Drugs of the Future 2008, 33(3): 235-239

CCC: 0377-8282/2008
DOI: 10.1358/dof.2008.033.03.1185822

Oculodentodigital dysplasia

E. Ferrer, J. Bozzo
Prous Science, P.O. Box 540, 08080 Barcelona, Spain

CONTENTS

Abstract

This month's Filling the Gaps in Drug Therapy focuses on oculodentodigital dysplasia (ODDD), a rare inherited disorder caused by mutations in the gap junction protein connexin 43 (Cx43). Patients with this disease suffer from malformations in the face, teeth, eyes and other body parts. There is currently no cure for this disorder. Advances in genetic studies have allowed the characterization of Cx43 mutations associated with ODDD. Recently, a suitable animal model was developed, encouraging further investigation on the pathogenesis of ODDD and the development of future therapies.

Introduction

Oculodentodigital dysplasia (ODDD) is a rare autosomal dominant disorder characterized by malformations in different body parts such as the face, teeth, eyes and limbs (1). Syndactyly, a digital malformation in which fingers or toes are joined together, is typical of ODDD. Syndactyly type III is common in ODDD patients, and involves fusion of the fourth and fifth finger (and sometimes the third finger and/or toes) by a tight osseous or cartilaginous union. Nevertheless, ODDD without syndactyly has also been described (2, 3). Ocular abnormalities found in ODDD patients include microphthalmos, microcornea, iris malformations, cataracts and glaucoma. Craniofacial abnormalities, such as a small, thin nose with hypoplastic nasal alae and anteverted nostrils, are also characteristic. Dentition abnormalities (hypoplasia of the tooth enamel), skeletal defects and hypotrichosis are also commonly seen (1, 4). Neurological manifestations have been described, with lower limb spasticity being the most commonly reported complaint, associated with high-signal-intensity brain magnetic resonance imaging (MRI) of subcortical white matter (4). Other neurological symptoms include loss of visual acuity and hearing, sphincter disturbances and gait ataxia.

ODDD syndrome is caused by mutations in the gap junction alpha-1 protein gene (*GJA1)* that encodes the gap junction protein connexin 43 (Cx43), predominantly expressed in cardiomyocytes and keratinocytes, but also found in at least 35 different human tissues. Connexins are transmembrane proteins that form intercellular gap junctions, or channels, that allow the transfer of small ions and molecules of up to 1 kDa between adjacent cells, which is essential for physiological and developmental processes (5). Connexins assemble into hexamers to form connexons or hemichannels (Fig. 1) that dock with apposing connexons from the adjacent cell, hence forming a gap junction channel. Gap junction channels cluster into plaques at the cell surface, an event that appears to be important for functional coupling (6).

There are 21 human connexin family members with a broad diversity of functions. Connexins can assemble into homomer or heteromer hexamers with other compatible connexins to form homomeric or heteromeric hemichannels, respectively, which may combine with other connexons to form homotypic or heterotypic gap junction channels, hence significantly increasing the variety of channel types (7).

There is currently no specific treatment for ODDD other than symptomatic management of the clinical picture.

Genetics

To date, more than 35 mutations have been identified in the human *GJA1* gene leading to ODDD. Initial genetic linkage analysis of six families with a history of ODDD allowed the mapping of the ODDD locus to the chromosome region 6q22-q24 (8), with further studies narrowing the chromosome region to 6q22-q23 (9) and finally identifying *GJA1* as the candidate gene for ODDD (1). This latter study analyzed 17 unrelated families with a total of 60 family members. ODDD-affected individuals exhibited *GJA1* missense mutations involving the cytoplasmic, transmembrane and extracellular domains of the Cx43

