

In Vivo Evidence for GABA_A Receptor Changes in the Sensorimotor System in Primary Dystonia

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ABSTRACT: Background: Preclinical and clinical evidence suggests that impaired gamma-aminobutyric (GABA) control, leading to disinhibition within the sensorimotor system, might play a role in dystonia. Aim of this study is the in vivo assessment of the GABAergic system in dystonia using positron emission tomography (PET) and ¹¹C-flumazenil, a selective GABA_A receptor ligand.

Methods: Fourteen subjects with primary dystonia (9 carriers of the DYT1 mutation and 5 sporadic cases) were compared to 11 controls, using a simplified reference tissue model to measure binding potential.

Results: Voxel-based analyses showed a reduction in GABA_A receptor expression/affinity both in DYT1 carriers and sporadic patients in primary motor and premotor cortex, primary and secondary somatosensory cortex, and in the motor component of the cingulate gyrus.

Conclusions: Dysfunction of GABA_A receptors in sensorimotor systems in primary (genetic and sporadic) dystonia supports the view that lack of GABAergic control may be associated with the generation of dystonic movements.

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Key Words: GABA; GABA_A receptors; dystonia; sensorimotor cortex; PET

Dystonia is characterized by abnormalities in the control of movements, with involuntary and sustained muscle contractions, frequently causing prolonged twisting and repetitive movements or abnormal postures.^{1,2}

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Preclinical and indirect clinical evidence suggests that changes in GABAergic control system might represent a key dysfunctional component leading to disinhibition of the sensorimotor system.^{3–16}

Noteworthy, magnetic resonance spectroscopy revealed a significant decrease in GABA levels in the sensorimotor cortex and lentiform nuclei contralateral to the affected side in patients with focal dystonia, an indirect evidence supporting the relevant role of GABAergic alterations.¹⁷

We used PET and ¹¹C-flumazenil (¹¹C-FMZ), a specific ligand for the benzodiazepine-binding site on the α subunit of the GABA_A receptor, to measure the GABA inhibitory system in primary dystonia in vivo. To evaluate GABA_A receptor changes independent from the specific disease etiology, we included both DYT1 carriers and patients presenting with sporadic dystonia. The former group has a known pathophysiology involving

the dysfunction of torsinA, a putative chaperone protein.¹ Previous data have consistently demonstrated structural and functional similarities in DYT1 carriers and sporadic dystonia patients.^{16,18,19} We hypothesized that this would hold also for PET ¹¹C-FMZ data.

Methods

Participants

We recruited 14 patients with primary dystonia (8 men, 6 women; mean age $\pm$ SD = 39.6 ± 7.8 years; range 28–56) and 11 healthy subjects (10 men, 1 woman; mean age: 31.4 ± 11.9 ; range 24–60).

All subjects gave written informed consent to take part in the study, approved by the Local Ethical Committees.

All patients underwent diagnostic DYT1 testing and the Fahn–Marsden Dystonia Rating Scale (FMDRS), a validated tool for assessment of dystonia.²⁰ Patients presenting with important tremor in the head and neck region, were not included to avoid movement artefacts on the imaging results.

The inclusion criteria for dystonia patients were (1) occurrence of clinically measurable dystonia²; (2) no neurological disorder other than dystonia; (3) no other cause for dystonia ascertained by clinical assessment, blood tests or neuroimaging; (4) no brain, spinal or peripheral nerve surgery for dystonia; and (5) no therapy, and no use of botulinum neurotoxin in the 4 months before the PET scan. All patients were tested for DYT1 gene mutations and their carrier status was annotated.

The control group was composed of 11 healthy subjects with no family history of dystonia and no DYT1 gene mutations. They were recruited from a departmental registry of volunteers. They all had a normal MRI brain scan and no history of neurological and psychiatric disorders, brain trauma, brain, spinal or peripheral nerve surgery.

¹¹C-FMZ PET Data Acquisition and Image Processing

The study included a bolus injection administration of 4 MBq/kg (320 ± 20 MBq) of ¹¹C-FMZ, measured by a 60-minute dynamic 3D PET scan with an 18-ring whole-body PET/CT scanner (GE Discovery, General Electric Medical System, Milwaukee, WI).

A detailed description of ¹¹C-FMZ PET acquisition has been provided elsewhere.²¹

¹¹C-FMZ PET Data Analysis

For quantitative analysis of the ¹¹C-FMZ data, we used the simplified reference region method.^{22,23}

Reconstructed images were processed and analyzed using SPM5 running under Matlab7 (MathWorks, Sherborn, MA).

