform gene therapy in this patient. Blood analyses and biochemical investigations revealed normal findings. The blood AADC enzyme activity was low in all patients. The genetic mutations in each patient are shown in Table 1 and Supplementary Fig. 1. A catecholamine metabolite analysis of the CSF revealed a very high concentration of L-DOPA and

very low concentration of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in all patients, findings that were compatible with AADC deficiency (Table 3). In addition, brain structural MRI showed no abnormalities. EEG showed no paroxysmal discharges in the patients, with the exception of Patient 4, who showed focal and diffuse epileptic discharges (Ito *et al.*, 2008; Ide *et al.*, 2010; Kojima *et al.*, 2016).

Before the gene therapy, the main drugs prescribed for patients were as follows: vitamin B<sub>6</sub> for Patients 1–5; L-DOPA for Patients 3 and 6; dopamine agonist for Patients 2, 4 and 5; MAOB inhibitor for Patients 3 and 6; and selective serotonin reuptake inhibitor for Patients 1 and 2. In addition to these, carnitine, folic acid, melatonin agonist and gastrointestinal drugs were prescribed for one to three patients, each.

## Ethics

This study was approved by the Ethics Committee for Gene Therapy at Jichi Medical University Hospital and the Science Council, Ministry of Health, Labor and Welfare. Conformance with the inclusion criteria was evaluated before each treatment, and the status of the treated patients was reported to the evaluation committee of the Ethics Committee for Gene Therapy at Jichi Medical University Hospital. Written informed consent was obtained from the parents of each patient. The study was conducted in compliance with the Japanese Guidelines on Gene Therapy and is registered with the UMIN Clinical Trials Registry (No. UMIN000017802).

## Vector

A recombinant AAV type 2 vector, AAV-hAADC-2, was prepared as described previously (Muramatsu *et al.*, 2010). The expression cassette consisted of a CMV promoter followed by human  $\beta$ -globin intron, human AADC complementary DNA and polyadenylation signal from human growth hormone. Clinical-grade AAV-hAADC-2 was manufactured, and quality control was tested in compliance with the current Good Manufacturing Practices at Takara Bio Inc.

**Table 1** Patient details

Patient No.	Gender	Age at GT (year)	Severity	Motor status	Intake	Respiratory support	DDC gene mutations	Follow-up period after GT
1	M	15	Severe	Bedridden	GS	Laryngo-tracheal separation	c.329C>A, p.(Ala110Glu) Not detected	2 years 7 months
2	F	12	Severe	Bedridden	GS	–	c.329C>A, p.(Ala110Glu) Not detected	2 years 6 months
3	F	5	Moderate	Walk with support	Oral	–	c.315G>C, p.(Try105Cys) c.385C>T, p.(Pro129Ser)	2 years 1 months
4	M	19	Severe	Bedridden	TF	NIPPV	c.1106A>G, p.(Tyr369Cys) IVS6+4A>T	2 years
5	M	10	Severe	Bedridden	GS	–	IVS6+4A>T IVS6+4A>T	1 years
6	M	4	Severe	Bedridden	Oral	–	c.236A>G, p.(Tyr79Cys) c.755A>G, p.(Asp252Gly)	6 months

GT = gene therapy; GS = gastrostomy; TF = nasogastric tube feeding; NIPPV = non-invasive positive pressure ventilation.

**Table 2** The clinical course of gene therapy

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5		Patient 6	
	BL	2y 7m	BL	2y 6m	BL	2y 1m	BL	2y	BL	1y	BL	6m
<b>Motor</b>												
Head control	–	+	–	+	+	+	–	+	–	+	–	+
Sit	–	±	–	±	+	+	–	±	–	±	–	±
Walk	–	±	–	±	±	++	–	–	–	±	–	–
Grip	–	+	±	++	+	++	–	+	–	+	–	±
<b>Involuntary movement</b>												
Dystonia	+++	–	+++	–	–	–	+	–	+++	–	+++	–
OGC	+++	±	+++	±	++	±	±	–	+++	±	+++	±
<b>Autonomic function</b>												
Sweat	++	±	++	–	±	–	++	–	++	–	+	±
Saliva	+++	–	+++	–	–	–	+++	–	++	+	+	–
Diarrhea	+	+	–	–	–	+	–	–	–	–	–	–
<b>Sleep disturbance</b>	++	–	++	±	–	–	±	±	++	+	++	–
<b>Mental status</b>												
Words	–	–	–	Bubbling	Few words	Conversation	–	±	–	–	–	Few words
Mood	Unstable	Good	Unstable	Smile	Stable	Very good	Stable	Smile	Unstable	Smile	Unstable	Smile
<b>Respiratory dysfunction</b>	±	–	–	–	–	–	+	–	±	–	–	–
<b>Feeding</b>	GS	Oral and GS	GS	Oral	Oral	Oral	TF	Oraland TF	GS	Oral and GS	Oral	Oral

BL = baseline; GS = gastrostomy; TF = nasogastric tube feeding. Plus/minus symbol in motor functions indicates that they are able to perform the function with support.

