Molecular Genetics and Metabolism 107 (2012) 229–233

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

journal homepage: www.elsevier.com/locate/ymgme

Neurotransmitter abnormalities and response to supplementation in SPG11

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ARTICLE INFO

Article history:
Received 25 May 2012
Accepted 26 May 2012
Available online 1 June 2012

Keywords:
Spastic paraparesis
Dopa responsive dystonia
Neurotransmitter disorder
SPG11

ABSTRACT

Objective: To report the detection of secondary neurotransmitter abnormalities in a group of SPG11 patients and describe treatment with L-dopa/carbidopa and sapropterin.
Design: Case reports.
Setting: National Institutes of Health in the Undiagnosed Disease Program; Children's National Medical Center in the Myelin Disorders Bioregistry Program.
Patients: Four SPG11 patients with a clinical picture of progressive spastic paraparesis complicated by extrapyramidal symptoms and maculopathy.
Interventions: L-Dopa/carbidopa and sapropterin.
Results: 3/4 patients presented secondary neurotransmitter abnormalities; 4/4 partially responded to L-dopa as well as sapropterin.
Conclusions: In the SPG11 patient with extrapyramidal symptoms, a trial of L-dopa/carbidopa and sapropterin and/or evaluation of cerebrospinal fluid neurotransmitters should be considered.

1. Introduction

Hereditary spastic paraplegias (HSP) are characterized by predominant lower limb pyramidal signs sometimes complicated by other neurological manifestations. SPG11, caused by mutations in *KIAA1840* encoding spatacsin [1], is characterized by childhood onset of progressive spastic paraparesis, cognitive deficits, and peripheral neuropathy [2]. Several patients with an early-onset primarily extrapyramidal phenotype have also been described [3–6].

We report here the prospective collection, based on a first observed patient, of the clinical findings of four patients with HSP due to mutations in *KIAA1840* and secondary neurotransmitter abnormalities. In pediatric neurotransmitter disorders (PNDs) abnormalities in cerebrospinal fluid (CSF) neurotransmitter metabolite concentrations provide the diagnostic hallmark. PNDs can be primary, due to mutations leading to enzymatic defects in the biosynthetic pathway of dopamine and serotonin, but neurotransmitter abnormalities may also be secondary to other neurological conditions [7]. In both cases, extrapyramidal signs are the most common finding. Supplementary treatment [7], which in primary causes of PND results in remarkable clinical improvement, may also have clinical utility in neurologic disorders with secondary neurotransmitter abnormalities.

2. Methods

2.1. Patient collection

Patients were collected prospectively, after identification of the index case, by evaluation of SPG11 patients in the Undiagnosed Disease Program at the National Human Genome Research Institute at the National Institutes of Health (NIH) and in the Myelin Disorders Bioregistry Project at Children's National Medical Center (CNMC). All activities were approved by the Institutional Review Board at both institutions.

2.2. Patient evaluation

Patients were evaluated at the Clinical Center at the NIH in a standardized fashion. Testing, including lumbar puncture, was performed

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doi:10.1016/j.ymgme.2012.05.020

according to standard clinical protocols. The 20 ft timed walk was measured according to a standardized protocol at the NIH.

2.3. Cerebrospinal fluid analysis

Lumbar puncture was performed in the morning prior to administration of pharmacologic treatment. Cerebrospinal fluid (CSF) was analyzed according to a standard protocol at a commercial laboratory, which includes fractionation of CSF aliquots due to the known gradient of neurotransmitters in this fluid compartment. CSF was immediately stored at −80 °C until use for the analysis. Neurotransmitters metabolites and pterins in CSF were investigated by high-performance liquid chromatography with electrochemical detection according to a codified procedure [8].

3. Results

3.1. Patient 1

Patient 1 is a 29 year old woman presenting with cognitive difficulties at age 7 and later onset progressive walking difficulties, incontinence, and dysarthria. Neurological evaluation noted spastic paraparesis combined with rigidity, dystonia in particular of the great toe, dysarthria, dysphonia, and dysesthesias in the lower limbs. Neuropsychological testing showed mild delays (IQ 65 on WAIS-IV). MRI imaging at 29 years showed characteristic findings for SPG11 (Fig. 1). Molecular analysis of *KIAA1840* for SPG11 revealed compound heterozygous mutations (Table 1). At age 26, CSF neurotransmitter analysis indicated low levels of tetrahydrobiopterin (BH4), 5-hydroxyindoleacetic acid (5H1AA) and homovanillic acid (HVA) (Table 2). Treatment with L-dopa/carbidopa led to an improvement in gait and speech. Escalation was stopped at the dose of 7 × 100 mg/25 mg/day because of side effects (tremors, dizziness and nausea). Sapropterin, introduced as an alternative (100 mg × 3), yielded no obvious clinical improvement, but in combination with lower L-dopa/carbidopa (maximal dose 4 × 50 mg/12.5 mg/day) resulted in improved tolerability, improved motor symptoms and reduced the frequency of falls. The patient is still on treatment. Retesting of CSF two years later, on treatment, demonstrated improvement of the initial CSF abnormalities (Table 2).

