GJA1 Variants Cause Spastic Paraplegia Associated with Cerebral Hypomyelination

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ABSTRACT

SUMMARY: Oculodentodigital dysplasia is an autosomal dominant disorder due to *GJA1* variants characterized by dysmorphic features. Neurologic symptoms have been described in some patients but without a clear neuroimaging pattern. To understand the pathophysiology underlying neurologic deficits in oculodentodigital dysplasia, we studied 8 consecutive patients presenting with hereditary spastic paraplegia due to *GJA1* variants. Clinical disease severity was highly variable. Cerebral MR imaging revealed variable white matter abnormalities, consistent with a hypomyelination pattern, and bilateral hypointense signal of the basal ganglia on T2-weighted images and/or magnetic susceptibility sequences, as seen in neurodegeneration with brain iron accumulation diseases. Patients with the more prominent basal ganglia abnormalities were the most disabled ones. This study suggests that *GJA1*-related hereditary spastic paraplegia is a complex neurodegenerative disease affecting both the myelin and the basal ganglia. *GJA1* variants should be considered in patients with hereditary spastic paraplegia presenting with brain hypomyelination, especially if associated with neurodegeneration and a brain iron accumulation pattern.

ABBREVIATIONS: Cx43 = connexin 43; Cx47 = connexin 47; ODDD = oculodentodigital dysplasia

Oculodentodigital dysplasia (ODDD, Online Mendelian Inheritance in Man, No. 164200) is an autosomal dominant disorder due to *GJA1* variants[1] and characterized by dysmorphic features involving the eyes (microphthalmia and microcornea), the nose (narrow, pinched nose with hypoplastic alae nasi), the teeth (small and carious), and limb extremities (syndactyly, camptodactyly). Some patients may present with neurosensory deficits such as spastic paraplegia, ataxia, decreased visual acuity, and hearing loss, possibly associated with white matter and/or basal ganglia signal abnormalities on brain MR imaging.[2,3] However, there is no comprehensive overview of the neuroimaging features of ODDD besides isolated case reports. Therefore, to improve the accuracy of clinical diagnosis and better understand the pathophysiology underlying neurologic symptoms in ODDD, we wished to define key brain imaging findings in 8 consecutive patients presenting with spastic paraplegia due to *GJA1* variants.

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MATERIALS AND METHODS

We retrospectively studied 8 patients from 5 families presenting with hereditary spastic paraplegia. Patients were referred to reference centers for neurogenetic and neurometabolic diseases. Patients were informed and gave their consent to this study.

GJA1 variants were suspected on the basis of the co-occurrence of hereditary spastic paraplegia with dysmorphic features in all patients. These dysmorphic features included ocular abnormalities (short palpebral fissures, microphthalmia), nasal abnormalities (long and narrow nose, hypoplastic alae nasi), dental abnormalities (microdontia, abnormal coloration of the enamel, multiple caries), and bone extremity abnormalities (syndactyly of the fourth and fifth fingers, clinodactyly, camptodactyly, and aphalangia). Clinical examination was performed by experts in rare neurologic diseases (P.C., C.V., M.C., and F.M.). Disease severity was estimated by a disability stage index: 0, no functional handicap; 1, no functional handicap but signs at examination; 2,

Table 1: Clinical and molecular characteristics of 8 patients with *GJA1* variants[a]

