Eye (2009) 23, 549–555

www.nature.com/eye

CLINICAL STUDY

Ocular manifestations in oculodentodigital dysplasia resulting from a heterozygous missense mutation (L113P) in *GJA1* (connexin 43)

FU Musa[1], P Ratajczak[2,3], J Sahu[2,3], S Pentlicky[2,3], A Fryer[4], G Richard[2,3] and CE Willoughby[5]

Abstract

Purpose **To characterize the ophthalmic findings, intrafamilial variability, and molecular genetic basis of oculodentodigital dysplasia (ODDD; MIM no. 164200).**
Methods **Ophthalmic examination included best-corrected visual acuity, slit-lamp biomicroscopy, direct and indirect ophthalmoscopy, Goldmann applanation tonometry and A-scan ultrasonography. Blood samples were taken for DNA extraction and mutation screening of *GJA1* (connexin 43).**
Results **All three affected individuals had characteristic features of ODDD. The ophthalmic features were epicanthus, microcornea, and the presence of glaucoma. The ocular phenotype resulted from a heterozygous T > C transition at nucleotide 338 in *GJA1* (L113P) that was not detected in 120 chromosomes of unaffected individuals. The L113P mutation results in a nonconservative substitution in the cytoplasmic loop of Cx43 (GJA1) and is predicted to disrupt the high-order structure of Cx43.**
Conclusions **This report describes the ocular phenotype in a molecularly characterized ODDD syndrome family. The ocular features in this family highlight the key role Cx43 plays in eye development and in the development of glaucoma. L113P represents a pathogenic mutation in GJA1 (Cx43) and results in ODDD with marked intrafamilial variation in glaucoma type and severity.**
Eye (2009) **23,** 549–555; doi:10.1038/eye.2008.77; published online 18 April 2008

Keywords: oculodentodigital dysplasia; glaucoma; genetics

Introduction

Oculodentodigital dysplasia (ODDD; MIM no. 164200) is a highly penetrant, autosomal-dominant congenital disorder characterized by developmental abnormalities of the face, eyes, limbs, and dentition, which shows marked intra- and interfamilial phenotypic variability. ODDD was first described in 1920 and Meyer-Schwickerath[1] introduced the term 'dysplasis oculodentodigitalis' in 1957. Characteristic craniofacial anomalies include a long, narrow nose with hypoplastic alae nasi, small, anteverted nostrils, and a prominent columnella and nasal bridge. ODDD can be classified as an ectodermal dysplasia syndrome as congenital defects occur in the hair, nails, and teeth. Brittle nails and hair abnormalities such as hypotrichosis and slow growth are present. The majority of cases also have abnormal primary and permanent dentition including microdontia, hypodontia, enamel hypoplasia, and accelerated dental caries. Bilateral complete syndactyly of the fourth and fifth fingers (type III syndactyly) is the characteristic digital malformation. The third finger may occasionally also be involved and camptodactyly, and clinodactyly due to hypoplasia or aplasia of the middle phalanges are common findings. Anterior segment ophthalmic features consist of micro-ophthalmia, microcornea, fine porous spongy iris abnormalities, and cataracts.[2] Glaucoma is the main cause of visual loss in

[1]Birmingham and Midland Eye Centre, City Hospital, Birmingham, UK

[2]Department of Dermatology and Cutaneous Biology and Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA, USA

[3]GeneDx Inc., Gaithersburg, MD, USA

[4]Department of Clinical Genetics, Royal Liverpool Children's Hospital, Liverpool, UK

[5]Ophthalmology and Vision Science, Queen's University Belfast, Northern Ireland, UK

Correspondence: FU Musa, Birmingham and Midland Eye Center, City Hospital, Dudley Road, Birmingham B18 7QH, UK. E-mail: fayyaz@doctors.net.uk

Received: 17 September 2007
Accepted in revised form: 4 February 2008
Published online: 18 April 2008

Financial/Proprietary interest: None.

