A novel mutation in \textit{GJA1} causing oculodentodigital syndrome and primary lymphoedema in a three generation family


Oculodentodigital syndrome (ODD; OMIM 164200) is a congenital condition with phenotypic features most commonly affecting the face, eyes, dentition and digits. The condition is caused by mutations in the \textit{GJA1} gene on chromosome 6. \textit{GJA1} codes for connexin 43, a gap junction protein important in providing cell to cell communication and is expressed in lymphatic valves. We present a patient with a clinical and molecular diagnosis of ODD and lower limb lymphoedema. Sanger sequencing of family members confirmed that the missense, p.K206R, \textit{GJA1} mutation segregated with the phenotype suggestive of causality. To our knowledge this association has not been reported previously. This is therefore the second connexin gene associated with a lymphoedema phenotype after the recent publication of \textit{GJC2} (connexin 47) as a cause of four limb lymphoedema.

Conflict of interest
All authors declare no conflict of interest.

Oculodentodigital syndrome (ODD; OMIM 164200) is a congenital condition with phenotypic features affecting the face, eyes, dentition and digits. Characteristic findings include hypotelorism, hypoplastic alae nasi, microphthalmia, microcornea, microdontia, enamel hypoplasia, and digital anomalies including syndactyly of the fourth and fifth fingers. Other reported features include glaucoma, poor hair growth, and conductive hearing loss. Neurological manifestations, e.g. spastic gait, incontinence and epilepsy have also been reported (1, 2).

The mode of inheritance is most often autosomal dominant, but rare autosomal recessive families and sporadic cases have been reported (2). ODD is caused by mutations in the connexin 43 gene (\textit{GJA1}), one of more than 20 connexins expressed in humans. Connexins code for gap junction proteins which assist in cell to cell adhesion and provide for direct intercellular communication by providing a low resistance intercellular passage for ions and small molecules (3). There are no distinct phenotype–genotype correlations but if an individual has two or less of the key ODD features then they are less likely to have a mutation in \textit{GJA1} (2).

To our knowledge, lymphoedema has not previously been reported in association with ODD syndrome. In a Mutation Update, Paznekas et al. (2) give a comprehensive overview of published \textit{GJA1} mutations, and all features that are associated with ODD. Lymphoedema is not reported. Likewise, a PubMed search failed to find any cases of ODD with lymphoedema. Here we present a family with a clear ODD phenotype along with lymphoedema, and molecular confirmation of a \textit{GJA1} mutation. This is therefore the first report of lymphoedema associated with a \textit{GJA1} mutation but, notably, the second connexin gene reported to produce lymphoedema.
Patients and methods

Patients

The proband (III:2, in Fig. 1a), first seen in the lymphoedema clinic aged 40, was born with syndactyly of the fourth and fifth fingers bilaterally, requiring surgery and skin grafting (Fig. 1b). From the age of 30 she was aware of bilateral lower limb swelling, which initially fluctuated in size. More recently, the oedema has remained constant in size despite overnight elevation.

On examination she had the characteristic facial features of ODD, with a flat face, pinched nose, hypoplastic alae nasi, and mild, bilateral ptosis. She had small, crowded teeth (Fig. 1c). There was bilateral below knee pitting oedema (Fig. 1d) with mild superficial venous disease. Stemmer sign was positive bilaterally. No clinically visible swelling of upper limbs.

Family history is significant. Her father (II:1, Fig. 1a), who lived overseas and therefore was not examined, was reported to have syndactyly but no lymphoedema. The proband’s paternal grandfather was also reported to have had syndactyly and lymphoedema. A paternal aunt (II:3, Fig. 1a) also has syndactyly and lymphoedema. A paternal cousin (III:5, Fig. 1a), the daughter of the affected paternal aunt, has syndactyly (fourth and fifth fingers, bilaterally), microphthalmia, microdontia, and poor enamel. She also reported bilateral lower limb oedema presenting at age 14.

Lymphoscintigraphy

The technique of lymphoscintigraphy relies on one of the major functions of the lymphatic system, i.e. the transport of large molecules from the interstitial space back to the vascular compartment. Our patient underwent lymphoscintigraphy using subcutaneous injection of radiotracer (99mTc-albumin nanocolloid) into the first interdigital space in each foot. Subsequently, the lower part of the body was scanned from the liver down to the feet and the radioactivity in a region of interest (ROI) around the injection site of each foot is calculated. A repeat scan is performed 2 h after injection. The 2 h scan not only calculates the radioactivity remaining in the ROI at each injection site but also the radioactivity accumulating in the ilio-inguinal glands. Normal values expect a drop to 80% or less at the injection site by 2 h with 8% or more of total tracer accumulating in the regional lymph nodes.

The same method can be applied to assess lymphatic drainage within the upper limbs. The radiotracer is injected into the first interdigital space of the hand, and a normal lymphatic system will drain at least 20% giving a 2 h retention figure of <80% at the injection site.

Fig. 1. Clinical features. (a) Pedigree with indication of which family members have the characteristic oculodentodigital syndrome feature and who also have associated lymphoedema. *The samples that were tested for GJA1. (b–d) Clinical features of proband (III:2), (b) fourth and fifth fingers after surgery and skin grafting for syndactyly, (c) small, crowded teeth, pinched nose, and (d) below knee oedema affecting toes, dorsum of foot, ankle and calf region.
site. More than 6% uptake in axillary nodes at 2 h is normal.

Venous duplex

Each of the nine segments of vein (upper and lower great saphenous vein; lesser saphenous vein; proximal and distal femoral vein; proximal and distal popliteal vein; saphenofemoral junction; and saphenopopliteal junction) were studied with an Acuson 128 ultrasound machine (Acuson, Stevenage, UK) at 7 MHz in a temperature-controlled room. The most common criteria for pathological reflux were used, namely, peak reflux velocity >10 cm/s and duration of reflux >0.5 s after release of distal compression.