	Patient 1#	Patient 2#	Patient 3	Patient 4*	Patient 5*	Patient 6*	Patient 7	Patient 8
Sex/age at examination (yr)	Male/64	Female/34	Female/25	Female/49	Female/22	Female/19	Female/49	Male/56
Family history	Dominant	Dominant	None	Dominant	Dominant	Dominant	None	Dominant
ODDD dysmorphia	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Age (yr)/symptom at onset	50/Gait	28/Gait	18/Urinary	14/Urinary	14/Urinary	16/Urinary	15/Gait	50/Gait
Current disability stage	1	3	3	3	1	1	6	1
LL reduced strength	None	Prox.	Prox.	Prox.	None	None	Prox./dist.	None
LL spasticity	Yes	Yes	Yes	Yes	No	No	Yes	Yes
UL/LL reflexes	↑/↑	↑/↑	↑/↑	↑/↑	↑/↑	↑/↑	↑/↑	↑/↑
Plantar reflexes	Indifferent	Extensor	Extensor	Extensor	Extensor	Extensor	Extensor	Extensor
UL/LL vibration sense	N/↓	N/↓	N/↓	↓/↓	N/N	N/N	N/↓	N/↓
Romberg sign	No	Yes	Yes	No	No	No	Yes	No
Oculomotor signs	Hypermetric saccades	Saccadic pursuit	None	Saccadic pursuit	Hypermetric saccades	None	None	Saccadic pursuit
Dysmetria/dysarthria	Yes/No	Yes/No	No/No	Yes/No	Yes/No	No/No	No/No	No/No
Urinary symptoms	No	+	+++	+++	++	++	++	No
Cognition	Dysexec.	Normal	Normal	Normal	Normal	Normal	Normal	Normal
GJA1 variant	*c.93T>G*	*c.93T>G*	*c.443G>A*	*c.428G>A*	*c.428G>A*	*c.428G>A*	*c.412G>A*	*c.634T>A*
Amino acid change	p.I31M	p.I31M	p.R148Q	p.G143D	p.G143D	p.G143D	p.G138S	p.F212I

Note:—* and # indicate patients belonging to the same family; UL, upper limbs; LL, lower limbs; Prox., proximal; dist., distal; ↑, increased; ↓, decreased; Dysexec., dysexecutive syndrome; N, normal; +, mild; ++, moderate; +++, severe.

[a] Disability stage index: 1, no functional handicap but signs at examination; 3, moderate, unable to run, limited walking without aid; 6, unable to walk, requiring wheelchair.

mild, able to run, walking unlimited; 3, moderate, unable to run, limited walking without aid; 4, severe, walking with 1 cane; 5, walking with 2 canes; 6, unable to walk, requiring a wheelchair; 7, confined to bed.

MR imaging was performed with a 1.5T (patients 1, 3, 5, 7, 8) or 3T (patients 2, 4, and 6) magnetic field and included at least 1 axial T1- and T2-weighted sequence and 1 sagittal sequence. CT scans were obtained in all patients except patients 1 and 3. Cerebral MR imaging and CT scans were qualitatively reviewed by 3 leukodystrophy experts (R.S., O.B.-T., and F.M.). Visual, brain stem auditory, and somatosensory and motor-evoked potentials were available for 4 patients.

RESULTS

Clinical findings are presented in Table 1. Most patients were women (6/8) and had a family history of the disease (5/8). The age at onset was variable, from 14 to 50 years of age, and symptoms at onset were gait difficulties (4/8) and urinary dysfunction (4/8). Disease severity was highly variable with a disability stage index ranging from 1 to 6. All patients presented with spastic paraplegia associated with reduced muscle strength in 4 patients and decreased vibration sense, more pronounced in the lower limbs, in 6 patients. Six patients presented with signs of neurogenic bladder with variable severity: mild (1/8), moderate (3/8), and severe (2/8). One patient required self-catheterization several times a day (patient 3), and 1 had a cystectomy with enterocystoplasty (patient 4). Five patients had mild cerebellar signs.