ODDD syndrome and can occur at different ages due to a variety of mechanisms.[3]

Linkage analysis of six families with ODDD in 1997 mapped the ODDD locus to a 28 cM region of the human chromosome 6q22–q24.[4] The location of the disease gene was further refined to a 1.9 cM interval between the markers D6S266 and D6S1639. Recent studies[2,5] have confirmed that mutations in *GJA1*, which encodes gap junction protein α1 (connexin 43; Cx43), are responsible for clinical condition of ODDD. We present a comprehensive assessment of the ocular phenotype in three affected members of a single family, with a molecular genetic diagnosis of ODDD. In this family a heterozygous missense mutation (L113P) in GJA1 (Cx43) results in ODDD with marked intrafamilial variation in glaucoma type and severity.

Materials and methods

Patient identification and specimen collection

The researchers followed the tenets of the Declaration of Helsinki in the treatment of the subjects reported herein and with the approval of the local research ethics committee, informed consent was obtained from family members. We ascertained biological material from three affected (I-2, II-1, and II-2) persons of a two-generation Caucasian family with ODDD originating from the United Kingdom (Figure 1). Genomic DNA was extracted from venous blood samples following standard procedures.

Clinical assessment

All three patients had a full medical examination by a clinical geneticist and a complete ophthalmic evaluation by an ophthalmologist. Intraocular pressure readings were obtained with Goldmann applanation tonometry. A-scan ultrasound (Storz CompuScan LT V2.00) was used to evaluate the anterior chamber depth, lens thickness, vitreous cavity length, and axial length. Microophthalmos was diagnosed if A-scan ultrasonography measurements showed axial lengths less than 2 SD below age-matched controls[6] or less than 18.5 mm in adults.[7] Microcornea was defined by a horizontal corneal diameter (HCD) of less than 11.00 mm.[8]

DNA amplification and mutation analysis

The coding region of *GJA1* and flanking intronic and 3′UTR sequences were PCR amplified from genomic DNA samples in two overlapping fragments for direct DNA sequence analysis (gene-specific PCR primer details available on request). PCR reactions were performed using 200 ng genomic DNA, 2.5 IU Taq DNA polymerase, 10% Q-solution (Qiagen Inc., Valencia, CA, USA) and standard PCR conditions for 60 μl total

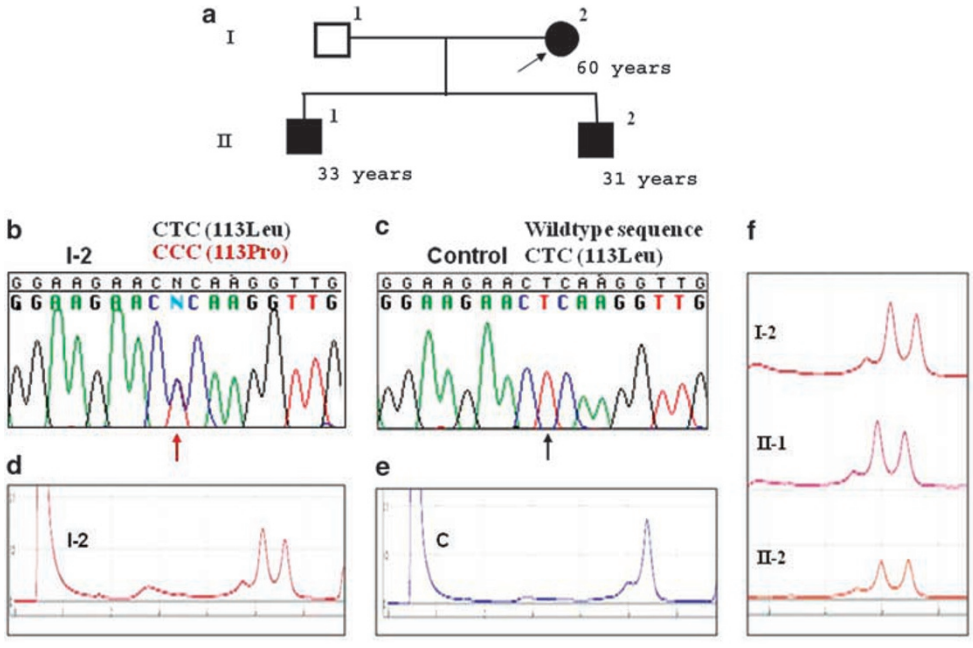

Figure 1 Two generation family with ODDD syndrome (a) caused by a heterozygous T>C transition at nucleotide 338 in *GJA1* in proband I-1 (b) compared with control wild-type sequence (c). L113P (c.338T>C) detected by dHPLC (d) was found in all affected family members (f) but was not detected in 120 chromosomes of unaffected individuals of Northern European origin (e).