**Table 3** Catecholamine metabolites in CSF

Patient No.	HVA, ng/ml		5-HIAA, ng/ml		MHPG, ng/ml		L-DOPA, ng/ml	
	BL	1m	BL	1m	BL	1m	BL	1m
1	1.1	1.2	<1.0	<1.0	<1.0	<1.0	8.2	9.8
2	2.1	3.9	<1.0	<1.0	<1.0	<1.0	14.5	13.2
3	10.7	15	1.5	1.4	1.6	1.3	34.7	31.8
4	3.5	4.1	<1.0	<1.0	<1.0	<1.0	12.1	9.6
5	0.1	0.1	0.1	0.1	<1.0	<1.0	11.2	5
6	2.1	6.8	1	1	<1.0	ND	371	173.4
Normal	4.4–15.1		1.8–6.1		6.5–51		1.2–2.2	

5-HIAA = 5-hydroxyindoleacetic acid; BL = baseline; L-DOPA, L-3,4-dihydroxyphenylalanine; MHPG = 3-methoxy-4-hydroxyphenylmethyleneglycol; ND = no data.

**Table 4** Adverse events

Adverse event	Number (%)
Transient choreic movements (limb dystonia)	6 (100)
Transient orofacial dyskinesia	6 (100)
Diarrhoea	1 (16)
Subdural haemorrhage with no clinical symptom	1 (16)
Serious adverse events	0 (0)

## Stereotactic neurosurgery

Patients received infusions of the vector into two target points (one each into the bilateral putamen) by stereotactic surgery

that was planned with the dedicated software program included with the StealthStation Treon Navigation System (FrameLink, Medtronic, Ireland) (Muramatsu *et al.*, 2010). Two target points that were sufficiently distant from each other in the dorsolateral direction, as confirmed by CT and MRI, were determined for each putamen. One burr hole was trepanned in each side of the cranial bone, and the vector was injected via a two-track insertion route. At each target point, 50 µl of the vector-containing solution was injected at a rate of 3 µl/min (a total of 200 µl containing  $2 \times 10^{11}$  vector genomes). For each injection, the needle was inserted into the deepest point of each target and then withdrawn 1 mm after each 10 µl injection. Thus, the vector was injected over a length of 5 mm at each point. All procedures were safely performed under general anaesthesia.

## Clinical evaluation of the gene therapy

The physical and neurological functions of the patients were evaluated. All patients were videotaped to track their clinical course. The Alberta Infant Motor Scale (AIMS) administered by paediatric neurologists was used to evaluate motor development (Piper *et al.*, 1992). The Kyoto Scale of Psychological Development 2001 was used to evaluate the mental status of the patients (Koyama *et al.*, 2009; Aoki *et al.*, 2016). We used the data for the Cognition-Adaptation and Language-Sociality score; the data from the Motor-Posture score were not used. The scores were rated by specially trained psychotherapists.

## PET

To detect the expression of AADC in the brain, FMT-PET was performed according to methods described previously (Muramatsu *et al.*, 2010). Prior to the emission scan, a 10 min transmission scan was obtained for attenuation correction. Subsequently, 0.12 mCi/kg of FMT in saline was infused into an antecubital vein and a 30–90 min static 3D acquisition was started simultaneously using a PET-CT (GEMINI GXL, Philips). Each subject also underwent 3.0-T MRI (Achieva 3.0 T, Philips) using an inversion recovery (IR) proton density (PD)-weighted pulse sequence to enhance the contrast of anatomical structures. The PET and MRI data were co-registered with a fusion processing program (Syntegra, Philips) to produce fusion images. This program provided manual and point-based image registration as well as automated methods of grey value-based image registration, including a mutual information algorithm (Maes *et al.*, 1999). In addition, an adaptive level set of segmentation was used for co-registration of CT and MRI data (Wells *et al.*, 1996; Muramatsu *et al.*, 2010).