Cerebral MR imaging revealed white matter abnormalities in all patients, consisting mainly of mild-to-moderate symmetric and diffuse hyperintensities of the corticospinal tracts on T2- and FLAIR-weighted sequences associated with hyper- or isointense T1-weighted signal (Table 2 and Fig 1), consistent with a hypomyelination pattern.[4] Most patients also presented with variable degrees of cerebral and cerebellar atrophy (Table 2 and Fig 1). Furthermore, all patients presented with basal ganglia abnormalities—that is, bilateral T2-hypointense signal of the pallidum and, in some instances, substantia nigra, red nucleus, and dentate nucleus, associated with bilateral hypointense signal of the pallidum on T2*- or magnetic susceptibility-weighted images (Table 2, Fig 2, and On-line Figure) as seen in neurodegeneration with brain iron accumulation diseases. One patient presented with a central region of hyperintensity within the T2-weighted hypointense signal in the globus pallidus, the so-called eye of the tiger sign (Table 2 and Fig 2). CT showed bilateral or unilateral calcifications of the basal ganglia in 2 of 5 patients (Table 2 and Fig 2). Patients with the more prominent basal ganglia abnormalities were the most disabled ones. When performed, visual, brain stem auditory, and somatosensory and motor-evoked potentials showed diffuse and pronounced central conduction anomalies (4/4), compatible with a hypomyelinating process.

Molecular analyses revealed 4 previously reported *GJA1* variants (*c.93T>G, c.412G>A, c.428G>A*, and *c.443G>A*)[5] and one novel heterozygous *GJA1* variant (*c.634T>A*). Of note, the mother of patient 4 carried the heterozygous *c.428 G>A* variant but without any ODDD symptoms, including normal bone extremities.

DISCUSSION

This series of 8 patients with ODDD presenting with hereditary spastic paraplegia shows that the neurologic symptoms associated with *GJA1* variants are related to a complex neurodegenerative process affecting both the white matter and the basal ganglia. Therefore, *GJA1* variants should be considered in all patients with hereditary spastic paraplegia presenting with brain hypomyelination, especially when associated with neurodegeneration with a brain iron accumulation pattern. The early occurrence of urinary symptoms in more than half of the patients suggests an ascending process affecting the corticospinal tracts. Of note, most patients were female, while men presented with a later onset of disease.

Indeed, the *GJA1* gene encodes connexin 43 (Cx43), a transmembrane protein acting in intercellular communication.[1] Cx43 is expressed in astrocytes and plays a role in astrocyte-oligodendrocyte communication by heterotypic Cx43/connexin 47 (Cx47) channels. Some studies have suggested that Cx43/Cx47 channels

Table 2: Brain imaging characteristics of 8 patients with *GJA1* variants[a]

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Age (yr)/disability stage	64/1	34/3	25/3	49/3	22/1	19/1	49/6	56/1
WM T1 signal (relative to the cortex gray matter)	Iso	Hyper	Hyper	Iso	Iso	Hyper	Iso	Hyper
WM T2-hyperintense signal								
Periventricular	−	++	+	++	+	+	++	+
Internal capsule (posterior limb)	+	+	+	+	+	+/−	+	+
Corpus callosum	−	−	−	+	−	+	−	−
Cerebellar peduncles	−	+	+	+	+	+	−	+
Ventral pons	−	+	−	+	−	−	−	+
Globus pallidus								
Hypointensity on T2*-/susceptibility-weighted imaging	ND	++	+	++	+/−	+	++	+
Eye of the tiger	−	−	−	+	−	−	−	−
Calcifications	ND	−	ND	+ (Unilat.)	−	−	++ (Bilat.)	−
Atrophy								
Ventricle/subarachnoid space	+++/+++	++/+	++/+	−/−	+/−	−/−	+++/++	+++/+
Corpus callosum	++	++	+/−	−	−	+/−	+++	+
Cerebellar vermis/hemisphere	+/−	++/+	−/−	+/−	+/−	+/−	++/+	++/+

Note:—Iso indicates isointense; Hyper, hyperintense; ND, not done; Unilat., unilateral; Bilat., bilateral; −, absence of abnormalities; +/−, very mild; +, mild; ++, moderate; +++, severe.

[a] Disability stage index: 1, no functional handicap but signs at examination; 3, moderate, unable to run, limited walking without aid; 6, unable to walk, requiring wheelchair.