Table 1
Patient genotype for mutations in *KIAA1840*.

Patient number	Mutation 1	Mutation 2
1	c.5470C>T; p.Arg 1824X; [13] (arginine > OPA)	c.5623C>T;p.Gln1875>X; [14] (glutamine > amber)
2	c. 2471–2472dupT; p.824fs [15]	c. 5456–5457delAG; p.1819fs [16]
3	c. 2471–2472dupT; p.824fs [15]	c. 5456–5457delAG; p.1819fs [16]
4	c.4222insA; p.1426X [STOPAA1426][a]	c.4777delA; p.1606X [STOPAA1606][a]

[a] Reported as a novel mutation.

3.2. Patient 2

Patient 2 is a 24 year old male with a history of childhood onset language and cognitive delay. At approximately 15–16 years, additional symptoms became apparent, including fine motor difficulties, gait disturbance leading to loss of independent ambulation at age 23, dysarthria and incontinence. Neurological exam showed a spastic paraparesis with rigidity, bradykinesia, dysmetria, and dysdiadochokinesia. Difficulties with gait initiation and gait freezing were prominent. Ophthalmologic exam showed subtle fleck-like macular pigmentary changes bilaterally (Fig. 2A). Electrophysiological examination suggested a length-dependent, chronic motor neuronopathy. Neuropsychological testing revealed mild cognitive delays (IQ of 59 on WAIS-IV). MRI showed characteristic findings for SPG11