volume. Amplicons were gel purified (QIAquick gel extraction kit, Qiagen Inc.) and directly sequenced using the BigDye terminator sequencing system on an ABI Prism 377 sequencer (PE Applied Biosystems, Foster City, CA, USA). The mutation was confirmed by bidirectional DNA sequencing and by denaturing high performance liquid chromatography (dHPLC; technical details available on request). This method was also used to exclude the mutation from 120 control chromosomes from unaffected individuals of Northern European origin.

Results

Clinical assessment

Proband

The proband (I-2) was a 60-year-old female who presented with left acute angle closure glaucoma at the age of 44. Intraocular pressure on presentation was 62 mmHg OD and 12 mmHg OS and on gonioscopy the angle was closed in the right eye (Shaffer grade 0) and narrow in the left eye (Shaffer grade 1). To control her intraocular pressures she required a right surgical peripheral iridotomy and a left trabeculectomy followed by a left phacotrabeculectomy and intraocular lens insertion 8 years later. Best-corrected Snellen visual acuity was 20/20 OU (OD: $+4.25/+0.50\times52^{\circ}$; OS: $+4.00/+1.00\times95^{\circ}$). There was bilateral microcornea with an HCD of 9 mm OU (Figure 2c). Anterior chambers were shallow (OD: 2.05 mm; OS: 1.84 mm) with normal axial lengths (OD: 21.93 mm; OS: 21.29 mm). Palpebral fissures were narrowed at 21 mm right and 22 mm left (Figure 2b). Normal palpebral fissure length in adults is reported to be approximately 25 mm.[9]

On the basis of the ocular, dental, and digital changes, the proband was clinically diagnosed with ODDD. The proband had fourth–fifth digital syndactyly and fifth digit clinodactyly in the upper limb with a normal lower limb. The characteristic facies of ODDD was present and the proband had a long, narrow nose with hypoplastic alae nasi, small, anteverted nostrils and a prominent columnella and nasal bridge (Figure 2a). There was a mild ectodermal dysplasia with hypotrichosis and enamel hypoplasia with associated dental caries. There was no significant cognitive impairment in either the two children, both of whom were clerical workers, or the proband. Following the diagnosis of ODDD in the proband both children were clinically assessed.

Child 1

This 33-year-old male offspring (II-1) had a history of congenital glaucoma. Glaucoma surgery was performed in early childhood but no operative details were available. He subsequently underwent bilateral trabeculectomies at the age of 11 for uncontrolled raised intraocular pressure. Visual acuities were count fingers at 50 cm in the right eye and 20/400 in the left eye. The left eye was buphthalmic with Haab striae (Figure 3d). Microcornea was present in the right eye (HCD = 9 mm) with a normal anterior chamber depth (2.25 mm) and axial length (21.71 mm). This individual had surgically