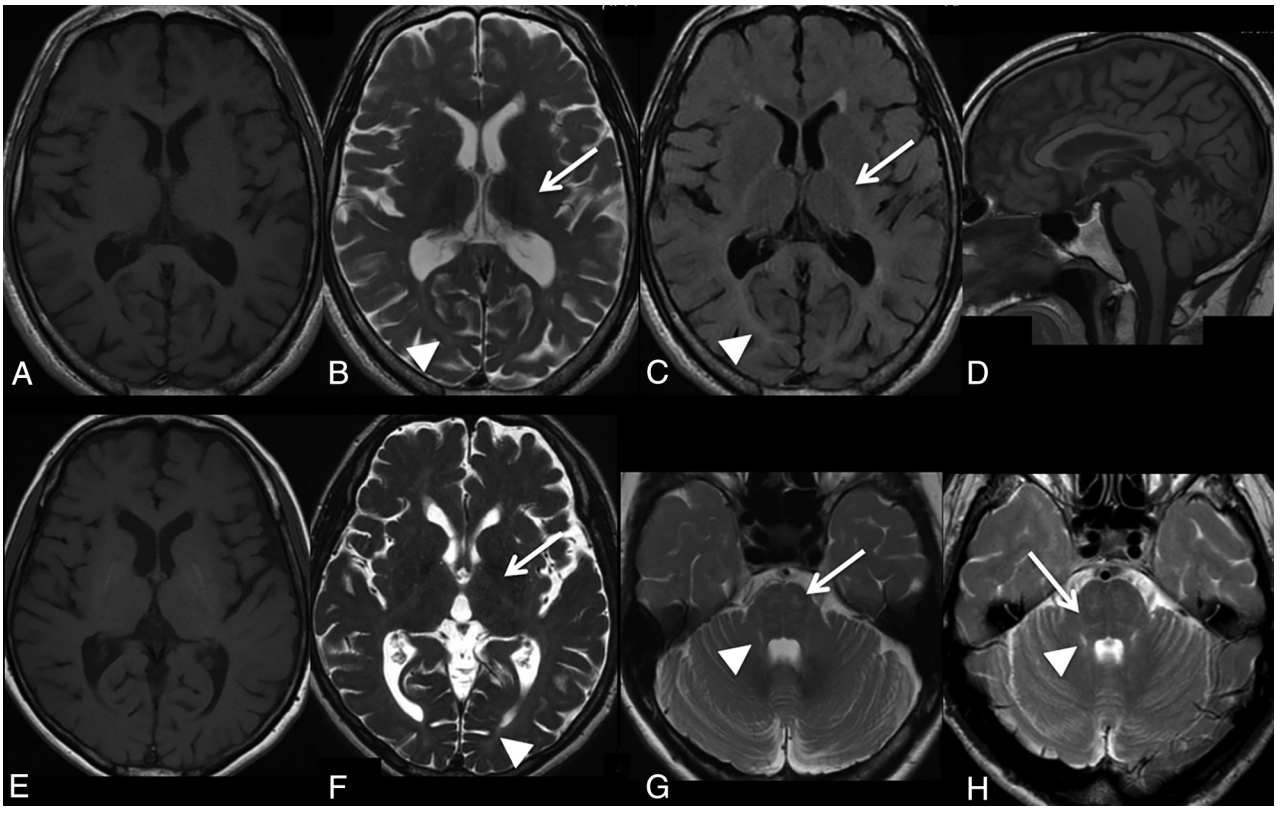

FIG 1. Axial scans of patients 8 (*A–C*) and 7 (*E* and *F*) show isointense-to-mild hyperintense T1-weighted signal (*A* and *E*) associated with mild-to-moderate hyperintense T2- (*B* and *F*) and FLAIR-weighted (*C*) signal of the white matter, especially the internal capsules (*arrows*) and optic radiations (*arrowheads*). Sagittal T1-weighted image of patient 8 (*D*) shows atrophy of the corpus callosum and the vermis. T2-weighted images of patients 2 (*G*) and 4 (*H*) show mild hyperintense signal of the corticospinal tract in the ventral pons (*arrows*) and cerebellar peduncles (*arrowheads*).