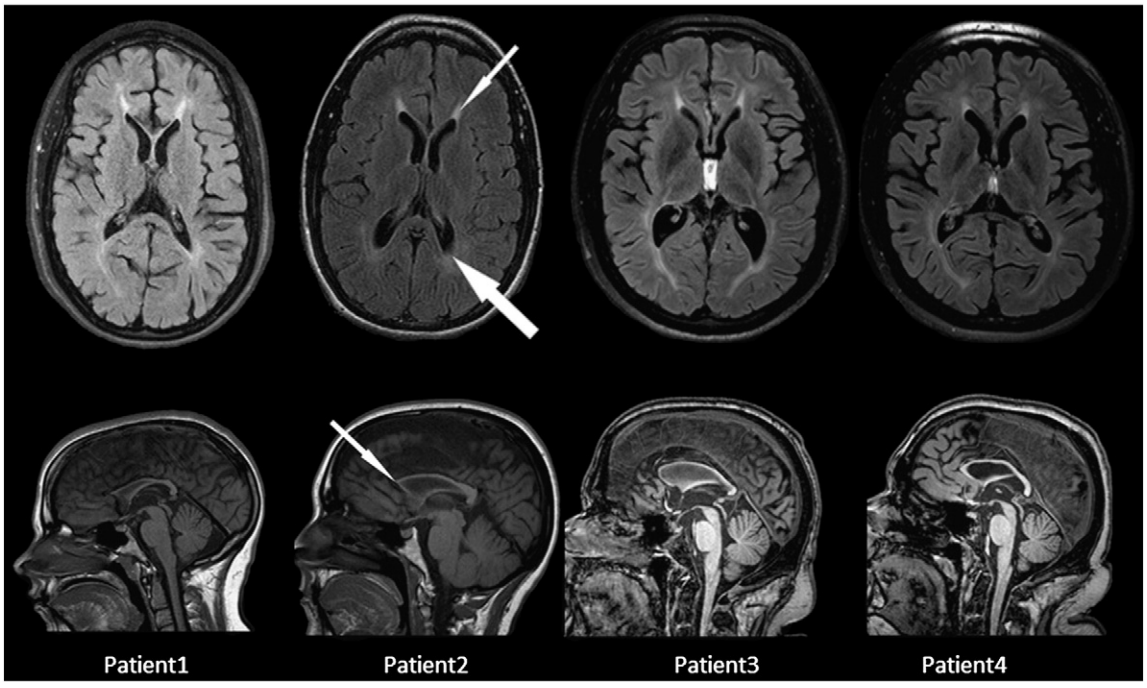

Fig. 1. Top portion: magnetic resonance imaging: MR flair-weighted axial scan. All patients showed the "ears of lynx" sign consisting of signal changes of periventricular white matter in the frontal region centered in the area of the forceps minor of the corpus callosum (thin white arrow). Patients also demonstrated white matter atrophy (thick white arrow). Bottom portion: magnetic resonance imaging: MR T_1-weighted sagittal scan. All patients had a thin corpus callosum more pronounced in the mid and anterior portions with relative sparing of the splenium. This abnormality results in a "beaked" appearance of the anterior portion of the corpus callosum considered characteristic of SPG11 (thin white arrow).

Table 2
Neurotransmitter values before and after treatment.

	Reference values (nmol/l)	Patient 1		Patient 2		Patient 3		Patient 4	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
HVA	145–324	**57**	143	**110**	ND	200	ND	**69**	557
5HIAA	67–140	**49**	63	120	ND	71	ND	106	106
3-OMD	<100	13	542	20	ND	26	ND	21	2500
Neo	8–28	16	14	17	ND	16	ND	27	14
BH4	10–20	**6**	14	**10**	ND	16	ND	**7**	17

Abnormal values are in bold. Pre and Post refer to treatment.
HVA = homovanillic acid; 5HIAA = 5-hydroxyindolacetic acid; 3-OMD = 3-O-methyldopa; Neo = neopterin; BH4 = tetrahydrobiopterin; MTHF = 5-methyltetrahydrofolate; ND = not done.

(Fig. 1). Molecular analysis of *KIAA1840* for SPG11 revealed compound heterozygous mutations (Table 1). CSF testing revealed low levels of HVA with normal 5HIAA and a BH4 concentration at the lower limit of the normal range (Table 2). The patient was started on L-dopa/carbidopa with a maximal dose of 4×150/37.5. Initially, the regimen resulted in substantial improvement in his capacity to reach the standing position and walk. The average of four consecutive attempts in a 20-foot timed walk was 29 s (range: 21 to 42 s) in the naïve state vs. 16.5 s (range: 14 to 19 s) 90 min after his morning L-dopa/carbidopa dose, in the first days after beginning of therapy. Over time, however, progressive lower extremity weakness negated any initial medication benefits and on re-evaluation after a year there was no sustained benefit. Repeat CSF testing was not performed because the patient did not consent to a second lumbar puncture.

3.3. Patient 3

Patient 3 is a 27-year-old male, brother of patient number 2, who presented for an evaluation for progressive gait impairment, incontinence and cognitive dysfunction that started at 19 years of age. Neurological exam revealed spastic paraparesis and rigidity, ataxia, dysmetria and dysdiadochokinesia, mild dysarthria and mild impairments with swallowing, decreased vibration and proprioceptive sensation. Ophthalmic exam showed subtle parafoveal flecks bilaterally (Fig. 2B). Electromyography suggested motor neuropathy and neuropsychological examination showed mild impairment (IQ 67 on WAIS-IV). Brain MRI showed characteristic findings for SPG11 (Fig. 1). Molecular analysis of *KIAA1840* for SPG11 showed the same mutations present in his younger brother (Table 1). CSF testing showed normal neurotransmitter metabolite levels (Table 2) but, because of the history of the other patients reported here, a trial of L-dopa/carbidopa (maximal dose 3×150 mg/37.5 mg) was initiated after gait symptoms worsened. There was improvement in overall ambulation and balance with onset immediately after initiation of therapy. The average of four consecutive attempts in a 20-foot timed walk was 19 s (range: 15 to 27 s) in the naïve state vs. 14 s (range: 12 to 18 s) 90 min after his morning L-dopa/carbidopa dose, in the first days of therapy. Repeat CSF testing was not performed because the patient did not consent to a second lumbar puncture; treatment is ongoing.