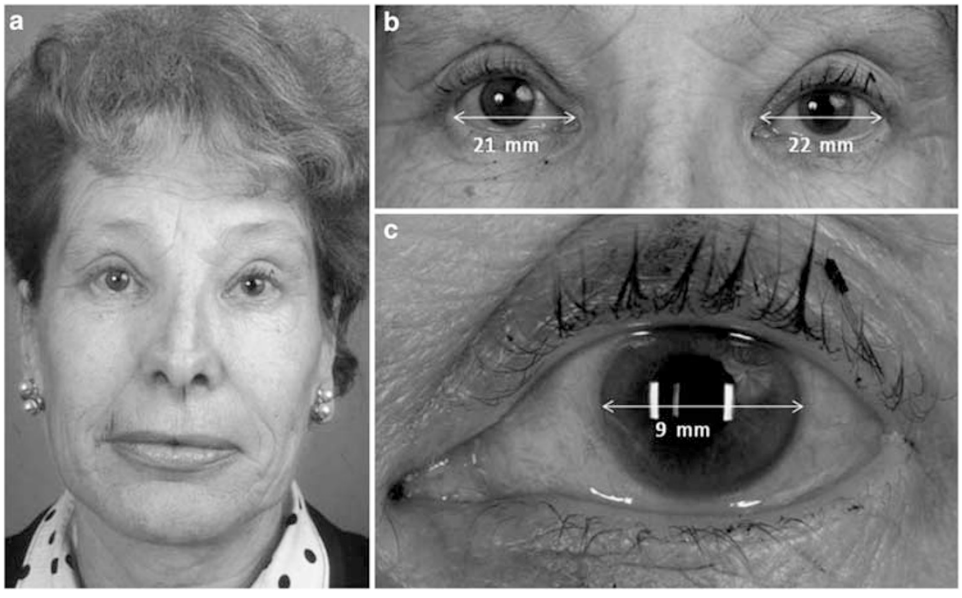

Figure 2 Characteristic facies of ODDD present in the proband with a long, narrow nose with hypoplastic alae nasi; small, anteverted nostrils; prominent columnella and nasal bridge (a); reduced palpebral fissure length (b) and microcornea (c).

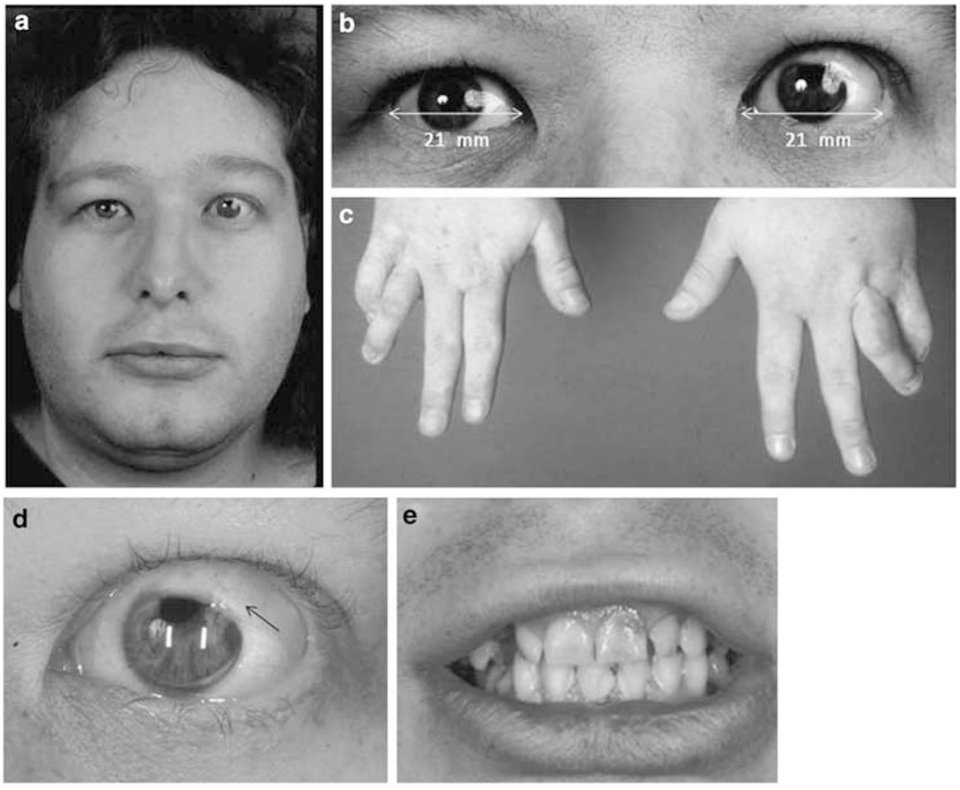

Figure 3 Characteristic facies of ODDD present in individual II-1 (a) with reduced palpebral fissure length (b). Surgically repaired syndactyly of the fourth and fifth fingers (c) with enamel hypoplasia (e). Buphthalmos and filtration bleb from previous trabeculectomy (arrow) plus epicanthus (d).

repaired syndactyly of the fourth and fifth fingers (Figure 3c), the characteristic facies of ODDD with hypoplastic alae nasi, small, anteverted nostrils, and a prominent columnella, including marked epicanthus (Figure 3a) and ectodermal dysplasia manifest as enamel hypoplasia (Figure 3e). Both palpebral fissures were narrow at 21 mm (Figure 3b).