Screening of GJA1

Due to the features of ODD, testing of connexin 43 (GJA1) was performed in a diagnostic genetic laboratory.

Results

Lymphoscintigraphy

Lower limbs
There was a delay in drainage and appearances were consistent with impaired lymphatic drainage in both legs. Two hour retention figures demonstrated 84.9% activity persisting in the right foot and 87.3% in the left foot. On the right side, 7.3% of activity reached the groin after 2 h and on the left 5.2%.

Upper limbs
There was impaired lymphatic drainage on the left. Appearances on the right were within normal limits. On the right side, activity in the hand after 2 h was 90.4% and in the left hand 96.1%. Uptake in the right axilla was 3.4% and in the left axilla 1.5% after 2 h.

Venous duplex ultrasound

On the left side, minor incompetence was noted in the short saphenous vein. All other deep and superficial veins were normal. Bilateral distal calf soft tissue oedema was noted and a few groin lymph nodes were seen, apparently normal in outline.

Genetic testing

A novel missense variant was found in exon 2 (c.617A>G, p.K206R). In silico analysis using ALNIN, SIFT, GVGDD and POLYPHEN predicts that this variant is pathogenic because it affects a highly conserved amino acid in a functional domain of the protein. The c.617A>G variant has not been detected in a control population (n = 600) of mainly European origin.

Further testing showed that the variant segregated with disease status, being present in the proband’s father and an affected paternal cousin (individual III:5), but not in an unaffected paternal cousin (individual III:6).

Discussion

ODD is a rare condition with a striking clinical phenotype affecting the face, eyes, teeth and digits. In addition to typical ODD features, our patient also presented with lymphoedema which was confirmed by lymphoscintigraphy and ultrasound scan. The lymphoedema seen in our patient was relatively mild and it is possible that other individuals with ODD syndrome also have unrecognized lymphatic abnormalities. Whilst the lymphoedema is unlikely to cause significant morbidity in this case, it is interesting that it was this, and not the ocular and skeletal manifestations of ODD, which brought proband to the attention of the genetics department. Despite the strong family history of characteristic signs of ODD syndrome no other family member had been offered genetic testing nor given a definitive diagnosis. We offered the family screening for mutations in the known causative gene for ODD, GJA1, which codes for connexin 43 and a novel missense change, c.617A>G, was identified in three affected family members. The change leads to an amino acid change: p.K206R. We suggest that the finding of a probable pathogenic GJA1 mutation in this patient and segregation in family members indicates that this gene is a rare cause of lymphoedema.

It may be significant that a related gene, connexin 47 (GJC2), has recently been reported to be a cause of four limb lymphoedema (4, 5). Lymph scans performed on patients with GJC2 mutations show a clear functional abnormality as lymph drainage routes appear normal on imaging but quantification reveals reduced lymph transport over the 2 h observation period (5). This is similar to the pattern seen in the proband.

The majority of GJA1 mutations reported to date are missense, occur in highly conserved amino acid residues among various species, and are also conserved among the human connexin protein family (2). The variant reported here sits in an important residue, the SRPTEK motif, of the second extracellular loop. Several patients with mutations at R202H in this motif have been reported (1, 2, 6), but this is the first report of a mutation at residue K206 in this motif. This particular motif is believed to be important for appropriate docking of the hemichannels, and mutations in the PTEK portion have been shown to decrease the formation of functional gap junctions (3). This is supported by in vivo and in vitro studies showing that mutant connexin 43 proteins are localized to the cell surface where they disrupt the formation of complete and functional channels. Levels of normal connexin 43 are also affected in those mutants, suggesting a dominant-negative mechanism (7). That this is a dominant-negative mechanism rather than loss of function is also supported by the mouse work by Paznekas et al. (1) who showed that the Cnx43 KO mouse did...
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not mimic ODD completely, leading to the conclusion that the deletion of one copy of the connexin 43 gene was insufficient to recapitulate the phenotype.

\textit{In situ} hybridization experiments in mouse embryos have demonstrated that connexin 43 plays an important role in facial and limb development (6), and it is further suggested that connexin 43 plays a major role in many developmental events in the chick (8). Kanady and colleagues (9) looked specifically at expression of connexins 37, 43 and 47 in developing and mature lymphatic vessels and found that the connexins 37 and 43 are necessary for valve formation in lymphatic collecting vessels. Mice deficient in these two connexins either have reduced numbers of valves, or lack them completely. Double knock-out mice (Cx37\textsuperscript{−/−}Cx43\textsuperscript{−/−}) also develop severe lymphoedema and chylothorax (9).

As several connexins have been demonstrated to be essential for normal development and function of the lymphatic vasculature, our finding of a \textit{GJA1} mutation associated with lymphoedema is perhaps not a surprise. The lack of previous reports of lymphoedema associated with ODD may be more surprising, but this could be a rare association or it could be overlooked in the context of the other more obvious phenotypic characteristics of the condition. Lymphoedema in general is under recognized, and frequently misdiagnosed (10). It would therefore be interesting to revisit some of the previously published ODD patients to specifically check for lymphoedema to determine if that feature should be included in the diagnostic criteria.

In conclusion, as the lymphoscintigraphy appears to indicate a functional reduction in lymph drainage without obvious anatomical abnormalities on the images of the lymphatic tracts, we provide evidence that a mutation in \textit{GJA1} leads not only to ODD as already described in the literature, but can also lead to lymphoedema as an associated feature. This may be a rare cause of lymphoedema, but it is the second connexin gene where mutations produce lymphoedema, and it is therefore possible that other members of the same family of gap junction proteins are candidates as causative genes for this condition.

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\section*{References}