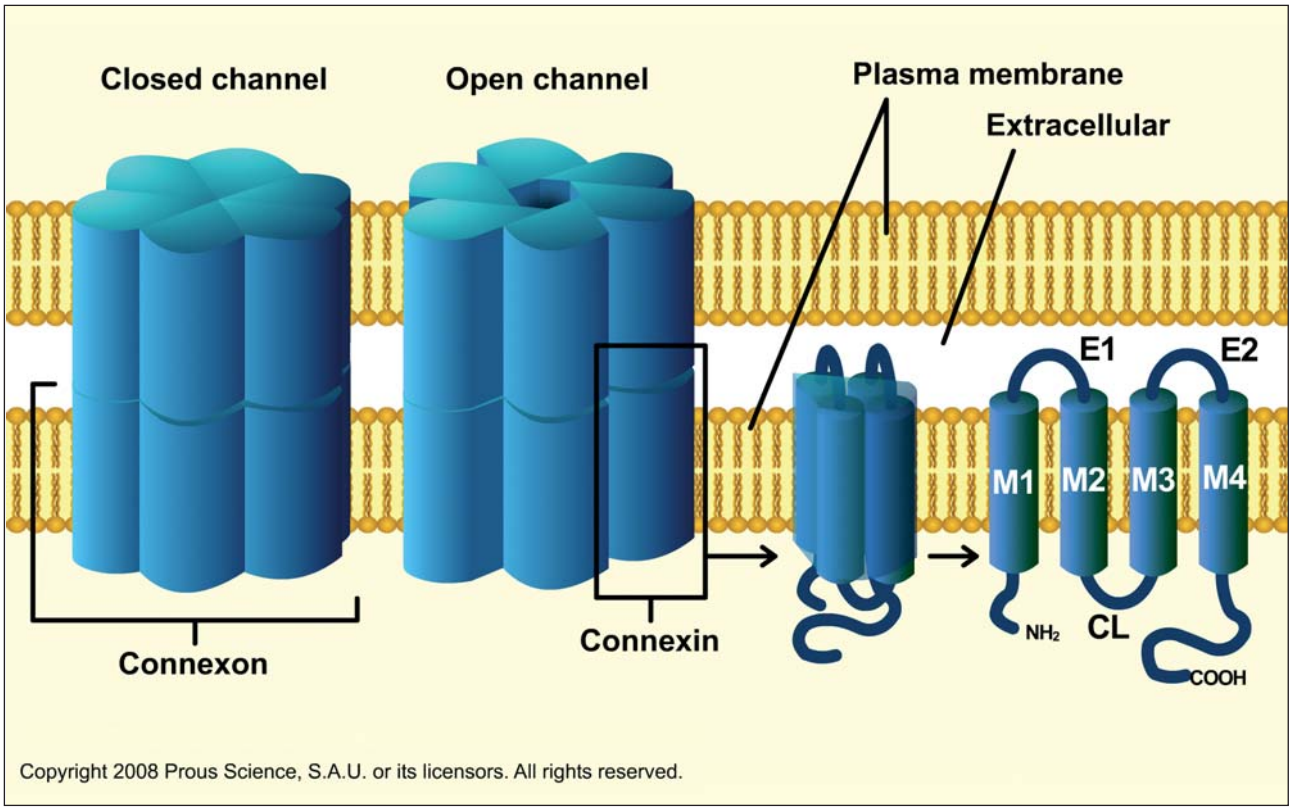

Fig. 1. Connexins form pore-forming hexamers that are known as connexons or hemichannels. Each connexon docks to an apposing connexon from the neighboring cell to form a functional gap junction channel. Connexins are integral membrane proteins with four transmembrane domains (M1-M4), two extracellular loops (E1, E2) and a cytoplasmic loop (CL). The *N*- and *C*-termini are both found in the cytoplasm.

protein. All mutations were found to affect amino acids highly conserved throughout the Cx43 sequence of different species. These studies demonstrated the high penetrance of ODDD, as all mutation carriers presented with typical craniofacial and limb malformations. Other major phenotypic features were syndactyly and neurodegeneration (leukodystrophy). Conductive hearing loss, cataracts, glaucoma, keratoderma and cardiac defects were also part of the phenotype displayed by ODDD families.