We examined the effect of the diagnostic group on binding potential (BP_{ND}) and on ¹¹C-FMZ relative per-

fusion index (R₁) by creating voxel-based, whole-brain statistical parametric maps using the theory of Gaussian random fields and the general linear model, by a one-way ANOVA design, with grand mean scaling. We controlled for age and gender effects introducing those factors in the analysis as “nuisance” variables.

We tested for significant group differences between:

1. all dystonic patients versus healthy controls;
2. DYT1 mutation patients versus healthy controls;
3. sporadic dystonic patients non-DYT1 carriers versus healthy controls.

Significance levels was set at $P < 0.05$ after family-wise error correction for multiple comparisons for whole-brain volume to minimize the risk of false positive findings.

The anatomical localization of statistically significant clusters was verified using cytoarchitectonically defined probabilistic maps and the SPM Anatomy toolbox.

Results

Clinical Characteristics

DTY1 Patients

The DYT1 GAG deletion was present in 9 patients (3 women/6 men) with a mean age of 39.6 ± 8.9 years (range 28–56 years) and mean disease duration of 20.3 ± 10.6 years (range 2–31 years). Mean age at onset was 19.3 ± 14.9 years (range 7–46 years).

At time of study evaluation, 6 patients had generalized dystonia, involving the neck in 3 cases and the larynx in 1 case; in the only patient with a focal onset, the condition remained limited to focal dystonia (writer’s cramp) without generalization, after 9 years. The remaining 2 DYT1 carriers + patients had multifocal dystonia with both upper limb involvement and associated right lower limb involvement in 1 case. Mean FMDRS scored 30.8 ± 20.4 (range 2–58).

Sporadic Patients

The 5 sporadic cases (3 women/2 men) had a mean age of 39.6 ± 6.1 years (range 31–46) at the time of evaluation. Mean age at onset was 28.0 ± 16.1 years (range 1–40 years), and disease duration was 11.8 ± 11.1 years (range 2–30).

At time of study, 1 patient had generalized dystonia, involving both upper limbs; 2 patients had segmental dystonia, involving both the neck and the left upper limb; the remaining 2 patients had focal dystonia, involving the neck in one case and the right upper limb in the other one. Mean FMDRS scored 17.0 ± 8.0 (range 8–29).

There were no significant differences for age, age at onset, disease duration, and disease severity between the two groups of patients.

