Original Article

Oral-facial-digital syndrome type 1 is another dominant polycystic kidney disease: clinical, radiological and histopathological features of a new kindred

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Abstract

Background. Oral-facial-digital syndrome type 1 (OFD1) is a rare disorder comprising malformations of the face, oral cavity, hands, and feet. Polycystic kidney disease (PKD) is a more recently recognized feature of the syndrome.

Subjects and methods. We now report on the clinical, radiological and histopathological features of an OFD1 and PKD kindred with five affected members in three subsequent generations.

Results. All patients were female and had accompanying PKD as assessed by ultrasound scans. The plasma creatinine was normal in three, but PKD caused end-stage renal failure in two of these individuals in the second and fifth decades. A histochemical analysis of renal tissue from one affected member of this kindred demonstrated a predominantly glomerulocystic kidney disease with a minor population of cysts derived from distal tubules as assessed by staining with Arachis hypogaea lectin. Cyst epithelia had a high level of mitosis as assessed by staining with antiserum to proliferating cell nuclear antigen, and distal cysts overexpressed PAX2 protein, a potentially oncogenic transcription factor. We detected multiple pancreatic cysts in one member affected by OFD1 although there were no symptoms of pancreatic disease; this constitutes a novel radiological feature of the syndrome.

Conclusions. This kindred illustrates the inheritance pattern of OFD1 and its accompanying PKD. Although the renal disease superficially resembles ADPKD with macroscopic cysts and a dominant inheritance pattern, histology shows a predominance of glomerular cysts and the syndrome is X-linked, with affected males dying before birth. The recognition of the accompanying dysmorphic features is the key to a diagnosis of OFD1 in a female child or adult who presents with PKD.

Key words: end-stage renal failure; histopathology; oral-facial-digital syndrome type 1; polycystic kidney disease; radiology; X-linked dominant disease

Introduction

The genetic causes of polycystic kidney disease (PKD), which are associated with macroscopic renal cysts, include the autosomal dominant PKDs [1,2], tuberous sclerosis [3,4], von Hippel-Lindau disease [5,6], and a recently recognized contiguous gene defect comprising deletions of PKD1 and TSC2 [7]. Other inherited disorders are associated with renal cysts which are generally microscopic, and these entities include autosomal recessive PKD, juvenile nephronophthisis, and medullary cystic disease [8,9]. In this report we wish to draw attention to the PKD associated with a rare disorder called the oral-facial-digital syndrome type 1 (OFD1; previously called orodigitofacial dysostosis or the Papillon-Leage-Psaume syndrome) [10,11]. Individuals affected by OFD1 have characteristic malformations of the face, oral cavity, hands, and feet but these signs may be relatively mild, especially since some of these defects are often surgically corrected in childhood [10,11]. The associated kidney disease superficially resembles ADPKD because of the presence of multiple macroscopic renal cysts and a dominant inheritance pattern [12–16]. Histological analysis of OFD1 kidneys, however, demonstrates a predominance of glomerular cysts, whereas this appearance is rare in ADPKD [13]. Moreover, a closer inspection of kindreds with OFD1 suggests that the mode of inheritance is X-linked dominant, in contrast to the ADPKDs which are autosomal dominant diseases [1,2]. Furthermore, OFD1 is almost exclusively diagnosed in females because males carrying OFD1 mutations die in utero, usually in first or second trimester [12,17]. These observations have major implications for genetic counselling of OFD1 patients with PKD who may be
investigated and treated in either paediatric or adult nephrological units. In the current report we describe the clinical, radiological and histopathological features of a three-generation kindred with OFD1 and PKD affecting five females, two of whom have required renal replacement therapy.

Subjects and methods

Subjects

The three-generation family which included five females affected by OFD1 and PKD (e.g. I-2; II-2 and II-4; III-1 and III-2) is depicted in Figure 1. The diagnosis of polycystic kidney disease in I-2, renal ultrasound were performed in her five children (II-2, II-4, II-6, II-8 and II-9) and, when II-2 and II-4 were found to be affected, their own children (III-1, III-2, III-3 and III-4) had renal ultrasound scans. In those individuals found to have renal cysts, the liver and pancreas were imaged to detect possible cystic involvement.