Another study associated a previously unreported *GJA1* mutation with the occurrence of open- and closed-angle glaucoma within the same family and suggested that structural modification in the Cx43 protein, which is expressed in the trabecular meshwork, could impair aqueous humor outflow (10). Classically considered a distinctive feature of ODDD, type III syndactyly has also been described as an isolated finding in patients with *GJA1* mutations (11), initially suggesting the existence of two separate conditions. However, the absence of hand and foot syndactyly reported in patients with *GJA1* mutations exhibiting other typical ODDD features (2, 3) has suggested that they likely represent a disease spectrum derived from *GJA1* mutations. In spite of multiple studies analyzing the distribution of mutations along the Cx43 protein, an obvious genotype-phenotype correlation for most mutations has not been identified (1, 2). Interestingly, uncommon skin manifestations linked to ODDD have been associated with certain Cx43 mutations. In particular, *C*-terminus-truncating nucleotide deletions in the *GJA1* gene have been associated with marked palmoplantar keratoderma (12, 13), while mutations affecting the *N*-terminus have resulted in discrete keratoderma (14). Investigating a developmental series of morphologically staged mouse embryos, another research group reported that the pattern of *Gja1* expression during mouse embryonic development correlated with the clinical phenotype of ODDD (15).

Recently, a new *GJA1* mutation affecting the *N*-terminal intracellular domain has been reported (16) in a patient of Mexican origin. This patient presented with typical ODDD features, in addition to previously undetected signs such as umbilical hernia and bilateral optociliary veins, which was associated with impaired drainage of retinal blood.

It is interesting to note that although Cx43 is the major connexin in the human heart and plays a role in normal cardiac conduction (5), ODDD patients do not consistently exhibit cardiac anomalies, although recurrent ventricular tachycardia and atrioventricular block have been reported (1), which would be consistent with altered Cx43 function.

Table I: Summary of functional characterization of ODDD-associated mutants.

Amino acid change	Cx43 domain	Effect	Ref.
G21R	Transmembrane 1	Nonfunctional* Dominant negative to wt Cx43	18-20
A40V	Transmembrane 1	Nonfunctional	19, 20
G138R	Intracellular loop	Nonfunctional Dominant negative to wt Cx43	17, 18
Y17S	Intracellular *N*-terminus	Nonfunctional	19, 20
F52dup	Extracellular E1 loop	Nonfunctional	19, 20
L90V	Transmembrane 2	Functional Dominant negative to wt Cx43	19-21
I130T	Intracellular loop	Functional**	17, 19, 20
K134E	Intracellular loop	Functional**	17, 19
R202H	Extracellular E2 loop	Nonfunctional Dominant negative to wt Cx43	19, 21
Q49K	Extracellular E1 loop	Nonfunctional Dominant negative to wt Cx43	21
V216L	Transmembrane 4	Nonfunctional Dominant negative to wt Cx43	21
I31M	Transmembrane 1	Nonfunctional	24
G143S	Intracellular loop	Nonfunctional	24
H194P	Extracellular E2 loop	Nonfunctional	24

*Refers to the inability to form nonfunctional gap junction channels. **Levels of channel activity were lower than those of wild-type Cx43 channels. G138R: Functional hemichannel activity according to Dobrowolski *et al.*, but nonfunctional gap junction intercellular communication. Wt, wild-type; A, alanine; R, arginine; N, asparagine; D, aspartic acid; C, cysteine; Q, glutamine; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; L, leucine; K, lysine; M, methionine; F, phenylalanine; P, proline; S, serine; T, threonine; W, tryptophan; Y, tyrosine; V, valine.

How do *GJA1* mutations affect Cx43 protein function?

