A Novel GJA1 Mutation in Oculodentodigital Dysplasia with Progressive Spastic Paraplegia and Sensory Deficits

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Abstract

Oculodentodigital dysplasia (ODDD) is a rare autosomal dominant inherited disorder mainly affecting the development of the face, eyes, dentition, limbs, hair and heart. GJA1 (the gap junction protein α-1) has been determined to be a causative gene of ODDD, mapped to chromosome 6q22-24 identified as the connexin 43 gene (Cx43). We found a novel GJA1 mutation (W25C) as the possible causative gene in this sporadic ODDD patient with neurological features of motor deficits by pyramidal tract signs, and sensory deficits due to peripheral nerve disturbance. It is also notable that the MRI of this patient demonstrated widespread aberrant signal lesions in the brain and brainstem.

Key words: oculodentodigital dysplasia (ODDD), GJA1, mutation, spastic paraparesis, peripheral sensory nerve deficits, MRI


Introduction

Oculodentodigital dysplasia (ODDD, OMIM 164200) is a rare autosomal dominant inherited disorder affecting the development of face, teeth, limbs, hair and heart. Affected individuals have a long, narrow nose with hypoplastic alae and a prominent nasal bridge, short palpebral fissures and bilateral microcornea, often with iris anomalies (1, 2). The characteristic digital abnormality is bilateral complete syndactyly of the fourth and fifth fingers (type III syndactyly); the third finger may also be involved (1). Additionally, microdontia and enamel hypoplasia, which tend to affect both the primary and secondary dentitions, are often observed (1, 3). Neurological symptoms observed in ODDD include dysarthria, neurogenic bladder disturbances, spastic paraparesis, ataxia, anterior tibial muscle weakness and seizures (4). Mild mental retardation occurs infrequently. In some cases, MRI studies of patients with ODDD have shown diffuse bilateral abnormalities in the subcortical cerebral white matter, which can define a slowly progressive leukodystrophy (4). Paznekas et al identified missense mutations of ODDD patients in the Cx43 gene, that is, the gap junction protein α-1 gene (GJA1), in all 17 studied families with ODDD (5). ODDD is usually inherited as an autosomal dominant trait with high penetrance (4), although autosomal recessive inheritance, sporadic cases have also been documented in some ODDD cases (4, 6, 7). These findings have been confirmed and extended by a series of studies that demonstrate clearly that the pleiotropic features observed in ODDD bearing missense mutations of GJA1 (8-13). Here, we report a sporadic ODDD patient with a novel GJA1 mutation, who presented with weakness and spasticity, along with sensory deficits that are rare manifestations. With regard to the sensory symptoms, we confirmed abnormal findings of peripheral sensory nerves by nerve conduction study.

Case Report

The patient, a 34-year-old-woman, had a congenital specific facial appearance including microgenia, hypoplastic alae nasi, internal epicanthus, tooth enamel dysplasia and syndactyly of the hands. Because of increasing unsteadiness of gait over the three years and the numbness of bilateral...
Foot bottoms, she was referred to our hospital for further investigation from another hospital. She is the second child of non-consanguineous healthy parents; she has an elder sister and a younger brother. Her father (68 years old), mother (65 years old) and other relatives have no such symptoms as this proband (Fig. 1). Growth and mental development were normal. She had surgery for bilateral syndactyly of fingers IV-V at 1 year old. Ocular anomalies comprised microcornea, microphthalmos and developmental glaucoma. She had lost her right visual field. At age 22, she had trabeculectomy because her left intraocular pressure was not controlled. Then she was clinically diagnosed with ODDD because of her specific facial appearance and other physical abnormalities.