Child 2

This 31-year-old male (II-2) had typical facial features of ODDD with hypoplastic alae nasi, small, anteverted nostrils, and a prominent columnella. In addition there was almost complete syndactyly of the third, fourth, and fifth fingers and early dental caries. There was no evidence of glaucoma with normal intraocular pressures, optic disc morphology, and Humphrey 24-2 full threshold visual fields. Unaided visual acuities were 6/6 in each eye. There was bilateral microcornea (HCD = 8 mm OU; Figure 4b) with normal anterior chamber depths (OD: 2.69 mm; OS: 2.80 mm) and axial lengths (OD: 22.26 mm; OS: 22.11 mm). Palpebral fissures were narrowed at 20 mm right and 22 mm left (Figure 4a).

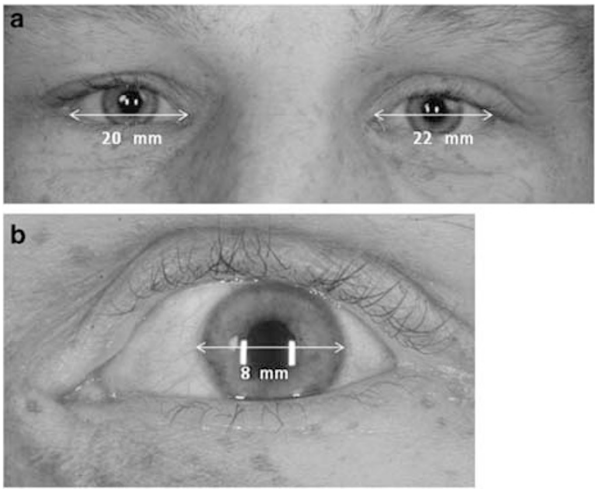

Figure 4 Individual II-2 with reduced palpebral fissure length (a) and microcornea (b).

Molecular genetic analysis

The presence of key features of ODDD syndrome in the proband and her children prompted mutation analysis of

the *GJA1* (Cx43) gene (Figure 1). The proband harboured a heterozygous T>C transition at nucleotide 338 (from ATG start site) in *GJA1* (Figure 1b). This point mutation is predicted to lead to a nonconservative replacement of leucine 113 (CTC) with a proline residue (CCC; L113P) in the cytoplasmic loop of Cx43. Proline is an amino acid that does not form hydrogen bonds and has restricted conformational space, hence this amino acid substitution might have significant consequences on the high-order structure of Cx43. As demonstrated by dHPLC, both affected children of the proband also carried this missense mutation, while it was not detected in 120 chromosomes of unaffected individuals of Northern European origin (Figure 1e). These findings exclude the possibility that L113P represents a nonconsequential sequence polymorphism and support the pathogenicity of this mutation. No sequence aberrations were found in the remainder of the coding sequence of *GJA1*.

Discussion

Mutations in various connexins have been implicated in a wide range of genetic disorders of the nervous system, ear, eye, skin, and heart. Oculodentodigital syndrome is primarily an autosomal dominant disorder with marked intra- and interfamilial phenotypic heterogeneity. Rarely ODDD can be inherited as an autosomal recessive trait[10,11] and homozygous *GJA1* (Cx43) mutations can result in Hallermann–Streiff syndrome (MIM no. 234100).[31]

The pleiotropic phenotype of ODDD results from heterozygous missense mutations in *GJA1*, the gene encoding the gap junction protein, Cx43.[2,5] Two other ectodermal dysplasias result from dominant connexin mutations affecting *GJB2* (connexin 26; Cx26) in keratitis-ichthyosis-deafness syndrome (MIM no. 148210)[13] and *GJB6* (connexin 30; Cx30) in hidrotic ectodermal dysplasia or Clouston syndrome (MIM no.129500).[14]