Several research groups have attempted the functional characterization of Cx43 mutations found in patients with ODDD by engineering Cx43 mutant constructs (Table I). Seki *et al*. first questioned whether mutations associated with ODDD had an impact on wild-type Cx43 function (17). They observed that point mutations affecting the cytoplasmic loop (G138R, I130T, K134E) resulted in either nonfunctional channels or channels with reduced open probability compared to wild-type channels, but their ability to form gap junction plaques was unaltered. Further work by Roscoe *et al.* revealed that mutations affecting the first transmembrane domain (G21R) and the intracellular loop of Cx43 (G138R) resulted in protein mutants that are transported to the cell surface and cluster into structures resembling gap junctions, but fail to form functional intercellular channels when transfected into different cell types (18). Moreover, when co-expressed with wild-type Cx43, ODDD mutants acted as dominant negatives compromising wild-type Cx43 function, a scenario that may mimic what occurs in ODDD patients. Similarly, Shibayama *et al.* (19) and Lai *et al*. (20) further characterized eight ODDD-related mutations (Table I) and found that they result in a wide spectrum of alterations in Cx43 gap junctions, ranging from protein mutants unable to reach the membrane to others unable to form functional intercellular channels. Studies by McLachlan *et al*. also described three additional mutations (R202H, Q49K, V216L) that yield nonfunctional channels and have dominant negative effects on wild-type Cx43 (21). Moreover, it also appeared that these ODDD-associated mutations do not interfere with osteoblast differentiation, as was initially hypothesized due to the presence of skeletal manifestations associated with abnormal bone development in ODDD patients. However, further studies need to be conducted using novel mouse ODDD models displaying similar bone deformities to those found in human patients. Cx43 is expressed in major bone cell types and plays a role in different signaling pathways that control osteoblast differentiation, bone formation and remodeling (reviewed in 22) and it thereby seems plausible that mutation-induced impaired signal transduction may result in the ODDD skeletal phenotype.

According to the above-mentioned observation, it has been suggested that the severity of the disease may depend on the impact that mutants may have as dominant negatives of wild-type Cx43 function and also on the ability of other co-expressed connexins to compensate for Cx43 loss of function. In fact, G21R and G138R mutants have shown differential potency in inhibiting wild-type Cx43, with the G21R mutant being 2-fold more potent than the G138R mutant (23), which could at least partially explain the different clinical manifestations exhibited by

ODDD patients bearing these mutations. It is not known whether Cx43 mutants would have dominant negative effects on other types of co-expressed connexins. Another study reported the inability of four ODDD-associated mutants (I31M, G138R, G143S, H194P) to form functional gap junction channels, but instead they could form open hemichannels featuring enhanced hemichannel activity (24).

Mouse models of ODDD

The first *in vivo* model of ODDD was characterized by Flenniken *et al*. a few years ago and consisted of a mouse carrying a point mutation in the *Gja1* gene (25). These mice constitutively express the G60S mutation, not described in ODDD patients so far but associated with a similar phenotype featuring syndactyly and craniofacial, ocular and dental abnormalities, as well as cardiac conduction abnormalities. In addition, mutant mice exhibited bone anomalies (osteopenia) and bone marrow atrophy, which have not been described in ODDD subjects.

Recently, Dobrowolski *et al.* have generated a targeted conditional mouse model expressing the G138R mutation (26), described in ODDD patients and extensively investigated in functional studies (see above). The most common phenotypic manifestations in these mutant mice included syndactyly, enamel hypoplasia and craniofacial defects. Interestingly, conditional expression of the G138R mutation in the heart increased embryonic lethality and mortality during the first 6 months postnatally. Mutant mice exhibited spontaneous ventricular tachycardia and other types of arrhythmias associated with broadening of the QRS complex and a decrease in the R wave. Enhanced ATP release associated with a positive chronotropic effect was also detected in mutant cardiomyocytes. It has been suggested that the increased frequency of cardiac abnormalities in mice compared to in humans bearing the same mutations may be explained by a differential sensitivity of the mouse heart compared to the human heart. In addition, it could be that a cardiac phenotype would manifest only in ODDD patients under conditions of hypoxic stress, known to aggravate defective conduction in G138R mutant mice (26), or that other connexins may compensate for the loss of function in Cx43.

Another research group successfully generated transgenic mice bearing human ODDD-linked mutants (I130T) and showing hind limb syndactyly, heart morphological abnormalities and an increased susceptibility to spontaneous and inducible ventricular arrhythmias (27). In this model, the total amount of Cx43 protein was reduced in the hearts of ODDD mutant mice. Moreover, mutant Cx43 proteins were found to be abnormally phosphorylated, resulting in reduced trafficking to the membrane and nonfunctional gap junction channels.