At age 31, she began to feel unsteadiness of gait and stiffness of the legs. During the past year, she reported episodes of falling. At age 34, because of increasing unsteadiness of gait, she was admitted to our hospital. She had a spastic tetraparesis with hyperreflexia, a pronounced scissor gait, and limb hyperreflexia. Foot clonus and patellar clonus were present. She complained of numbness of bilateral foot bottoms. In her clinical history, she had not suffered from diabetes mellitus, spinal cord or vertebral diseases, or collagen diseases presenting peripheral neuropathies. Cranial nerves and autonomic nervous systems were normal. Deep senses of toe position and vibration were intact. General examination revealed bilateral blepharoptosis, short, scarce eyelashes, converged strabismus, epicantus and blepharoptosis of both eyes, and micrognathia (small chin), with curly, soft and very fine hairs (Fig. 2A). Her teeth were abnormal with different degrees of enamel hypoplasia and taurodontism (Fig. 2B). She had bilateral camptodactyly of fingers IV and V, and clinodactyly of finger V, with scars of hand surgery (Fig. 2C) as also shown in X-P (Fig. 2D).

The laboratory data of serum and cerebrospinal fluid was normal. Nerve conduction studies were undertaken. Motor conduction velocity and the amplitudes of median, ulnar, peroneal and tibial nerves were normal. Sensory conduction velocity of bilateral median, ulnar, and sural nerves was delayed as well as the amplitude was low and distal latency was delayed. Sensory conduction velocity of the sural nerve was not detected at all (Table 1).

Brain MRI showed low signal changes in the bilateral globus pallidus internal segment (GPI) on T1- and T2-weighted images (T1WI, T2WI) (Fig. 3A, B, C). Abnormal high signal on T2WI was observed in the bilateral posterior limbs of internal capsula, corona radiata and centrum semiovale (Fig. 3A, D, E). Brain CT revealed bilateral dense calcification of the GPI (Fig. 3F). In midbrain on T2WI MRI, bilateral cerebral peduncle showed high signals (Fig. 3G: ). An abnormal high signal of transverse fibers (→) and longitudinal fibers (→) was observed in the mid-pons (Fig. 3H), as well as a high signal of transverse fibers (→) and longitudinal fibers (→) was observed in the lower pons (Fig. 3I). Aberrant high signal changes in the internal capsula, cerebral peduncle in the midbrain and in the ventral side of the pons corresponded to the corticospinal tract, that is, the pyramidal tract.

After obtaining informed consent for this genetic test approval by the Ethics Committees in Gunma University, we determined mutation analysis. We purified genomic DNA from lymphocytes in the peripheral blood of the proband. Purified genomic DNA was sequenced according to a previous method (14, 15). One microgram of genomic DNA isolated from leukocytes was mixed with 0.5 μg of each primer, followed by amplification through 30 cycles under the following conditions: 94°C for 30 s for denaturation, 60°C for 30 s for annealing, and 72°C for 30 s for elongation.

The GJA1 coding exon was amplified in two overlapping fragments using the primers 5′-AAT ACG TGA AAC CGT TGG TAG-3′ and 5′-CTC TTT CCC TTA ACC CGA TC-3′, which amplified a product of 856 bp, and 5′-TCT TTG AGG TGG CCT TCT TG-3′ and 5′-TAA GGC TGT TGA GTA CCA CC-3′, which amplified a product of 773 bp (16). The PCR product was excised from 2.5% Nusieve (FMC Bioproducts, Rockland, ME) agarose gel, purified, and treated with T vector cloning system pGEM-T (PROMEGA). Then, the plasmid clones were subjected to sequencing using an ABI 3130XL genetic analyzer DNA sequencer (Applied Biosystems, Foster City, CA, USA) as specified by the manufacturer. The sequence result revealed that heterozygous with “TGG” for normal allele and “TGT” for mutant allele, namely, W (tryptophan) to C (cysteine) at the third base of codon 25 (W25C) in the GJA1 gene (Fig. 4). No similar mutation was observed in 100 normal alleles of 50 subjects providing informed consent. Unfortunately, we could not examine the same mutation in GJA1 gene for her parents and siblings because they refused the genetic test. W25 is conserved between ten species from human to mycobacteria (human, bovine, dog, rat, mouse, chicken, zebrafish, drosophila, saccharomyces and mycobacteria). In the present patient’s recent physical and neurological examinations, no particular change was observed. Her gait is still instable, but she is somehow able to walk by herself.
Figure 2. Appearance of this case. (A) This patient showed a thin and tapered nose, hypoplastic alae nasi, epicanthal folds and ocular asymmetry. Her hair was very thin and curly. (B) Her teeth were abnormal with enamel hypoplasia of a brown color and an irregular size for each tooth. (C) She had bilateral camptodactyly of fingers IV and V, and clinodactyly V. (D) X-ray showed clinodactyly of bilateral fingers V after operation.