Histochemistry

Chemicals were obtained from Sigma (Poole, Dorset, UK) unless otherwise stated. Directly after autopsy, PKD kidneys from individual I-2 (see Figure 1) were fixed in 4% paraformaldehyde and embedded in paraffin wax. Sections (10 μm) were placed on glass slides and were dewaxed through HistoClear (National Diagnostics, Atlanta, USA) twice for 10 min, followed by dehydration through 100% ethyl alcohol twice for 5 min and then stepwise through 95%, 75%, 50% and 30% alcohol for 3 min each. They were further processed for immunohistochemistry [18]. After washing in phosphate-buffered saline (PBS, pH 7.4) for 5 min and tap water for 10 min, they were immersed in citric-acid buffer (2.1 g/l, pH 6.0) and boiled in a microwave for 8 min. They were allowed to cool, washed in tap water and PBS, then incubated in 3% hydrogen peroxide for 15 min to quench endogenous peroxidase activity. Non-specific antibody binding was blocked by preincubation of the slides with fetal calf serum (10% volume/volume in PBS). Primary antibodies were rabbit polyclonal antibody raised against amino acids 188–385 in the carboxyterminus domain of PAX2 used as previously described [18]; a rabbit polyclonal IgG fraction raised against an epitope in the carboxyterminus of human WT1 protein (C-19: Santa Cruz Biotechnology, Inc., CA, USA) used as previously described [18]; mouse monoclonal antibody to proliferating cell nuclear antigen (PCNA Ab-1; Oncogene Science Inc., Cambridge, MA, USA) used as previously described [18]. PCNA is a DNA-polymerase α-associated protein expressed at high levels during S phase [19]. Primary antibodies were detected using a streptavidin biotin peroxidase system (Dako, ABC Kit) followed by diamino benzidine. Sections were counterstained with 0.5% methyl green for 10 min and washed three times with water.

Fig. 1. Family tree of OFD1 kindred. The three generations are designated I, II and III while individual members in each generation are designated I-1, I-2 etc. Female heterozygotes affected by OFD1 are indicted by filled circles. I-2 is the index case who required dialysis and renal transplantation but subsequently died from a myocardial infarction.

Results

Clinical and radiological features of the affected members are summarized in Table 1, renal histology of I-2 is shown in Figures 2 and 3, and selected dysmorphic and ultrasonographic features of certain individuals are depicted in Figure 4.

The index case (I-2; Figure 1), presented in her fifth decade with end-stage renal failure. She had a pseudocleft of the upper lip, a broad nasal bridge, and an asymmetrical face, characteristic features of OFD1.

At autopsy her kidneys were polycystic with cysts <1 cm in diameter. Cysts were not noted in other organs. As expected for an ‘end-stage’ kidney, there was marked fibrosis between cysts (Figure 2A and B). Most cysts were lined by flat epithelial cells and were enclosed by fibrotic walls. In approximately 5–10% of such structures in any single section, a glomerular tuft was seen to be attached to the cyst lining (Figure 2A and B). A subset of nuclei in these tufts stained with antibody to WT1 (Figure 2C), consistent with identit-
Table 1. Clinical and radiological features of affected individuals.