3.4. Patient 4

Patient 4 is a 25-year-old woman with progressive gait disturbance. This patient had congenital left leg lymphedema resulting in leg length discrepancy. At age 12 she developed impaired balance and frequent falls after elective orthopedic surgery. Gait impairment progressed; and she was confined to a wheelchair by age 21. Over time she also noted weakness in her hands, difficulty with fine motor skills, urinary incontinence, dysarthria and insomnia. Neurologic examination revealed a spastic paraparesis, distal muscular atrophy, extrapyramidal symptoms including rigidity of upper extremities and forced dystonia of toes, and psychomotor slowness. An ophthalmologic anterior segment exam was positive for lagophthalmos and mild punctate keratopathy, while fundus exam showed macular flecks involving the macular and perimacular areas (Fig. 2C). Neuropsychological testing revealed a significant decline in cognitive ability (IQ of 76, a 33-point decline from documented testing ten years prior). EMG, NCV and CMCT revealed mild sensory neuropathy, diffuse neurogenic changes consistent with anterior horn cell neuronopathy and demyelination of the corticospinal tracts. MRI imaging showed characteristic findings for SPG11 (Fig. 1). Mutation

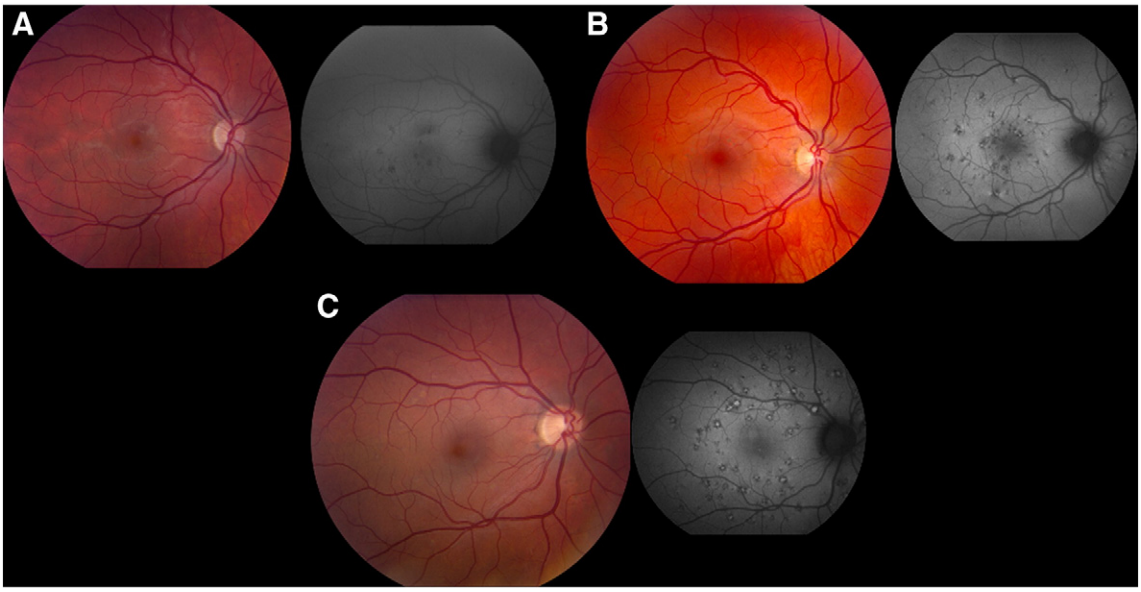

Fig. 2. Retinal images of the patients 1, 2 and 3 (A/B/C) show the retinal changes in the right eyes of the patients as noted on color fundus photos and on fundus auto-fluorescence imaging. The finding of retinal abnormalities is consistent with Kjellin syndrome, and was previously felt to distinguish the clinically similar SPG15 (caused by mutations in *KIAA0321* encoding spastizin) from SPG11, but recently described in SPG11 patients, [17,18]. Kejellin syndrome is additionally characterized by cerebellar signs, and amyotrophy [2], also seen clinically in some of our patients.

testing of *KIAA1840* for SPG11 sequencing revealed novel compound heterozygous mutations (Table 1). CSF neurochemical testing revealed low levels of HVA and BH4 (Table 2). Treatment with L-dopa/carbidopa (maximal dose 4×50 mg/100 mg) and sapropterin (20 mg/kg) resulted in notable improvement and stabilization in motor abilities, with maintenance of improvement over a 2 year period. Repeat CSF testing 3 months after initiation of therapy showed normalized values (Table 2). Attempts to convert to monotherapy by gradually discontinuing sapropterin were unsuccessful with substantial clinical deterioration at doses lower than 700 mg daily. Patient continues to be maintained on therapy.