Table 1. Nerve Conduction Examination

<table>
<thead>
<tr>
<th>Name (Nerve)</th>
<th>R/L</th>
<th>MCV/SCV</th>
<th>Prox. (ms)</th>
<th>Distal lat. (ms)</th>
<th>Distance (mm)</th>
<th>Velocity (m/s)</th>
<th>Amp. (Distal)</th>
</tr>
</thead>
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<tr>
<td>Median</td>
<td>R</td>
<td>MCV</td>
<td>5.92</td>
<td>2.82</td>
<td>180</td>
<td>58.1</td>
<td>18.65</td>
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<tr>
<td>Median</td>
<td>L</td>
<td>MCV</td>
<td>5.66</td>
<td>2.54</td>
<td>170</td>
<td>54.5</td>
<td>13.66</td>
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<tr>
<td>Ulnar</td>
<td>R</td>
<td>MCV</td>
<td>6.36</td>
<td>2.28</td>
<td>190</td>
<td>46.6</td>
<td>8.73</td>
</tr>
<tr>
<td>Ulnar</td>
<td>L</td>
<td>MCV</td>
<td>5.72</td>
<td>1.98</td>
<td>170</td>
<td>45.5</td>
<td>4.21</td>
</tr>
<tr>
<td>Peroneal</td>
<td>R</td>
<td>MCV</td>
<td>8.94</td>
<td>4.48</td>
<td>245</td>
<td>54.9</td>
<td>4.22</td>
</tr>
<tr>
<td>Peroneal</td>
<td>L</td>
<td>MCV</td>
<td>8.02</td>
<td>3.12</td>
<td>250</td>
<td>51</td>
<td>2.19</td>
</tr>
<tr>
<td>Tibial</td>
<td>R</td>
<td>MCV</td>
<td>10.94</td>
<td>3.74</td>
<td>340</td>
<td>47.2</td>
<td>10.53</td>
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<tr>
<td>Tibial</td>
<td>L</td>
<td>MCV</td>
<td>9.54</td>
<td>3.06</td>
<td>330</td>
<td>50.9</td>
<td>19.18</td>
</tr>
<tr>
<td>Median</td>
<td>R</td>
<td>SCV</td>
<td>9.1</td>
<td>n.d.</td>
<td>250</td>
<td>31.8</td>
<td>0.093</td>
</tr>
<tr>
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<td>SCV</td>
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<td>n.d.</td>
<td>220</td>
<td>37.7</td>
<td>0.139</td>
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<tr>
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<td>200</td>
<td>36.2</td>
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<tr>
<td>Ulnar</td>
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<td>SCV</td>
<td>7.78</td>
<td>3.74</td>
<td>190</td>
<td>47</td>
<td>0.19</td>
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</table>

Nerve conduction examination revealed normal results in motor conduction velocity and amplitude of median, ulnar and peroneal nerves. Sensory conduction velocity of bilateral median, ulnar, the tibial nerves was delayed, while the amplitude was low and distal latency was delayed. Sensory conduction velocity of the sural nerve was not detected.
Discussion