Treatment

The discovery of animal models exhibiting a clinical phenotype similar to that of ODDD patients (26) will allow us to test potential experimental therapies. For example, in experimental models of cardiac disease, transplantation of embryonic cardiomyocytes (eCMs) or Cx43-expressing skeletal muscle cells (SM-*Cx43*$^{+}$) has successfully resulted in improvement in cardiac conduction. When transplanted into mice suffering from left ventricular infarcts, eCMs and SM-*Cx43*$^{+}$ increased the electrical stability and reduced the incidence of ventricular tachycardia, similar to in noninfarcted controls. Moreover, eCM grafts showed coupling with native, noninfarcted cardiomyocytes *in vivo*, decreased the incidence of conduction block and suppressed border zone focal activity, which is characteristic of postinfarct ventricular arrhythmia (27).

Conclusions

Functional characterization of mutations in Cx43 associated with ODDD has greatly contributed to elucidating some of the pathogenic mechanisms of this disease. The latest advances in generating an adequate animal model that mimics ODDD features may provide further understanding of how *GJA1* mutations result in such varied clinical features and will also allow the investigation of future therapies.

Online link

Subscribers to the online version of *Drugs of the Future* and/or Prous Science Integrity® can access the animation: Structure and Function of Gap Junctions.

References

1. Paznekas, W.A., Boyadjiev, S.A., Shapiro, R.E. et al. *Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia.* Am J Hum Genet 2003, 72(2): 408-18.

2. Vitiello, C., D'Adamo, P., Gentile, F., Vingolo, E.M., Gasparini, P., Banfi, S. *A novel GJA1 mutation causes oculodentodigital dysplasia without syndactyly.* Am J Med Genet A 2005, 133(1): 58-60.

3. van Es, R.J., Wittebol-Post, D., Beemer, F.A. *Oculodentodigital dysplasia with mandibular retrognathism and absence of syndactyly: A case report with a novel mutation in the connexin 43 gene.* Int J Oral Maxillofac Surg 2007, 36(9): 858-60.

4. Loddenkemper, T., Grote, K., Evers, S., Oelerich, M., Stogbauer, F. *Neurological manifestations of the oculodentodigital dysplasia syndrome.* J Neurol 2002, 249(5): 584-95.

5. Saez, J.C., Berthoud, V.M., Branes, M.C., Martinez, A.D., Beyer, E.C. *Plasma membrane channels formed by connexins: Their regulation and functions.* Physiol Rev 2003, 83(4): 1359-400.

6. Bukauskas, F.F., Jordan, K., Bukauskiene, A., Bennett, M.V., Lampe, P.D., Laird, D.W., Verselis, V.K. *Clustering of connexin 43-enhanced green fluorescent protein gap junction channels and functional coupling in living cells.* Proc Natl Acad Sci USA 2000, 97(6): 2556-61.

7. Laird, D.W. *Life cycle of connexins in health and disease.* Biochem J 2006, 394(Pt. 3): 527-43.

8. Gladwin, A., Donnai, D., Metcalfe, K. et al. *Localization of a gene for oculodentodigital syndrome to human chromosome 6q22-q24.* Hum Mol Genet 1997, 6(1): 123-7.

9. Boyadjiev, S.A., Jabs, E.W., LaBuda, M. et al. *Linkage analysis narrows the critical region for oculodentodigital dysplasia to chromosome 6q22-q23.* Genomics 1999, 58(1): 34-40.

10. Vasconcellos, J.P., Melo, M.B., Schimiti, R.B., Bressanim, N.C., Costa, F.F., Costa, V.P. *A novel mutation in the GJA1 gene in a family with oculodentodigital dysplasia.* Arch Ophthalmol 2005, 123(10): 1422-6.

11. Brueton, L.A., Huson, S.M., Farren, B., Winter, R.M. *Oculodentodigital dysplasia and type III syndactyly: Separate genetic entities or disease spectrum?* J Med Genet 1990, 27(3): 169-75.