4. Discussion

We describe four SPG11 patients with confirmed *KIAA1840* mutations and a clinical picture of a progressive spastic paraparesis complicated by cognitive deterioration, cerebellar and extrapyramidal signs, peripheral neuropathy/neuronopathy, and central retinal degeneration. All four patients had characteristic MRI abnormalities previously described in SPG11 [9]. In addition, 3 of our 4 patients showed abnormalities in CSF neurotransmitter metabolite concentrations: low concentration of HVA, the main metabolite in the catabolic pathway of dopamine; and low levels of BH4. While other SPG11 patients with mainly extrapyramidal signs have been described [4], CSF neurotransmitter metabolite analysis has not been reported in these patients, although one study illustrated functional dopamine deficits on SPECT imaging [3,4].

It is unlikely that these four subjects with established SPG11 mutations additionally had a primary neurotransmitter disorder [7]. The CSF profile of neurotransmitters of the patients is not classical for any known primary disorder. Specifically, aromatic L-amino acid decarboxylase (AADC) deficiency would be expected to have elevated 3-O-methyldopa with normal BH4, and tyrosine hydroxylase (TH) deficiency would be expected to have normal BH4 in the presence of decreased HVA [10]. In addition, other abnormalities of biopterin metabolites might be seen, such as decreases in neopterin in addition to BH4 (GTP cyclohydrolase deficiency), or increases in neopterin (sepiapterin reductase deficiency or 6-pyruvoyltetrahydropterin synthase or PTPS deficiency) [8,10,11].

The mechanism leading to neurotransmitter abnormalities in SPG11 patients is not known. The function of spatacsin itself is not well understood, although the protein has been associated with cytoskeleton, endoplasmic reticulum and vesicles involved in protein trafficking, suggesting a role in axonal transport [12]. We speculate that the disruption of these systems could also disturb the physiological neurotransmitter synthesis and transport toward synapses leading to the secondary neurotransmitter abnormalities, although this could also be due to effects of non-specific neurodegeneration and cellular loss. 123I-ioflupane SPECT imaging in two cases reported by Anheim et al. [3,4] demonstrated bilateral reduction of striatal ligand uptake consistent with dysfunction of nigro-striatal innervation.

Although further studies are necessary to understand the origin of neurotransmitter abnormalities in SPG11, this finding in combination with extrapyramidal signs led us to start supplementation of dopamine and tetrahydrobiopterin pathways with L-dopa/carbidopa and sapropterin. We saw at least temporary improvement of motor symptoms in 4/4 patients. Benefits from L-Dopa therapy in SPG-11-associated juvenile parkinsonism have been previously described [3,4]. Our cases further support the idea of an imbalance of the normal dopaminergic circuits in SPG11 patients and the potential benefit of supplemental treatment, in particular in the presence of extra pyramidal features. The observations in this report cover a period of approximately two years, thus, the effects over a longer time span remain unclear for this patient group, and larger, adequately controlled studies are need to determine the benefits of this type of therapy.

Of note, the frequency of neurotransmitter abnormalities in SPG11 is unknown, and the relationship between identified secondary neurotransmitter abnormalities and a response to supplementation has not been explored. Our patient 3, who showed normal CSF neurotransmitters concentrations but responded to supplementation, presented milder symptoms than his brother (patient 2). It is unknown why his neurotransmitters differed from the other patients. It is conceivable that measurable deficits of dopamine and tetrahydrobiopterin pathways are a late finding, and earlier undetectable deficits may result in clinical manifestations and be amendable to supplementation.

5. Conclusion

Although extrapyramidal symptoms have been previously identified in SPG11, this is the first description of neurotransmitter abnormalities in the dopamine and tetrahydrobiopterin pathways in these patients. A trial of L-dopa/carbidopa and sapropterin for the treatment of extrapyramidal signs and symptoms, even in the setting of normal neurotransmitter levels, should be considered in SPG11. The impact of supplementation on motor function and side effects should be carefully monitored until the long term benefits of such therapies are better established. The role of CSF monitoring of neurotransmitters in this disorder, and its relationship to therapeutic monitoring, are unknown.

Acknowledgments

Patients 1, 2 and 3 were enrolled via the Myelin Disorders Bioregistry Program and all patients were evaluated as part of the Undiagnosed Disease Program at the National Institutes of Health (NIH). Thanks are given to the administrative staff of the Undiagnosed Disease Program (UDP) for their logistical efforts. Thanks are also given to referring physicians and patients described in this report, in particular Dr. Steven Vernino for his assistance on outpatient care in one case. Interpretation of neurotransmitter abnormalities was assisted by Dr. Philip Pearl at Children's National Medical Center. The contributions of A.V. were supported by the intramural program at the National Human Genome Research Institute, NIH. Assistance in description of mutation status was provided by Khalida Liaquat, MS, CGC, Genetic Counselor at Athena Diagnostics.

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