Since Paznekas et al has found causative missense mutations in GJA1 (5), more than 50 different mutations have been described (13). GJA1 or Cx43, like other connexin proteins, consists of an intracellular N-terminus, four transmembrane domains, two extracellular loops, one cytoplasmic loop and an intracellular C-terminus (5). Six connexins can form a connexon, a specialized intracellular structure surrounding a pore (17). Two connexons in apposing cell membranes can align to form an intercellular gap junction. These channels provide a direct low-resistance intercellular pathway for the passage of ions and small molecules that confer distinct physiological properties. Gap junctions have been found in the majority of mammalian tissues. Most tissues express more than one type of connexin, and multiple types of connexins can assemble to form gap junctions between cells, with the diversity of combinations influencing the nature of the cell-to-cell communication (18-20). In more than 240 reported cases of ODDD, most of these substitutions in GJA1 have been located in the second intracellular and first transmembrane domains (13). Indeed, our novel GJA1 mutation W25C is located in the first transmembrane domains. Among affected individuals, sensory deficits are very rare. Previous reports describe one patient with pain in the lower limbs (21), three patients with paresthesia (22, 23) and one patient with decreased vibration sensation in L90V muta-
widely spread neurodegeneration as an aberrant high signal lesions in cerebral white matter (25, 27) as well as peripheral neuropathies. The disruption of gap junction communication between oligodendrocytes and astrocytes may involve the cellular mechanism by which GJB1 (Cx32) mutations cause a “CNS phenotype” (25).

In this inter-relationship between the same family proteins (connexins), mutant connexin Cx43 might have a trans-dominant effect to Cx32 due to functional changes of intercellular channels. Laird reported that Cx26 and Cx43 might link genotypes to phenotypic outcomes by aberration of gap junctions (26). To date, in some reported cases of ODDD, abnormal white matter high signal lesions on T2WI in nine cases were particularly marked in the periventricular parieto-occipital region and in the temporal lobe extending to the corticospinal tracts (4). In previous reports, other findings were also observed including a low signal in the globus pallidus, substantia nigra, red nucleus (25, 27) and thalamus (28). Including these above-mentioned findings, the current ODDD patients presented with aberrant high signal findings in widespread areas from cerebral subcortical regions to brainstem (midbrain and pons). Roscoe et al (29) showed in the first functional study of two missense mutations, one in the first transmembrane (TM1) domain (p.G21R) and the other in the cytoplasmic loop (p.G138R), that the mutant proteins were able to reach cell-cell interfaces, but neither formed functional gap junction channels (30). In this study, the mutant proteins also interfered with the function of the wild-type protein. It is likely that the function of mutant Cx43-mediated gap junctions is reduced due to the dominant-negative effect on the wild-type protein.

Figure 4. Molecular analysis. Sequences of the PCR product spanning codon 25 of genomic GJA1 DNA. The patient was heterozygous with “TGT” for normal allele and “TGTT” for mutant allele, namely, to W (tryptophan) to C (cysteine) at the first base of codon 25 in the GJA1.

Physiologically, Cx43 is expressed in the peripheral nerves, Schwann cells as well as other Cx family members of Cx26, 32, 37, 40, 45 and 46 (24). Mutant Cx43 may play aberrant roles in peripheral nerves and Schwann cells among gap junctions, resulting in demyelination. MRI of some Cx32 mutation patients (CMT-X: X-linked Charcot-Marie-Tooth disease) was reported to show diffuse high signal lesions in cerebral white matter (25, 27) as well as peripheral neuropathies. The disruption of gap junction communication between oligodendrocytes and astrocytes may involve the cellular mechanism by which GJB1 (Cx32) mutations cause a “CNS phenotype” (25).

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calcium.

In addition to physical anomalies, widespread aberrant high signal lesions of cerebral white matter, basal ganglia and brainstem may imply neurodegeneration of the corticospinal tract, which might be caused by some aberrant gap junctions between cells. Mutant Cx43 may have some correlation between the expression of a CNS phenotype and peripheral nerves. To date, no pathology and no postmortem data have been reported, which could help in the search for the gene product responsible for ODDD.

The authors state that they have no Conflict of Interest (COI).

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