Autosomal Recessive Oculodentodigital Dysplasia: A Case Report and Review of the Literature

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Established Facts

- Oculodentodigital dysplasia (ODDD) is generally inherited as an autosomal dominant disorder, but in few cases recessive forms of the disease have been identified.
- Gap junction channels such as Cx43 play an essential role in the cardiomyocyte interconnection that provides synchronized cardiac contraction. Impairment in gap junction trafficking may result in cardiac complications such as arrhythmias and sudden cardiac death.

Novel Insights

- p.Arg148Ter, detected in our patient, is the third mutation described so far in the literature leading to the autosomal recessive form of the disease.
- A possible arrhythmia may have caused sudden death in our patient. Therefore, routine cardiac examination should be suggested for patients with ODDD.

Keywords

GJA1 · Oculodentodigital dysplasia · ODDD · Microphthalmia · Syndactyly

Abstract

Oculodentodigital dysplasia (ODDD) is a rare condition characterized by a typical facial appearance and variable findings of the eyes, teeth, and fingers. ODDD is caused by mutations in the *GJA1* gene in chromosome 6q22 and inherited in an autosomal dominant manner in the majority of the patients. However, in recent clinical reports, autosomal

recessive ODDD cases due to by *GJA1* mutations were also described. Here, we report on a 14-year-old boy with microphthalmia, microcornea, narrow nasal bridge, hypoplastic alae nasi, prominent columnella, hypodontia, dental caries, and partial syndactyly of the 2nd and 3rd toes. These clinical findings were concordant with the diagnosis of ODDD, and a novel homozygous mutation (c.442C>T, p.Arg148Ter) was determined in the *GJA1* gene leading to a premature stop codon. His phenotypically normal parents were found to be carriers of the same mutation. This is the third family in the literature in which ODDD segregates in an autosomal recessive manner.

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Oculodentodigital dysplasia (ODDD) is an inherited disorder with high penetrance and variable expressivity with involvement of the eyes, teeth, and limbs [Paznekas et al., 2003]. In addition to the autosomal dominant form of the disease, a recessive form was also defined recently (MIM 257850). In this form, besides ocular manifestations like microphthalmia, microcornea, iris abnormalities, and persistent pupillary membrane, hypoplastic alae nasi, long narrow nose, low-set ears, hypoplastic teeth, malocclusion, prominent upper incisors, delayed bone age, digital abnormalities, and sparse fine hair can also be seen. The digital malformations include syndactyly involving the 3rd, 4th, and 5th fingers and 2nd to 4th toes, camptodactyly, and clinodactyly due to absent phalanx of the toes. Abnormal gray and white matter differentiation in frontal, occipital, and cerebellar regions on CT scan and neurologic symptoms such as hypotonia at birth and psychomotor retardation were also described [Traboulsi et al., 1986; Pizzuti et al., 2004; Richardson et al., 2006]. Phenotypic features observed in both types of ODDD arise from mutations of the gap junction alpha 1 gene (*GJA1*; MIM 121014) located in chromosome 6q22. The gene has 2 exons separated by an 11-kb intron, and the complete coding region is included in the last exon [Paznekas et al., 2009]. *GJA1* encodes the transmembrane protein connexin 43 (Cx43) [Willecke et al., 2002]. The human connexin family comprises 21 members of connexins [Laird, 2006]. Like all other connexin proteins, Cx43 consists of an intracellular N-terminal region, 4 transmembrane regions, 2 extracellular loops, 1 cytoplasmic loop, and an intracellular C-terminal region [Skerrett and Williams, 2017]. Six connexins generate a connexon. A connexon of one cell lines up with a connexon of an opposing cell, building a gap junction that mediates the intercellular transport of ions and molecules up to 1 kDa without exposure to the extracellular environment [Alexander and Goldberg, 2003; Esseltine and Laird, 2016].