Connexin proteins consist of an intracellular N-terminus, four transmembrane domains, two extracellular loops, one cytoplasmic loop, and one intracellular C-terminus.[15] Gap junctional intercellular communication (GJIC) controls and coordinates cellular activities by allowing the direct exchange of ions, metabolites and secondary messengers in physiological and developmental processes. Gap junctions are formed by a polygenic family of more than 20 different connexin proteins with complex and overlapping patterns of expression. Six connexin protein subunits oligomerize to form a connexon hemichannel, which then associates to form intercellular gap junction channels between adjacent cells.[15,16] These channels can be composed of similar or different connexin proteins, forming homotypic or heterotypic channels, providing functional diversity by conferring distinct physiological properties that influence the nature of cell-to-cell communication.[17] Over 20 mutations in GJA1 have been reported in ODDD.[5,18–21]

The L113P mutation detected in the family reported here is located in the cytoplasmic loop of Cx43. The functional characterization of other *GJA1* mutations in this domain (I130T, K134E, and G138R) demonstrated that these connexin mutants were capable of forming gap junction-like plaques; however, their channel function was compromised, consistent with loss-of-function mutations.[22,23] Moreover, when mutant Cx43 protein (G138R and G21R) was co-expressed with wild-type Cx43, endogenous gap junctional communication was inhibited, indicating functionally relevant dominant-negative effects.[23] As cells express more than one member of the connexin family, the functional consequences of a mutant Cx43 in ODDD will likely depend on other co-expressed connexins and the degree to which GJIC is compromised. This phenotypic variability is demonstrated by the G143S mutation in the cytoplasmic loop of *GJA1*, which can result in syndactyly type III alone or ODDD. Bigenic mutations in *GJA1* (V41L) and *GJB2* (R127H) have been reported in a patient with hidrotic ectodermal dysplasia with abortive features of ODDD providing direct evidence that *GJA1* mutations can produce variable phenotypes when combined with sequence variants in other connexins. It can be proposed that the pleiotropic phenotype in ODDD results from the co-expression and functional interactions of wild-type and mutant Cx43 in adult and developing tissues (Figure 5).

L113P has previously been reported in ODDD but no phenotypic data and in particular no ocular features were reported.[5] Ocular involvement in ODDD has been described with the mutations Y17S, G22E, and L90V in *GJA1*, although the phenotypic data was limited.[2] Y17S was associated with cataract and glaucoma while G22E and L90V were associated with glaucoma.[2] Heterozygous missense mutations in *GJA8* (connexin 50) and *GJA3* (connexin 46) have been associated with autosomal dominant congenital cataracts.[24–27] Ocular findings have been reported in other patients with ODDD but without molecular genetic characterization.[3,28]

The ocular features in this family highlight the key role Cx43 plays in eye development and in the development of glaucoma. Cx43 (*GJA1*) is expressed in the developing and adult eye.[5,29] Cx43 is localized in the apical side of the outer pigmented cell layer of the ciliary body epithelial bilayer and pairs with different connexin proteins in the nonpigmented cell layer to form heterotypic junctions.[29] Defective developmental ocular expression of mutant Cx43 can result in an anterior segment dysgenesis with trabeculodysgenesis producing

Figure 5 Sequence alignment (Clustal X38; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/) of Cx43 proteins across species (labelled on left) visualized in GENEDOC (http://www.nrbsc.org/gfx/genedoc/index.html). Identical residues are highlighted with a dark background. The arrow indicates the position of the L113P mutation where there is a nonconservative replacement of leucine (L) with a proline residue (P) (L113P) in the cytoplasmic loop of Cx43. This leucine is highly conserved in connexin 43 across species.

a development glaucoma which manifests as congenital glaucoma as in II-1 or late childhood onset glaucoma.[3] The anterior segment dysgenesis with microcornea and narrow drainage angles combined with age-related lenticular growth predisposes to the development of angle closure glaucoma, which occurred in the proband (I-2) and other literature. ODDD can also result in adult-onset open angle glaucoma although the mechanism is undetermined. Glaucoma is the most common cause of visual loss in ODDD, however the type, severity, and age of onset are variable even within the same family harbouring identical *GJA1* mutations and our study illustrates the pleiotropic ocular phenotype of ODDD. The inter- and intrafamilial variability of ODDD illustrated by the ocular phenotype in this family likely results from the complexity of functional interactions of mutant Cx43 with wild-type Cx43 and other connexin proteins at different developmental stages and in different tissue groups. Additionally, there may be modifiers which alter the degree of pleiotropy and variability of expression observed in ODDD. A recent study of patients with the Axenfeld–Rieger syndrome reported patients with both *FOXC1* and *GJA1* mutations had a less severe ocular phenotype than those with the *FOXC1* mutation alone suggesting that mutations in the *GJA1* may act as a modifier gene.[30] Multiple gene–gene interactions are involved in anterior segment development[32] and the ocular features in this family with ODDD highlight the key role Cx43 plays in eye development and in the development of glaucoma.