## Analyses of CSF neurotransmitter metabolites

The levels of HVA, 5-HIAA, dopamine, 3-methoxy-4-hydroxyphenylglycol (MHPG) and L-DOPA in the CSF were measured by high-performance liquid chromatography (HPLC), followed by electrochemical detection of monoamine neurotransmitter metabolites at SRL, Inc.

## The quantification of vector-derived genome copies in the blood and urine

Blood and urine samples were obtained before and for 3 days subsequent to surgery, and the vector-derived genome was titrated. DNA was extracted from blood samples using a QIAamp<sup>®</sup> DNA Blood Midi kit, and 100 ng of DNA was used as a template. DNA was extracted from urine samples using a QIAamp<sup>®</sup> circulating nucleic acid kit, and 1 µl of sample was used as a template. The primer sequences for AADC were 5'-GGCAACGTGCTGGTCTGTGT-3' (forward) and 5'-CGTCCCTCAATGCCTTCCATGT-3' (reverse). Quantitative PCR was carried out as described previously using a Thermal Cycler Dice Real-Time System (TAKARA BIO Inc.).

## Titration of neutralizing antibodies against AAV2 capsid in serum

The sera from patients before and 6 months after the operation were measured to quantify the presence of neutralizing antibodies against AAV2 capsid. The procedure for measuring the neutralizing antibodies was performed as described previously (Mimuro *et al.*, 2013, 2014). The titres are shown as the actual dilution factor.

## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary material.

## Results

### Gene therapy

Gene therapy was performed from 29 June 2015 to 14 July 2017. At the time of surgery, the patients were between 4 and 19 years of age. The observation period ranged from 6 months to 2 years 7 months.

### Adverse events

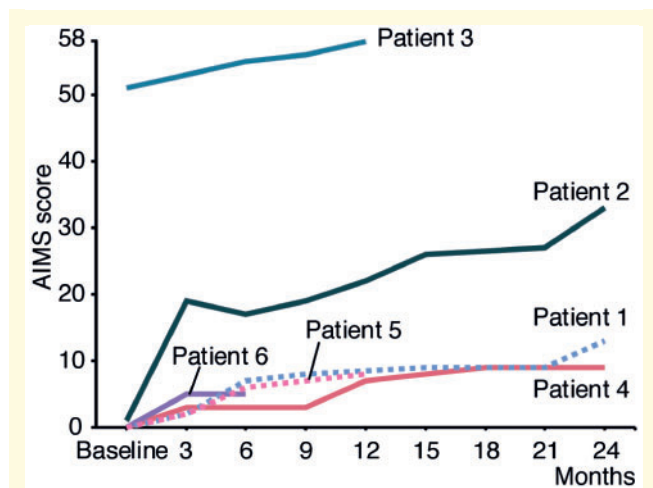
All surgeries were completed safely (Table 4). One patient (Patient 6) showed subdural haemorrhaging on brain CT at 3 days after surgery. However, no associated clinical symptoms were observed. No cases of intracerebral haemorrhaging or brain oedema were observed during the study period, and there were no vector-related adverse events. In all patients, transient choreic movements in the extremities and mouth started at around 2 weeks after therapy, increased until 2 months after therapy, and then gradually diminished from 3 to 6 months after therapy. Risperidone was used for two patients (Patients 4 and 5); however, its effectiveness was limited. One patient (Patient 3) showed diarrhoea at 1 year and 3 months after treatment, just after influenza virus infection. Apnoea was not noted in any of the patients.

## Summary of the clinical effects

### The motor functions and evaluation using AIMS

All patients showed improved motor functions (Fig. 1, Table 2 and Supplementary Videos 1–3). The severe patients began to move voluntarily at 1 to 2 months after treatment. All severe patients were able to control their heads from 2 to 8 months and could sit with support from 4 to 8 months. Patients 1, 2 and 5 were able to stand with support from 1.5 years, 4 months and 7 months, respectively. Patient 2 started to walk with a walker from 4 months and gradually became able to walk long distances. Patients 1, 4, 5 and 6 started to move their arms from 2 to 3 months after the treatment.





**Figure 1 AIMS.** The AIMS score of each patient was plotted. Before gene therapy, Patients 1, 4, 5 and 6 had a score of 0, Patient 2 had a score of 1, and Patient 3 had a score of 52. The scores of all patients improved. At 12 months, the score of Patient 3 reached 58, which represents the maximum score on this scale.