12. Vreeburg, M., Zwart-Storm, E.A., Schouten, M.I. et al. *Skin changes in oculo-dento-digital dysplasia are correlated with C-terminal truncations of connexin 43.* Am J Med Genet A 2007, 143(4): 360-3.

13. van Steensel, M.A., Spruijt, L., van der Burgt, I., Bladergroen, R.S., Vermeer, M., Steijlen, P.M., van Geel, M. *A 2-bp deletion in the GJA1 gene is associated with oculo-dento-digital dysplasia with palmoplantar keratoderma.* Am J Med Genet A 2005, 132(2): 171-4.

14. Kelly, S.C., Ratajczak, P., Keller, M., Purcell, S.M., Griffin, T., Richard, G. *A novel GJA 1 mutation in oculo-dento-digital dysplasia with curly hair and hyperkeratosis.* Eur J Dermatol 2006, 16(3): 241-5.

15. Richardson, R., Donnai, D., Meire, F., Dixon, M.J. *Expression of Gja1 correlates with the phenotype observed in oculodentodigital syndrome/type III syndactyly.* J Med Genet 2004, 41(1): 60-7.

16. de la Parra, D.R., Zenteno, J.C. *A new GJA1 (connexin 43) mutation causing oculodentodigital dysplasia associated to uncommon features.* Ophthalmic Genet 2007, 28(4): 198-202.

17. Seki, A., Coombs, W., Taffet, S.M., Delmar, M. *Loss of electrical communication, but not plaque formation, after mutations in the cytoplasmic loop of connexin43.* Heart Rhythm 2004, 1(2): 227-33.

18. Roscoe, W., Veitch, G.I., Gong, X.Q. et al. *Oculodentodigital dysplasia-causing connexin43 mutants are non-functional and exhibit dominant effects on wild-type connexin43.* J Biol Chem 2005, 280(12): 11458-66.

19. Shibayama, J., Paznekas, W., Seki, A., Taffet, S., Jabs, E.W., Delmar, M., Musa, H. *Functional characterization of connexin43 mutations found in patients with oculodentodigital dysplasia.* Circ Res 2005, 96(10): e83-e91.

20. Lai, A., Le, D.N., Paznekas, W.A., Gifford, W.D., Jabs, E.W., Charles, A.C. *Oculodentodigital dysplasia connexin43 mutations result in non-functional connexin hemichannels and gap junctions in C6 glioma cells.* J Cell Sci 2006, 119(Pt. 3): 532-41.

21. McLachlan, E., Manias, J.L., Gong, X.Q. et al. *Functional characterization of oculodentodigital dysplasia-associated Cx43 mutants.* Cell Commun Adhes 2005, 12(5-6): 279-92.

22. Jiang, J.X., Siller-Jackson, A.J., Burra, S. *Roles of gap junctions and hemichannels in bone cell functions and in signal transmission of mechanical stress.* Front Biosci 2007, 12: 1450-62.

23. Gong, X.Q., Shao, Q., Langlois, S., Bai, D., Laird, D.W. *Differential potency of dominant negative connexin43 mutants in oculodentodigital dysplasia.* J Biol Chem 2007, 282(26): 19190-202.

24. Dobrowolski, R., Sommershof, A., Willecke, K. *Some oculodentodigital dysplasia-associated cx43 mutations cause increased hemichannel activity in addition to deficient gap junction channels.* J Membr Biol 2007, 219(1-3): 9-17.

25. Flenniken, A.M., Osborne, L.R., Anderson, N. et al. *A Gja1 missense mutation in a mouse model of oculodentodigital dysplasia.* Development 2005, 132(19): 4375-86.

26. Dobrowolski, R., Sasse, P., Schrickel, J.W. et al. *The conditional connexin43G138R mouse mutant represents a new model of hereditary oculodentodigital dysplasia in humans.* Hum Mol Genet 2008, 17(4): 539-54.

27. Roell, W., Lewalter, T., Sasse, P. et al. *Engraftment of connexin 43-expressing cells prevents post-infarct arrhythmia.* Nature 2007, 450(7171): 819-24.