participate in myelin maintenance.[6,7] Cx47, encoded by *GJA12*, is deficient in Pelizaeus-Merzbacher-like disease,[8] a hypomyelinating disorder characterized by nystagmus, delayed psychomotor development, and cerebellospastic signs. Some data suggest that the total loss of function of Cx47/Cx43 is implicated in the pathophysiology of part of *GJA12* variants.[6,7] Similarities among neurologic and imaging characteristics between Pelizaeus-Merzbacher-like disease and ODDD could underlie common molecular mechanisms involving the Cx43/Cx47 channels. However, unlike most hypomyelinating disorders such as Pelizaeus-Merzbacher-like disease, the perception of neurologic symptoms by patients with *GJA1* variants usually occurs in adulthood, which

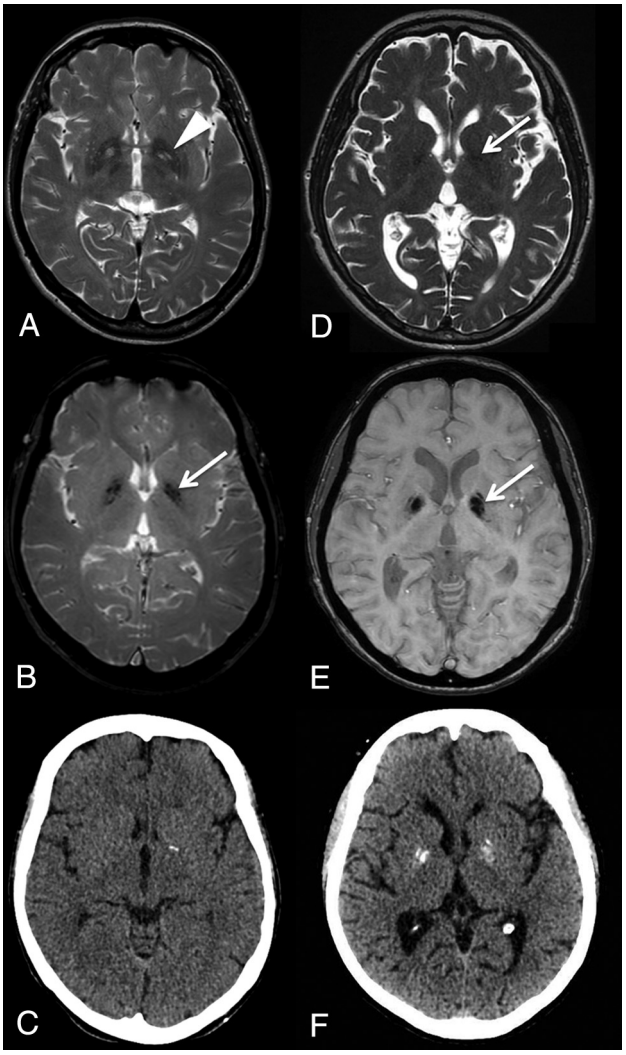

FIG 2. Basal ganglia abnormalities of patients 4 (*A–C*) and 7 (*D–F*). Axial T2- (*A* and *D*) and magnetic susceptibility- (*B* and *E*) weighted images show bilateral hypointensities of the pallidum (*arrows*) and the eye of the-tiger sign (*arrowhead*). Axial CT scans (*C* and *F*) show unilateral (*C*) and bilateral (*F*) calcifications.

may be related to the haploinsufficiency (instead of a loss of function) of Cx43 in ODDD.

In our series, all patients had abnormal MR imaging signal of the basal ganglia, as seen in neurodegeneration with brain iron accumulation disorders. We also observed calcifications of the basal ganglia as previously reported.[9,10] One patient even had an eye of the tiger sign described as a hallmark of pantothenate kinase–associated neurodegeneration.[11] Basal ganglia involvement is observed in other hypomyelinating leukodystrophies, including patients with *POLR3A*/*B* and *TUBB4* variants.[12,13] In our series, the extent of basal ganglia abnormalities was associated with the degree of the patient's disability. Given our limited number of patients, this observation requires further validation in a larger group of patients.