They were able to reach out and grasp objects, although they had difficulty controlling their hand movement well. Patient 2 was able to grasp objects from 4 months and hold food and bring it to her mouth after 1.5 years. Respiration and swallowing were also improved in all patients. Patient 4 was weaned from non-invasive positive pressure ventilation. Four patients had required tube feeding, but all started to eat orally after therapy. Patient 2 was able to eat all foods orally and was weaned from tube feeding. All patients were still hypotonic. The muscle atrophy in the severe patients showed mild improvement but was not resolved.

Regarding the AIMS scores of the patients (Fig. 1), the scores of Patients 1, 4, 5 and 6 gradually improved from almost 0 to 13 (2 years), 9 (2 years), 8 (1 year) and 5 (6 months), respectively, which was similar to the reported course of the Taiwanese patients (Hwu *et al.*, 2012). Patient 2 showed better improvement than the other patients. Her score increased to 20 after 3 months and 33 at 2 years. On comparing their scores at 6 months, younger patients tended to show superior improvement to older patients with the same or a similar mutation. Patient 2 (age 12 years) improved faster than Patient 1 (age 15 years). Patient 5 (age 10 years) showed better improvement than Patient 4 (age 19 years), despite both having IVS6+4A>T. However, the patients with a missense mutation (Patients 1, 2 and 6) all showed earlier improvement than those with the frameshift mutation IVS6+4A>T (Patients 4 and 5). Patient 3, who had a moderate phenotype, showed marked improvement, being able to walk independently at 6 months, ride a bicycle at 10 months, and play on a swing at 1 year and 6 months after treatment. She was hypotonic but otherwise looked like a healthy young girl.

### Dystonia attacks and oculogyric crisis

Dystonia attacks disappeared by 2 months post-treatment in all patients. OGC was decreased markedly after 1 month, but a mild degree of the condition remained. Patients 1, 2, 5 and 6 showed OGC several times a day lasting for several hours before the treatment. After treatment, they suffered from OGC several times a week lasting for several minutes to a few hours. The OGC in Patients 3 and 4 that occurred several times a week lasting for several dozen minutes before the treatment almost completely disappeared and was only observed when the patients were tired, lasting for several minutes.

### Evaluation of cognitive, social and verbal development

Patient 3 showed a remarkable improvement in her Developmental Quotient score (Fig. 2). Before treatment, her Cognition-Adaptation score was 50, and her Language-Sociality score was 40; after 1 year, they improved to 66 and 84, respectively. She was able to speak words after 3 months, started to make conversation after 6 months, and was able to speak fluently after 1 year. She was able to perform simple arithmetic after 2 years. Patient 6 started to speak several words after 6 months. However, other severe patients spoke no words at all. The Cognition-Adaptation and Language-Sociality scores of Patients 1, 2 and 4 also showed mild improvement. All patients became able to respond, smile, or utter sounds and showed improvement regarding their ability to understand language, particularly Patient 2, who was able to quickly respond to spoken orders.