Gap junction channels, such as Cx43, have principal roles in excitable tissues by easing rapid transport of action potentials between neighboring cells. Cardiac rhythm synchronization is organized by these ion channels at the cardiomyocyte cell-cell boundary. Impairment in gap junction trafficking leads to hazardous complications in the heart such as arrhythmias and sudden cardiac death [Epifantseva and Shaw, 2018].

Mutations in the *GJA1* gene mostly result in an autosomal dominant pattern of ODDD [Paznekas et al., 2003]. In functional studies, most of these mutations were suggested to disrupt the gap junction assembly by a dominant negative effect leading to reduced channel function

[Flenniken et al., 2005]. On the other hand, recent reports have shown *GJA1* mutations in 2 patients with ODDD (R76H and R33X) inherited in an autosomal recessive manner. In the current study, we present a 14-year-old boy with microphthalmia, microcornea, narrow nasal bridge, hypoplastic alae nasi, prominent columnella, hypodontia, dental caries, and partial syndactyly of the 2nd and 3rd toes caused by a novel nonsense mutation in the *GJA1* gene. Unfortunately, the patient suddenly died at the age of 15.

Case Report

The proband was a 14-year-old male, the first child of consanguineous parents who had a second 6-year-old healthy son. He was born at term by cesarean section with a birth weight of 3,700 g (50–75th percentile) and a length of 52 cm (50–75th percentile). The perinatal history was uneventful, but he was noted to be macrocephalic and microphthalmic by 1 month of age. His psychomotor milestones were normal as he walked unaided at the age of 9 months. He was referred to our department because of his dysmorphic findings such as microphthalmia, microcornea, hypodontia, partial syndactyly of the 2nd and 3rd toes, and gait disturbance (Fig. 1a, b). His height was 171 cm (75–90th percentile), weight was 46 kg (25–50th percentile), and head circumference was 56 cm (75–90th percentile). His outer and inner canthal distance was 9 cm (50–75th percentile) and 4 cm (>2 SD), respectively, and midpupillary distance was 6 cm (75–97th percentile). His dysmorphic features were noted as flat face, wide forehead, bilateral epicanthus, microphthalmia, microcornea, hypertelorism, septal deviation, hypoplastic alae nasi, narrow nasal bridge, prominent columnella, high-arched palate, microdontia, hypodontia, misalignment of teeth, dental caries, low-set ears, pectus carinatum, flexion contracture of elbows, cubitus valgus, and partial syndactyly of the 2nd and 3rd toes. He had severe visual loss. MRI scan of the brain showed white matter alterations particularly in frontal, occipital and parietal regions as well as increased calvarium bone thickness. These findings were considered to result from myelination defects. Hearing tests were normal. The patient had no cardiac or renal complaints. EMG analysis which was performed due to gait disturbance was also normal. Despite the absence of cardiac complaints, the patient suddenly died at the age of 15. His parents' and brother's general physical examinations were normal, and the family history was negative.

Materials and Methods

Genomic DNA was isolated from peripheral blood samples of the patient, his unaffected parents, and his brother using MagnaPure LC DNA Isolation Kit-Large Volume and MagnaPure LC instrument (Roche Applied Science, Mannheim, Germany). The *GJA1* coding exon (exon 2) was amplified by PCR, and sequence analysis was performed using BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI PRISM 3130 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

Fig. 1. a Frontal appearance of the patient. Note microphthalmia and dysmorphic facial features. **b** Bilateral syndactyly of the 2nd and 3rd toes of the patient.

Fig. 2. Pedigree of the family and partial electropherograms of the individuals. The father (I.1) and mother (I.2) are heterozygous for the p.Arg148Ter mutation; the proband (II.1) is homozygous for the mutated allele (arrows). The proband's brother (II.3) is homozygous for the normal allele.

Results

We found a novel homozygous c.442C>T, p.Arg148Ter mutation in the *GJA1* gene of the patient. While his sibling had wild-type alleles, the mother and father were found to be heterozygous for the same mutation (Fig. 2). This variant was neither listed in the 1000 Genomes (<http://browser.1000genomes.org/index.html>) nor in the ExAC database (<http://exac.broadinstitute.org/>). The impact of the mutation on Cx43 structure and function predicted by in silico analysis was described as damaging.