Disease severity was extremely variable with symptom onset from adolescence to adulthood and pyramidal symptoms ranging from very mild to very disabling. In addition, the mother of patient 4 - bearing the *c.428 G>A* variant - had a complete normal phenotype despite the penetrance of ODDD is classically qualified as high.[1] These findings emphasize that intra- and interfamilial expression of the disease is highly variable, without genotype-phenotype correlation. Therefore, it appears difficult to predict the risk of developing neurologic symptoms in patients with *GJA1* variants, a major pitfall for genetic counseling.

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REFERENCES

1. Paznekas WA, Boyadjiev SA, Shapiro RE, et al. **Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia.** *Am J Hum Genet* 2003;72:408–18 CrossRef Medline
2. Gutmann DH, Zackai EH, McDonald-McGinn DM, et al. **Oculodentodigital dysplasia syndrome associated with abnormal cerebral white matter.** *Am J Med Genet* 1991;41:18–20 CrossRef Medline
3. Loddenkemper T, Grote K, Evers S, et al. **Neurological manifestations of the oculodentodigital dysplasia syndrome.** *J Neurol* 2002; 249:584–95 CrossRef Medline
4. Vanderver A, Prust M, Tonduti D, et al; GLIA Consortium. **Case definition and classification of leukodystrophies and leukoencephalopathies.** *Mol Genet Metab* 2015;114:494–500 CrossRef Medline
5. Paznekas WA, Karczeski B, Vermeer S, et al. **GJA1 mutations, variants, and connexin 43 dysfunction as it relates to the oculodentodigital dysplasia phenotype.** *Hum Mutat* 2009;30:724–33 CrossRef Medline
6. Orthmann-Murphy JL, Salsano E, Abrams CK, et al. **Hereditary spastic paraplegia is a novel phenotype for GJA12/GJC2 mutations.** *Brain* 2009;132(Pt 2):426–38 CrossRef Medline
7. May D, Tress O, Seifert G, et al. **Connexin47 protein phosphorylation and stability in oligodendrocytes depend on expression of connexin43 protein in astrocytes.** *J Neurosci* 2013;33:7985–96 CrossRef Medline
8. Uhlenberg B, Schuelke M, Rüschendorf F, et al. **Mutations in the gene encoding gap junction protein alpha 12 (connexin 46.6) cause Pelizaeus-Merzbacher-like disease.** *Am J Hum Genet* 2004;75: 251–60 CrossRef Medline
9. Furuta N, Ikeda M, Hirayanagi K, et al. **A novel GJA1 mutation in oculodentodigital dysplasia with progressive spastic paraplegia and sensory deficits.** *Intern Med* 2012;51:93–98 CrossRef Medline
10. Tumminelli G, Di Donato I, Guida V, et al. **Oculodentodigital dysplasia with massive brain calcification and a new mutation of GJA1 gene.** *J Alzheimers Dis* 2016;49:27–30 CrossRef Medline
11. Hayflick SJ. **Unraveling the Hallervorden-Spatz syndrome: pantothenate kinase-associated neurodegeneration is the name.** *Curr Opin Pediatr* 2003;15:572–77 CrossRef Medline
12. La Piana R, Tonduti D, Gordish Dressman H, et al. **Brain magnetic resonance imaging (MRI) pattern recognition in Pol III-related leukodystrophies.** *J Child Neurol* 2014;29:214–20 CrossRef Medline
13. Curiel J, Rodríguez Bey G, Takanohashi A, et al. **TUBB4A mutations result in specific neuronal and oligodendrocytic defects that closely match clinically distinct phenotypes.** *Hum Mol Genet* 2017;26: 4506–18 CrossRef Medline