### Other symptoms

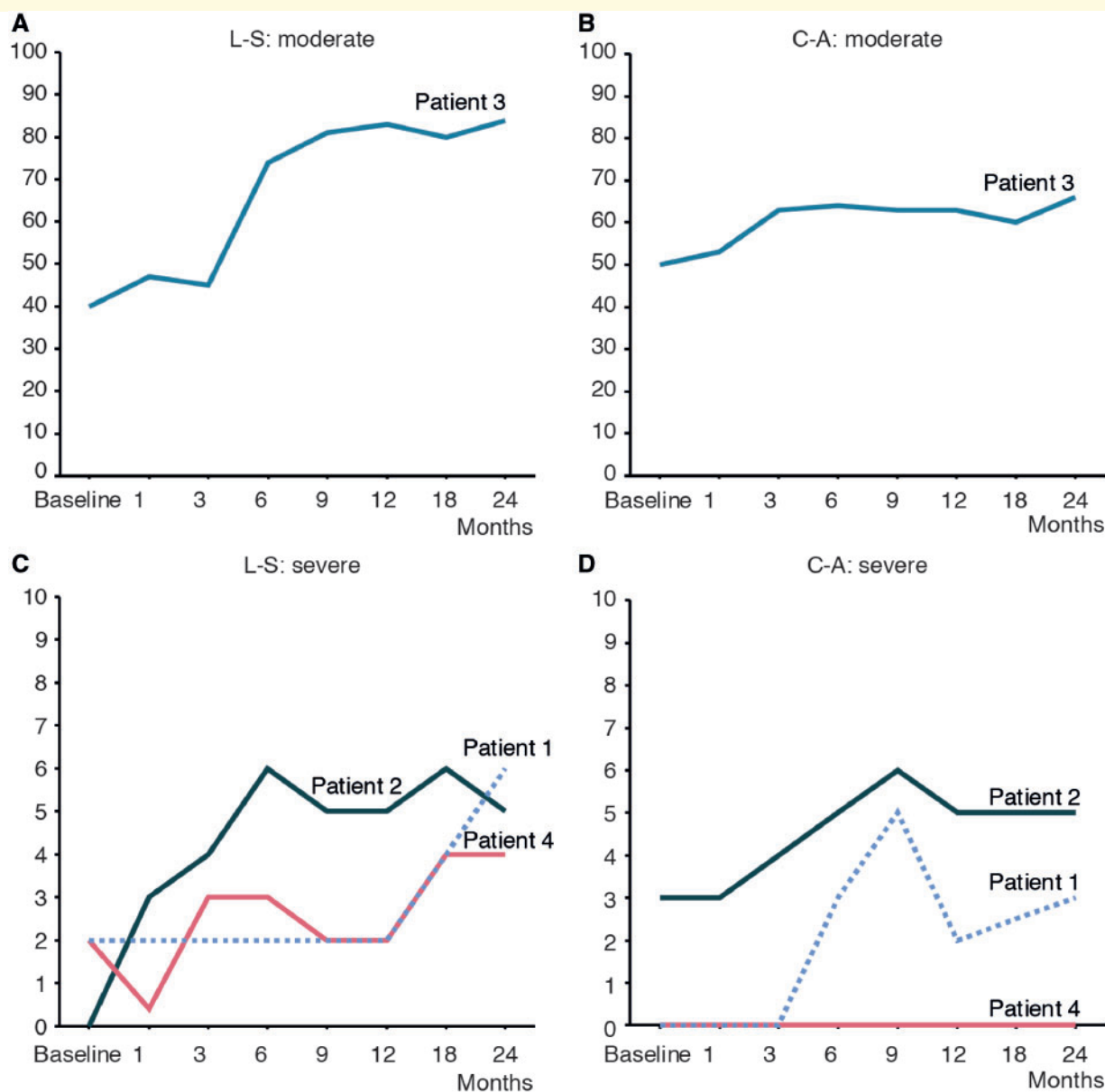
Autonomic dysfunction, such as hyperhidrosis, improved after 3 to 6 months. Hypersalivation appeared to be improved but was observed under anaesthesia. Thus, the improvement in hypersalivation seems to result from improved swallowing.

All patients had been in an unstable emotional state and cried frequently before the treatment. After treatment, they were always smiling and became emotionally stable. Because we did not imagine this emotional change previously, we were able to not evaluate it systematically. The patients suffered from sleep disturbance, difficulty falling asleep, and difficulty remaining asleep; these factors showed mild improvement after treatment.

Three patients (Patients 1–3) who were followed for over 2 years gained 3.1 kg (2.2–4.4 kg) in body weight (data not shown). Regarding the drugs after the treatment, Patient 2 became drug-free, and the other patients were taking small doses of MAOB inhibitor.

### FMT-PET findings

Before treatment, patients showed no signal uptake in the putamen. Patients 1–4 completed FMT-PET at 6 months and 2 years, Patient 5 completed it at 3 months, and Patient 6 completed it at 6 months. All examinations



**Figure 2** The evaluation of the mental development using Kyoto Scale of Psychological Development. The Cognition-Adaptation (C-A) and Language-Sociality (L-S) scores for the Developmental Quotient before and after gene therapy in Patient 3: moderate phenotype (A and B), and Patients 1, 2 and 4: severe phenotype (C and D). (A) Before treatment, the Language-Sociality score of Patient 3 was 42; this improved to 84. A Developmental Quotient score over 70 is in the normal range. (B) The Cognition-Adaptation score of Patient 3 improved from 50 to 66. (C and D) The pretreatment scores of the severe patients were almost 0 but showed mild improvement.

showed high signal intensity in the bilateral putamen, reflecting the expression of the AADC enzyme in the putamen (Fig. 3).