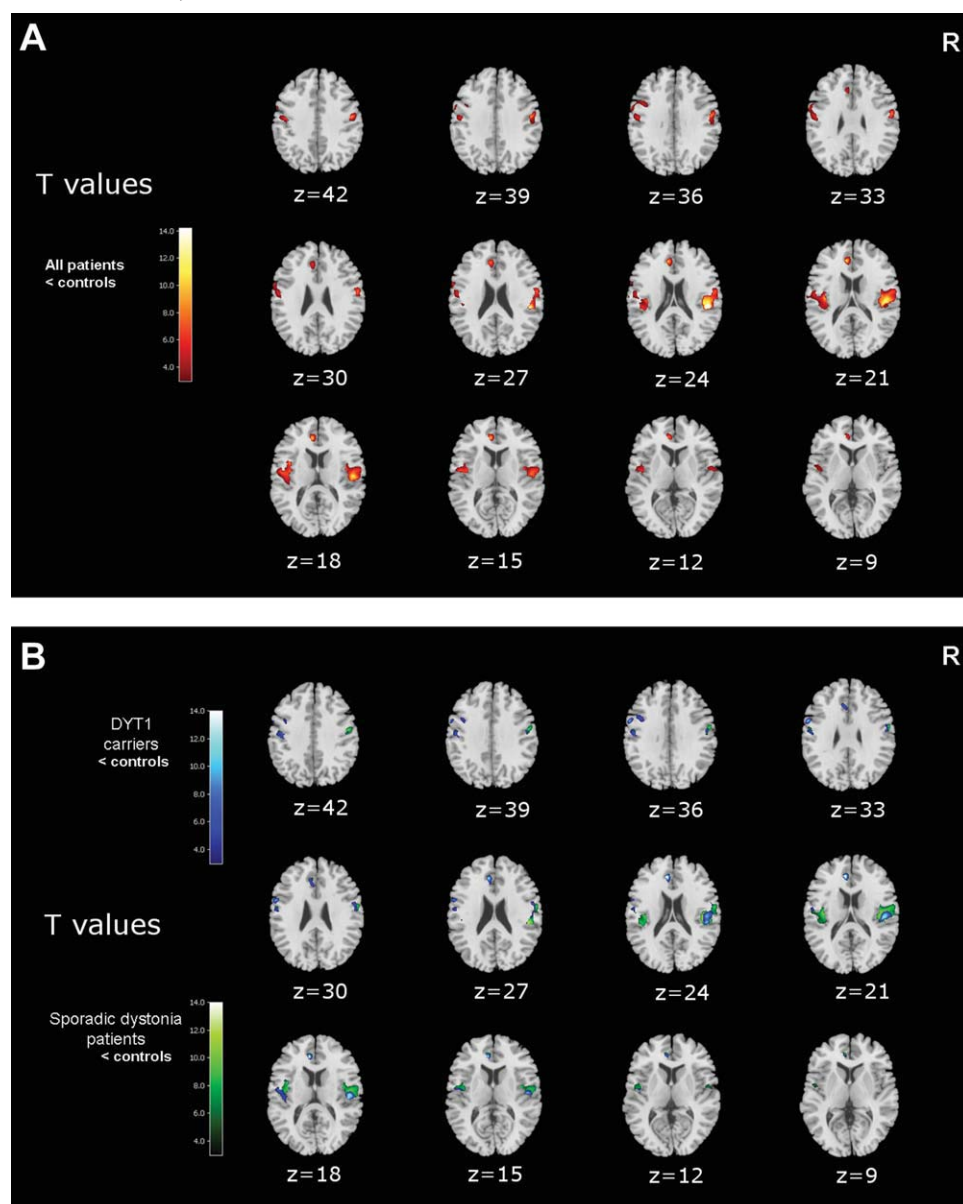


FIG. 1. A. Results of SPM5 group analysis showing GABA_A binding potential (BP_{ND}) reduction in the whole patients group, superimposed on a standard template (see text for details). **B.** Results of SPM5 group analysis showing GABA_A BP_{ND} reduction separate for in DYT1 carriers (blue) and sporadic (green) patients, superimposed on a standard template (see text for details). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

¹¹C-FMZ PET

Group Analysis

The ANOVA revealed a significant ¹¹C-FMZ BP_{ND} reduction in the whole dystonia group as compared to healthy controls, involving, bilaterally, the primary motor and premotor cortex, the primary and secondary somatosensory cortex (S2), the posterior insula and the motor component of the left anterior cingulate gyrus, as classically defined²⁴ (see Fig. 1). The stereotactic coordinates and statistical significance of each cluster of ¹¹C-FMZ BP_{ND} are reported in Table 1.

No significant differences were observed in the basal ganglia or in the cerebellum.

The reverse comparison failed to identify any focal BP_{ND} increase in the dystonia group.

The separate analysis of DYT1 carriers and sporadic patients, each versus controls, confirmed the focal ¹¹C-FMZ BP_{ND} reductions, as shown in Figure 1. Consistent with the latter findings, the direct comparison between DYT1 carriers versus sporadic patients showed no significant between-group differences.

Discussion

In this study, we provide in vivo PET evidence of central GABA_A receptor dysfunction in the sensorimotor systems of patients with primary dystonia,

TABLE 1. Anatomical localization of significant GABA_A binding potential (BP_{ND}) reduction according to standard stereotactic coordinates (Montreal Neurological Institute)

Anatomical localization and corresponding cytoarchitectonic areas	Coordinates of cluster maximum			Cluster extent (voxel number)	T value	P value
	X	Y	Z			
Right primary sensorimotor cortex (area 3a, 3b, 4a, 4p); right secondary somatosensory cortex – S2 (areas OP1, OP2, OP3, OP4)	51	–28	20	655	13.2	<0.000
Right premotor cortex (area 6)	32	–6	55	4	4.6	0.004
Left primary sensorimotor cortex (areas 4a, 4p, 3a, 3b)	–42	–16	38	62	6.5	<0.000
Left secondary somatosensory cortex – S2 (areas OP1, OP2, OP3, OP4)	–38	–16	18	210	7.2	<0.000
Left premotor cortex (area 6)	–62	6	22	15	4.1	0.008
Left anterior cingulate gyrus	–8	38	16	145	8.2	<0.000

including carriers of DYT1 mutations and sporadic non-DYT1 carriers.