Acknowledgements

This study was supported by Financial Support—British Medical Association.

Conflict of interest
None.

References

1 Meyer-Schwickerath G, Gruterich E, Weyers H. Mikrophthalmussyndrome. *Klin Mbl Augenheilk* 1957; **131**: 18–30.
2 Paznekas WA, Boyadjiev SA, Shapiro RE, Daniels O, Wollnik B, Keegan CE *et al*. Connexin 43 (GJA1) mutations cause the pleiotropic phenotype of oculodentodigital dysplasia. *Am J Hum Genet* 2003; **72**(2): 408–418.
3 Traboulsi EI, Parks MM. Glaucoma in oculo-dento-osseous dysplasia. *Am J Ophthalmol* 1990; **109**: 310–313.
4 Gladwin A, Donnai D, Metcalfe K, Schrander-Stumpel C, Brueton L, Verloes A *et al*. Localization of a gene for oculodentodigital syndrome to human chromosome 6q22-q24. *Hum Mol Genet* 1997; **6**: 123–127.
5 Richardson R, Donnai D, Meire F, Dixon MJ. Expression of Gja1 correlates with the phenotype observed in oculodentodigital syndrome/type III syndactyly. *J Med Genet* 2004; **41**: 60–67.
6 Weiss AH, Kousseff BG, Ross EA, Longbottom J. Simple microphthalmos. *Arch Ophthalmol* 1989; **107**: 1625–1630.
7 Warburg M. Classification of microphthalmos and coloboma. *J Med Genet* 1993; **30**: 664–669.
8 Kaye SB, Rao P. Developmental abnormalities of the cornea and anterior segment. In: Easty DL, Sparrow JM (eds). *Oxford Textbook of Ophthalmology*, 1st ed. Oxford University Press: Oxford, UK, 1999.
9 Dollfus H, Verloes A. Dysmorphology and the orbital region: a practical clinical approach. *Surf Ophthalmol* 2004; **49**(6): 547–561.
10 Frasson M, Calixto N, Cronemberger S, de Aguiar RA, Leao LL, de Aguiar MJ. Oculodentodigital dysplasia: study of ophthalmological and clinical manifestations in three boys with probably autosomal recessive inheritance. *Ophthalmic Genet* 2004; **25**(3): 227–236.
11 Joss SK, Ghazawy S, Tomkins S, Ahmed M, Bradbury J, Sheridan E. Variable expression of neurological phenotype in autosomal recessive oculodentodigital dysplasia of two sibs and review of the literature. *Eur J Pediatr* 2008; **167**(3): 341–345.
12 Pizzuti A, Flex E, Mingarelli R, Salpietro C, Zelante L, Dallapiccola B. A homozygous GJA1 gene mutation causes a Hallermann-Streiff/ODDD spectrum phenotype. *Hum Mutat* 2004; **23**(3): 286.
13 Richard G, Rouan F, Willoughby CE, Brown N, Chung P, Ryyanen M *et al*. Missense mutations in the *GJB2*