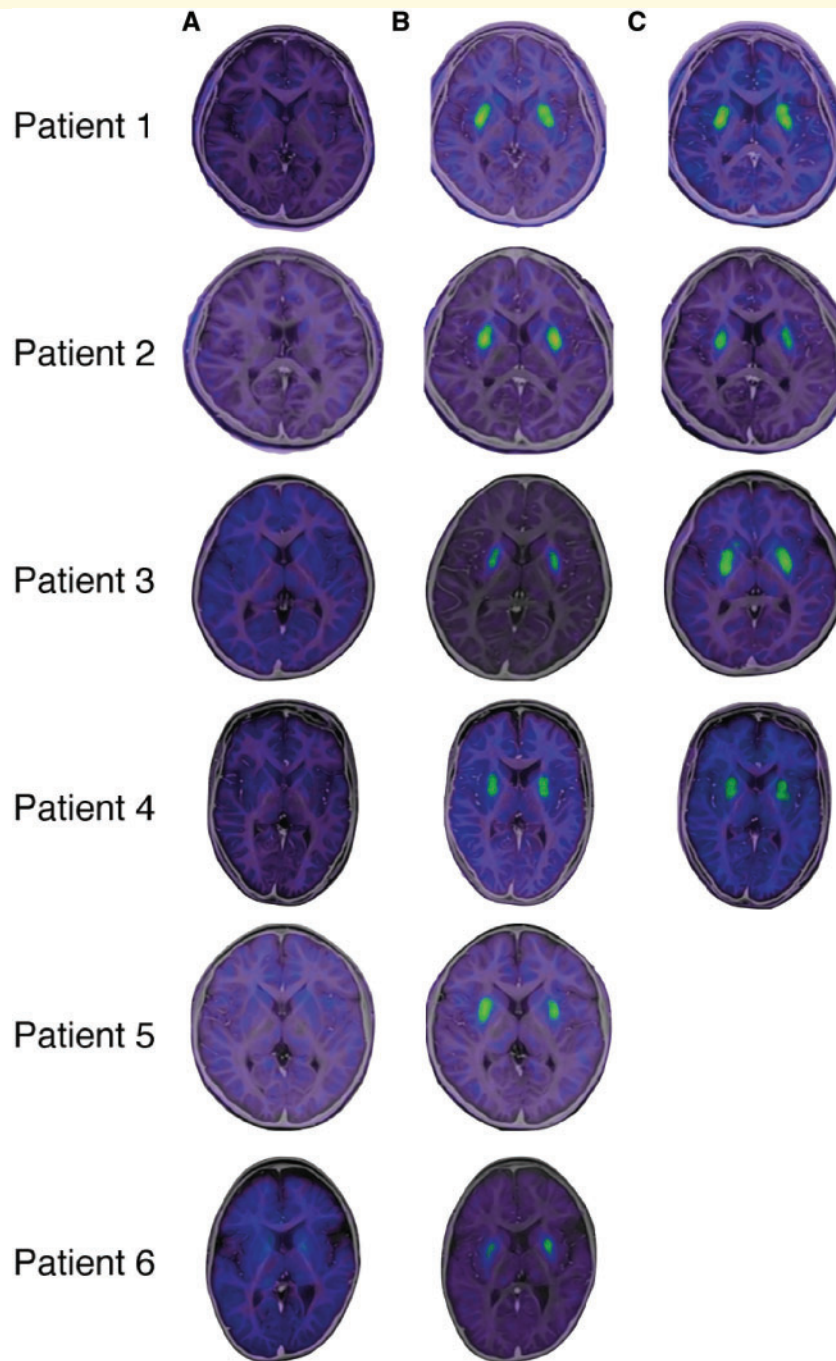
### Analysis of catecholamine metabolites in CSF

Before treatment, the levels of dopamine and the catecholamine metabolites HVA and MHPG as well as the serotonin metabolite 5-HIAA were low, and the L-DOPA level was elevated (Table 3). The level of HVA at 1 month after gene

therapy was mildly elevated in Patients 2, 4 and 6 but not in the other patients. Almost normal levels of dopamine and serotonin metabolites were detected in Patient 3 at baseline, which might indicate remnant enzyme activity.

### The detection of vector-derived genomes in serum and urine after treatment

No vector sequence was detected in the serum or urine by PCR in any of the patient samples after vector injection.



**Figure 3 FMT-PET findings.** The expression of AADC was detected by PET after the injection of FMT, the specific tracer for AADC. **(A)** The co-registered PET and MRI data before treatment. No signal was detected in their brains. **(B)** At 6 months after treatment (at 3 months after in Patient 5 alone), a high-intensity signal was detected in the bilateral putamen in all patients. **(C)** The signal in the putamen was maintained after 2 years in all patients.