>Studies in animal models of primary dystonia, using pharmacologic, neurochemical, and autoradiographic measurements, reported alterations in GABA levels.^{11,12}

The preclinical studies focusing specifically on GABA_A receptors in animal models of dystonia have provided discordant results, showing both decreased and increased receptor density and affinity in different brain regions.^{4,3,14,25}

The present observed reduction in GABA_A BP_{ND} in patients with DYT1 mutation and non-DYT1 sporadic dystonia could be explained either by a primary alteration of GABA_A receptors, e.g. changes in receptor density, affinity, subunit composition, or by secondary changes associated with the imbalance of GABA neurotransmitter synthesis. The first hypothesis is supported by the observation of changes in the structure of GABA_A receptors, with the prevalence of the closed conformation of the GABA-gated chloride ionophore, in animal models of dystonia.¹³ The second hypothesis, postulating a change of GABA_A receptors subsequent to GABA synthesis defects, is supported by indirect experimental evidence. Preclinical work has shown a peculiar behavior of the GABA_A receptor system, suggesting that increased GABA levels enhance the affinity of GABA_A receptors for benzodiazepine ligands through a conformational change (the so-called “GABA-shift”).^{26,27} Accordingly, a reduction in GABA levels should induce a reduced GABA_A receptor affinity and a consequent reduction of ¹¹C-FMZ binding, as supported by recent in vivo imaging studies in baboons.^{28,29} Thus, a disease condition associated with a reduction in GABA concentration/synthesis would be associated with a decrease of the ¹¹C-FMZ BP_{ND}.

In addition, we found a significant ¹¹C-FMZ BP_{ND} reduction in both DYT1 carriers and noncarriers, in line with previous studies, showing that the clinical expression of dystonia depends on widespread deficits,

and the presence of the DYT1 gene mutation itself leads only to a subset of these changes, probably through a pathogenetic mechanism common to different dystonia forms.⁷

The present findings cannot discriminate whether the observed BP_{ND} changes are causative of the dystonia phenotype or whether they represent an epiphenomenon associated with abnormal motor circuitry. On the one hand, a dysfunction of GABA_A receptors might arise from the disorders of sensorimotor systems primarily involved in the pathophysiology of primary dystonia. Already, there is evidence not only from manifesting but also from nonmanifesting carriers of the DYT1 mutation that GABA_A activity (as measured by short intracortical inhibition – SICI) is abnormal.¹⁹ This suggests that GABA_A abnormality may pre-exist overt clinical manifestations and represent a subclinical endophenotypic trait of dystonia. Alternatively, GABA_A deficits may represent a secondary phenomenon associated to dysfunctions of an extended neuro-anatomical and neurotransmission network in dystonia.

It is important to emphasize that the GABAergic system is part of a plastic neurotransmitters network. Amelioration of dystonic symptoms by GABAergic drugs is by no means complete or universally effective: abnormalities of dopamine and acetylcholine have also been implicated in dystonia, consistent with the concept that neurochemical defects at multiple points along the motor circuit might be required for dystonia to manifest.³⁰ Deficient GABA levels could also be secondary to a reduced dopaminergic input in the putamen, as D₂ dopamine receptors mostly inhibit the GABAergic striatal medium spiny neurons.¹⁷ Symptoms of dystonia can also be treated effectively with anticholinergic medications.³⁰ Acetylcholine is deeply intertwined with dopaminergic function and dysfunction. Cholinergic interneurons branch extensively onto GABAergic medium spiny neurons and have been shown to modulate responses of these neurons to cortical and thalamic excitation.³¹

A deficit in GABAergic function might then result in abnormalities of neuronal inhibition affecting both the motor and somatosensory systems. The role played by GABAergic neurotransmission in the modulation of motor function is well known, and is mainly associated with SICI and with cortical plasticity.³² GABAergic inhibition has been shown to play a role in the generation of cortical spatiotemporal patterns of muscle activity.³³ A reduction of the intracortical inhibitory GABA system is in agreement with the robust finding in dystonia patients that sensorimotor plasticity is enhanced and spreads outside the topography of the target muscle in paired associative stimulation studies.^{34,35}

The finding of reduced inhibitory GABA control in somatosensory cortex is also of special interest as the GABA system also plays a crucial role in the modulation of the afferent signal in the somatosensory cortex in dystonia.^{16,36}

A deficit in GABAergic function might then result in abnormalities of neuronal inhibition affecting both the motor and somatosensory systems.³⁷ Previous studies have provided evidence both for spatial dedifferentiation of somatosensory representation and for a disturbance of sensorimotor integration in dystonia, that may contribute to spatial dedifferentiation in the motor cortex.³⁸ ■

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