Discussion

Here, we present a case with recessively inherited ODDD and a novel homozygous nonsense mutation in the *GJA1* gene that encodes the transmembrane protein Cx43. The vast majority of ODDD cases described in the literature show an autosomal dominant inheritance pattern and high phenotypic variability [Shapiro et al., 1997; Loddenkemper et al., 2002]. Up to date, 11 patients from 6 families with autosomal recessive ODDD have been reported [Gillespie, 1964; Beighton et al., 1979; Traboulsi et al., 1986; Frasson et al., 2004; Pizzuti et al., 2004; Richard-

Table 1. Cases of autosomal recessive inheritance of ODDD and clinical findings of the patients

	Gillespie, 1964	Beighton et al., 1979	Traboulsi et al., 1986	Frasson et al., 2004	Pizzuti et al., 2004	Richardson et al., 2006	Present case
Number of patients	2 (siblings)	2 (1st cousins)	1	3 (siblings)	1	2 (siblings)	1
Gender	M/F	M/F	F	M/M/M	M	F/F	M
Parental consanguinity	no	no (isolated ethnic group)	yes	yes	no	yes	yes
Short stature	NC	NC	NC	NC	+	+/-	-
Microphthalmia	+/+	+/+	+	+/+/+	-	+/+	+
Ocular examinations	NC	cataract	PHPV and dysplastic iris	PHPV and glaucoma	not done	PHPV	severe visual loss
Fine hair	+/+	+/+	+	NC	+	+/+	+
Hypoplastic alae nasi	+/+	+/+	+	NC	+	+/+	+
Dental anomalies	hypoplastic enamel +/-	hypoplastic enamel +/+	hypoplastic enamel +	NC/enamel defects/NC	hypoplastic enamel and malocclusion +	hypodontia / no teeth at 1 year	hypodontia and microdontia
Digital anomalies	syndactyly of 4th and 5th fingers +/-	5th finger clinodactyly +/-	syndactyly of 4th and 5th fingers, tissue syndactyly of toes 2, 3, 4	bilateral absence of the middle phalanx, bilateral cutaneous syndactyly of the 2nd and 3rd toes / bilateral absence of the middle phalanx of the 2nd-5th toes / bilateral cutaneous syndactyly of the 5 toes, with absence of the middle phalanx of the 2nd-5th toes	bilateral 5th finger clinodactyly	skin syndactyly of the left ring and little fingers and the right middle, ring, and little fingers / bilateral syndactyly of her ring and little fingers	partial syndactyly of 2nd and 3rd toes
Developmental state	NC	normal IQ / delayed walking	normal	mild psychomotor retardation (1/3)	mild psychomotor retardation	psychomotor retardation / normal	normal
Brain imaging	not done	not done	no basal ganglion calcification	NC/IC /IC	not done	poor myelination / not done	myelination defects and white matter differentiation
Other	cleft lip and palate	mandibular overgrowth	-	cardiomyopathy	-	deceleration of head growth	gait disturbance
<i>GJA1</i> mutation	not done	not done	not done	not done	homozygous missense R76H mutation	homozygous nonsense R33X mutation	homozygous nonsense p.Arg148Ter mutation
Death	-/-	-/6 years	-	2 years/-/-	-	-	15 years

F, female; IC, intracranial calcifications; M, male; N, normal; NC, no comment; PHPV, persistent hyperplastic primary vitreous.

son et al., 2006], but only 2 of these cases were confirmed by molecular analysis. Pizzuti et al. [2004] detected the R76H homozygous missense variant in one of these patients, while Richardson et al. [2006] defined the R33X homozygous nonsense variant in the *GJA1* gene in the second case. The p.Arg148Ter mutation identified in our patient is the third mutation reported so far in autosomal recessive ODDD cases. Clinical findings of the patients with autosomal recessive ODDD described in the literature and of the present case are summarized in Table 1.