### Neutralizing antibodies against AAV2 capsid

None of the subjects exhibited neutralizing antibodies against AAV2 capsid before operation. All patients had elevated titres (up to 1:56–1:28 000) at 6 months after vector injection.

### Discussion

All six of the patients in our study showed marked improvement in their motor function after gene transfer. Although five patients with the severe phenotype had been bedridden before treatment, one patient (Patient 2) became able to walk using a walker, and two patients



(Patients 1 and 5) were able to stand with support. The pattern of improvement after gene therapy for elder patients differed from that of normal infantile development. Therefore, the AIMS score and actual motor function of the patients were not correlated in some aspects. However, AIMS scores gradually increased in all patients, suggesting that the AIMS score has some utility for evaluating the motor improvement. The disappearance of dystonia, stabilized respiration and decreased salivation improved the patients' quality of life.

Differences in the results of our study and the Taiwanese study were (i) that older patients (>8 years of age) also showed improvement, although the treatment was more effective in younger patients than in older ones; (ii) that the genetic background of our patients was heterogeneous and some patients showed better improvement than the Taiwanese patients; and (iii) that the cognitive and verbal functions were markedly improved in one patient with the moderate phenotype (Patient 3). The patients who underwent gene therapy in Taiwan ranged in age from 1 to 8 years (Hwu *et al.*, 2012, 2018). In our study, Patients 1, 2, 4 and 5 were older than the Taiwanese patients, but Patients 1, 4 and 5 showed similar degrees of motor improvement to the Taiwanese patients (Hwu *et al.*, 2012). This indicates that neuronal cells and the dopaminergic tracts can still be activated in older patients, up to at least 19 years of age. However, older patients were slower to recover than younger patients.

Another point to note is the effect of the genetic background. Our patients were genetically heterogeneous (Table 1), and only Patient 5 had IVS6+4A>T homoplasmism like the Taiwanese patients, although one allele of Patient 4 was IVS6+4A>T. The mutation IVS6+4A>T is a splice site mutation resulting in the formation of a truncated DDC protein (Lee *et al.*, 2009). The other mutations were base substitutions inducing amino acid changes; thus, some enzyme activity would be expected, and the position of the mutation might influence the severity of the disease (Supplementary Fig. 1). A relationship between the position of the mutation and the enzyme catalytic activity in AADC has been reported previously (Montioli *et al.*, 2014). Mutations Ala110Glu in Patients 1 and 2, Trp105Cys in Patient 3, Pro129Ser in Patient 3, Tyr369Cys in Patient 4 and Asp252Gly in Patient 6 localized outside of the catalytic domain (Supplementary Fig. 1) were thought to have a milder effect than other mutations (Kojima *et al.*, 2016). The moderate phenotype of Patient 3 may be attributed to the localization of both mutations outside of the catalytic domain (Kojima *et al.*, 2016).

Details concerning the mechanisms underlying the effects of gene transfer, including what circuits of dopamine systems are activated and how the dopamine that is produced works, remain to be elucidated. The most prevalent feature of AADC deficiency is the loss of voluntary movement. Dopamine plays an important role in the proper regulation of voluntary movement. The main pathway for dopaminergic control of the motor function system is from the