encoding connexin-26 cause the ectodermal dysplasia keratitis-ichthyosis-deafness syndrome. *Am J Hum Genet* 2002; **70**(5): 1341–1348.
14 Lamartine J, Munhoz Essenfelder G, Kibar Z, Lanneluc I, Callouet E, Laoudj D *et al*. Mutations in GJB6 cause hidrotic ectodermal dysplasia. *Nat Genet* 2000; **26**: 142–144.
15 Bruzzone R, White TW, Paul DL. Connections with connexins—the molecular basis of direct intercellular signalling. *Eur J Biochem* 1996; **238**: 1–27.
16 Simon AM, Goodenough DA. Diverse functions of vertebrate gap junctions. *Trends Cell Biol* 1998; **8**: 477–483.
17 Bevans CG, Kordel M, Rhee SK, Harris AL. Isoform composition of connexin channels determines selectivity among second messengers and uncharged molecules. *J Biol Chem* 1998; **273**: 2808–2816.
18 Pizzuti A, Flex E, Mingarelli R, Salpietro C, Zelante L, Dallapiccola B. A homozygous GJA1 gene mutation causes a Hallermann-Streiff/ODDD spectrum phenotype. *Hum Mutat* 2004; **23**: 286.
19 van Steensel MA, Spruijt L, van der Burgt I, Bladergroen RS, Vermeer M, Steijlen PM *et al*. 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**: 171–174.
20 Kjaer KW, Hansen L, Eiberg H, Leicht P, Opitz JM, Tommerup N. Novel connexin 43 (GJA1) mutation causes oculo-dento-digital dysplasia with curly hair. *Am J Med Genet* 2004; **127A**: 152–157.
21 Vitiello C, D'Adamo P, Gentile F, Vingolo EM, Gasparini P, Banfi S. A novel GJA1 mutation causes oculodentodigital dysplasia without syndactyly. *Am J Med Genet A* 2005; **133A**: 58–60.
22 Shibayama J, Paznekas W, Seki A, Taffet S, Jabs EW, Delmar M *et al*. Functional characterization of connexin43 mutations found in patients with oculodentodigital dysplasia. *Circ Res* 2005; **96**: 83–91.
23 Roscoe W, Veitch GIL, Gong X-Q, Pellegrino E, Bai D, McLachlan E *et al*. Oculodentodigital dysplasia-causing connexin43 mutants are non-functional and exhibit dominant effects on wild-type connexin43. *J Biol Chem* 2005; **280**(12): 11458–11466.
24 Willoughby CE, Arab S, Gandi R, Zeinal S, Arab S, Billingsley G *et al*. A novel R23T mutation in GJA8 encoding connexin 50 in an Iranian family with autosomal dominant congenital cataract. *J Med Genet* 2003; **40**: e124.
25 Mackay D, Ionides A, Kibar Z, Rouleau G, Berry V, Moore A *et al*. Connexin 46 mutations in autosomal dominant congenital cataract. *Am J Hum Genet* 1999; **64**: 1357–1364.
26 Shiels A, Mackay D, Ionides A, Berry V, Moore A, Bhattacharya S. A missense mutation in the human connexin 50 gene (GJA8) underlies autosomal dominant 'zonular pulverulent' cataract, on chromosome 1q. *Am J Hum Genet* 1998; **62**: 526–532.
27 Berry V, Mackay D, Khaliq S, Francis PJ, Hameed A, Anwar K *et al*. Connexin 50 mutation in a family with congenital 'zonular nuclear' pulverulent cataract of Pakistani origin. *Hum Genet* 1999; **105**: 168–170.
28 Sugar HS. Oculodentodigital dysplasia syndrome with angle-closure glaucoma. *Am J Ophthalmol* 1978; **86**(1): 36–38.
29 Vaney DI, Weiler R. Gap junctions in the eye: evidence for heteromeric, heterotypic and mixed-homotypic interactions. *Brain Res Rev* 2000; **32**: 115–120.
30 Melo MB, Vasconcellos JP, Cella WP, Kneipp B, Rocha MN, Richeti F *et al*. Axenfeld–Rieger syndrome: coexistence of mutations in the FOXC1 and GJA1 genes. *Invest Ophthalmol Vis Sci* 2005; **46**, E-Abstract 46.
31 Gould DB, John SW. Anterior segment dysgenesis and the developmental glaucomas are complex traits. *Hum Mol Genet* 2002; **11**(10): 1185–1193.
32 Polyakov AV, Shagina IA, Khlebnikova OV, Evgrafov OV. Mutation in the connexin 50 gene (GJA8) in a Russian family with zonular pulverulent cataract. *Clin Genet* 2001; **60**: 476–478.