As in autosomal dominant ODDD patients, phenotypic variability was also observed in the autosomal recessive form of the disease. Together with the classical findings including facial, dental, and limb defects, cardiac and

neurological findings were also seen in some patients. For instance, there was cardiomyopathy in the cases described by Frasson et al. [2004]. Similar to our patient, a myelination defect was detected in the case of Richardson et al. [2006]. Psychomotor retardation was observed in only 3 of 11 cases with autosomal recessive ODDD described in the literature. Our patient's psychomotor development was normal. Similar to the digital anomalies in our patient, partial syndactyly of the 2nd and 3rd toes was present in the patients reported by Frasson et al. [2004] and Traboulsi et al. [1986].

In the literature, loss of the Cx43 protein was shown to be associated with sudden cardiac death. Studies demonstrated that mice with both cardiac-specific and global

Fig. 3. Schematic presentation of the Cx43 protein with nonsense mutations (indicated by red lines) associated with the autosomal recessive form of ODDD. The Cx43 protein consists of 4 transmembrane domains, 2 extracellular loops, 1 intracellular (cytoplasmic) loop, and intracellular amino and carboxy termini. Cysteine residues are shown in purple and grey, respectively.

knockout of Cx43 exhibited structural cardiac anomalies, resulting in sudden death [Eckardt et al., 2006; Dobrowolski and Willecke, 2009]. Further, Thibodeau et al. [2010] found that isolated atrial fibrillation was associated with somatic Cx43 mutation in a patient. Unfortunately, we do not have more detailed information about a potential cardiac involvement in this condition as an autopsy was not done. However, in view of the above described studies, possible arrhythmia may have caused the sudden death in our patient and, therefore, cardiac screening should be considered for patients with ODDD diagnosis.

Most of the autosomal dominant ODDD cases described up to now have missense variants, and the disease occurs through 2 mechanisms: gain of function and dominant negative effect [Paznekas et al., 2003]. On the other hand, mutations found in patients with the recessive form of the disease resemble loss of function of the protein product, as expected. In a functional study on gap junctional intercellular communication-deficient HeLa cells, Huang et al. [2013] showed that while the R76H mutant protein trafficked to the plasma membrane to build gap junction-like plaques, the R33X mutant protein diffusely localized throughout the cell and failed to form functional channels. Although we did not perform functional studies, the homozygous nonsense mutation identified in our patient is also predicted to lead to a truncated non-functional protein. In the R33X mutation, the protein is truncated at the first transmembrane domain, whereas the first 2 transmembrane domains can be synthesized in the p.Arg148Ter mutation (Fig. 3). But, in our opinion, the p.Arg148Ter mutant protein can still fail to traffic to

the cell surface and form functional channels due to the loss of the last 2 transmembrane domains. In addition, both extracellular loops include 3 cysteine residues that are highly conserved in the connexin family. The first extracellular loop (EL1) has a CX₆CX₃C motif, and the second extracellular loop (EL2) has CX₄CX₅C. Extracellular cysteines are substantial for the formation of the connexon construction by the disulfide link between EL1 and EL2 [Warner et al., 1995]. In our opinion, connexon formation will probably be disrupted since the second extracellular loop will be lost in the presence of the p.Arg148Ter mutation. On the other hand, the mutation may also cause nonsense-mediated decay leading to complete loss of the protein. All these effects of the mutation on the protein product result in the loss of function, consistent with a recessive inheritance pattern. That the parents, heterozygous for the p.Arg148Ter mutation, have no phenotype of ODDD, suggests that the remaining Cx43 is sufficient for organ development and regulation, once again supporting autosomal recessive inheritance.

In summary, we identified a novel nonsense mutation in exon 2 of the *GJA1* gene in a patient with ODDD syndrome. This nonsense mutation causes a premature stop codon and probably leads to nonfunctional protein which is responsible for the clinical features. This mutation is the third mutation described so far in the literature leading to the autosomal recessive form of the disease.

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Statement of Ethics

Informed consent was obtained from the patient and his family. The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have no conflicts of interest to declare.

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