substantia nigra to the striatum (Bolam *et al.*, 2000; Gerfen *et al.*, 2011). Others are from the ventral tegmentum to the frontal area and nucleus accumbens, which are associated with higher brain functions and satisfaction, respectively. Cortico-basal ganglia loops, such as motor, prefrontal, limbic and oculomotor loops, have been proposed as functions of the basal ganglia (Alexander *et al.*, 1990; Hikosaka *et al.*, 2000; Middleton and Strick, 2000). In the motor loop, the motor area in the cerebral cortex projects into the putamen (Gerfen *et al.*, 2011). In patients with AADC deficiency, dopamine depletion in the striatum leads to the failure of the motor loop function, resulting in the loss of voluntary movement (Gerfen *et al.*, 2011). For the vector injection site, we targeted the putamen, which is the site that receives dopaminergic projections from the substantia nigra, in order to ensure improvement in the motor function. In preclinical studies in a non-human primate model of Parkinson disease, more than 85–90% of transduced cells were medium spiny neurons (Muramatsu *et al.*, 2002; Daadi *et al.*, 2006). We suspect that dopamine was synthesized and secreted from these postsynaptic neurons in the putamen and then functioned as a neurotransmitter to stimulate postsynaptic dopaminergic receptor directly or that it was secreted from the presynaptic terminal after the uptake by dopamine transporters. The mechanism underlying dystonia in patients with AADC deficiency is not well understood. Patients with several diseases associated with low levels of dopamine, such as Segawa disease, show dystonia (Segawa *et al.*, 1976). An imbalance between the direct and indirect pathways caused by dopamine depletion has been proposed to be associated with the course of dystonia (Wichmann *et al.*, 1998; Vitek *et al.*, 1999; Sanger, 2003). Providing a sufficient amount of dopamine to the striatum would help control dystonia. However, the most prominent adverse event observed after the gene therapy was transient dyskinesia: choreic movements that were observed in all patients after their dystonia improved. In contrast to dystonia, excessive dopamine is considered to be involved in the course of dyskinesia (Wichmann *et al.*, 1998; Sanger, 2003). Because the L-DOPA level was excessive and the dopamine receptors were assumed to be hypersensitive based on the low level of dopamine before gene transfer, a transient hyper-dopamine state was induced. Hypersensitivity of DRD2 might strongly but transiently activate the pathways. Furthermore, as dopamine receptors are reported to exist all over the dendrites and not in the postsynaptic membrane alone, these receptors might be stimulated, thus allowing a sufficient dopamine effect to be obtained (Yung *et al.*, 1995; Uchigashima *et al.*, 2016). Limbic and oculomotor loops were also considered to be activated since patients showed improvement in their emotion as well as saccadic eye movements and OGC. Dopamine is reported to play a role in synaptic plasticity, modulating glutamatergic neurons in order to enhance the synaptic connection to create a neuronal network (Calabresi *et al.*, 2007; Shen *et al.*, 2008). The loss of dopamine resulted in

poor synaptic and neuronal network formation. Patients with some remnant enzyme activity may—to some extent—have a synaptic and neuronal network that shows a stronger and faster response to AADC induction.

Another promising result of our study was the high degree of improvement observed in the cognitive and verbal function of Patient 3. Our target of gene therapy was to improve the motor function of patients, and improvement in cognitive and verbal function were not expected. After gene therapy, the patient's Language-Sociality score improved to the normal range, and she became able to converse normally. The contribution of dopamine to the higher brain function is mainly achieved by projection from the ventral tegmentum to the frontal area, which is associated with the executive function. This projection was not expected to be activated. The prefrontal cortex and striatum (mainly caudate and partially rostral part of putamen) contribute to cognition, memory and other functions via their connection as the prefrontal loop (Middleton and Strick, 2000). Furthermore, the frontal area of the putamen is rich in neural connectivity between many areas of the cortex. The dopamine produced following gene therapy likely activated these networks.

The AADC enzyme expression after gene transfer was clearly visualized by an FMT-PET analysis, and the enzyme was still expressed at almost the same intensity at 2 years after gene transfer treatment. This confirmed that the transduced DDC gene and production of AADC enzyme were maintained for long periods in neuronal cells with no cell division (Bankiewicz *et al.*, 2006). The long-term enzyme expression was also observed in the post-mortem analysis of a monkey; AAV2-AADC that had been injected into the putamen was still detectable after 15 years (Sehara *et al.*, 2017). The level of catecholamine and serotonin metabolites in the CSF did not change markedly after the gene transfer therapy, except for a mild elevation of HVA in Patients 2–4 and 6. However, this HVA elevation was mild and not confirmed to be related to the increase in dopamine synthesis. The slightness of this change may have been because of the small number of gene copies injected into a restricted area of the brain or because the analysis was performed too soon (1 month after injection) to reflect the gene transfer.

Although the present patients were older than the previously studied Taiwanese patients, they were treated with the same dose of vector and showed similar improvements in their motor performance and putaminal tracer uptake on PET. These findings provide independent confirmation of the safety, tolerability and potential efficacy of AADC gene therapy. Future studies focusing on the optimal vector dose and defining the relationship between the vector dose and clinical effects are necessary. In conclusion, these data indicate that the AAV vector-mediated gene transfer of AADC is safe and that it may benefit patients with AADC deficiency.

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## Competing interests

S.M. and T.S. own equity in a gene therapy company (Gene Therapy Research Institution) that commercializes the use of AAV vectors for gene therapy applications. To the extent that the work in this manuscript increases the value of these commercial holdings, they have a conflict of interest. The other authors declare no conflicts of interest in association with the present study.

## Supplementary material

Supplementary material is available at *Brain* online.

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