<table>
<thead>
<tr>
<th>Case</th>
<th>I-2</th>
<th>II-2</th>
<th>II-4</th>
<th>III-1</th>
<th>III-2</th>
</tr>
</thead>
<tbody>
<tr>
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<td>female</td>
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<tr>
<td>Age at diagnosis</td>
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<td>3rd decade</td>
<td>3rd decade</td>
<td>15 years</td>
<td>13 years</td>
</tr>
<tr>
<td>Renal ultrasound</td>
<td>Bilateral polycystic kidneys</td>
<td>Unilateral polycystic kidney</td>
<td>Bilateral polycystic kidneys</td>
<td>Bilateral polycystic kidneys</td>
<td>Bilateral polycystic kidneys</td>
</tr>
<tr>
<td>Renal status</td>
<td>End-stage renal failure; renal transplant</td>
<td>Normal plasma creatinine</td>
<td>Plasma creatinine 104 μM</td>
<td>End-stage renal failure</td>
<td>Plasma creatinine 54 μM</td>
</tr>
<tr>
<td>Facial features</td>
<td>Pseudocleft upper lip; broad nasal bridge; asymmetrical face</td>
<td>Pseudocleft upper lip; broad nasal bridge; asymmetrical face</td>
<td>Pseudocleft upper lip; broad nasal bridge; asymmetrical face</td>
<td>Pseudocleft upper lip; broad nasal bridge; asymmetrical face</td>
<td>Pseudocleft upper lip; broad nasal bridge; asymmetrical face</td>
</tr>
<tr>
<td>Oral features</td>
<td>Not documented</td>
<td>Tongue hamartoma</td>
<td>Tethered tongue cleft palate</td>
<td>High arched palate, oral frenulae; absent lateral incisors</td>
<td>High arched palate, tethered and cleft tongue</td>
</tr>
<tr>
<td>Digital features</td>
<td>Not documented</td>
<td>Not documented</td>
<td>Soft-tissue syndactyly; middle and index finger left hand; left hallux varus and duplication</td>
<td>Brachydactyly left index finger and 4th toes</td>
<td>Brachydactyly 4th toes</td>
</tr>
<tr>
<td>Other features</td>
<td>Learning difficulties</td>
<td>Learning difficulties 2nd trimester miscarriages</td>
<td>Learning difficulties</td>
<td>Learning difficulties pancreatic cysts</td>
<td>Learning difficulties</td>
</tr>
</tbody>
</table>

The majority (>90%) of cysts failed to stain with either FITC-conjugated *Tetragonolobus lotus* and *Arachis hypogaea* lectins (Figure 3A), consistent with a glomerular origin. Mildly dilated tubules of up to 100–200 μm were also noted (Figure 2G and H). These stained with *Arachis hypogaea* lectin (Figure 3B) and never contained glomerular tufts, suggesting a distal tubule rather than a glomerular origin. The epithelial cells which lined the distal tubule cysts stained with PAX2 antibody (Figure 2G and H). Glomerular cysts did not express PAX2 (Figure 2E and G), while dilated distal tubules showed no immunoreactivity with WT1 antibody (data not shown). Up to 50% of nuclei in dilated distal tubules stained with PCNA antibody (data not shown).

After presentation of the index case, her family were assessed clinically and by ultrasound scanning (Table 1). Two (II-2 and II-4) of her three daughters had dysmorphic features of OFD1 and two granddaughters (III-1 and III-2) were similarly affected. Dysmorphic features included tongue tethering by frenulae, abnormal dentition and digital abnormalities (Table 1 and Figure 4A–D). No live-born males in this kindred were affected by OFD1 (Figure 1) but there was a family history of miscarriages in OFD1 individuals I-2, II-2, and II-4. The inheritance pattern in this kindred is consistent with an X-linked dominant pattern in which female heterozygotes exhibit the clinical syndrome whereas males who carry a single, mutated gene die before birth. As well as the dysmorphic features, mild to moderate learning difficulties were prominent in this OFD1 kindred.

All affected members had PKD as assessed by ultrasound scans, although renal lengths were within normal limits and the contours were not irregular. Renal cysts were <1 cm across (Figure 4E) and were bilateral in all but II-2. At initial assessment at 15 years, III-1 was found to be in chronic renal failure, but the other three affected members (II-2, II-4, and III-2) had normal plasma creatinine levels. Within 2 years of diagnosis III-1 required treatment with chronic ambulatory peritoneal dialysis and she is awaiting transplantation. We also detected multiple pancreatic cysts (<5 cm) in this individual (Figure 4F) although she reported no symptoms of pancreatic disease. Ultrasound scans did not detect pancreatic cysts in other OFD1 individuals.

**Discussion**

OFD1 is a rare syndrome, occurring in approximately 1,250,000 live births [10,11,17]. The dysmorphology of OFD1 is characteristic, with facial features which include frontal bossing, facial asymmetry, hypertelorism (widely spaced eyes), a broadened nasal bridge, and facial milia. Oral features include pseudoclefting of the upper lip, cleft palate and tongue, high arched palate, tethering of the tongue (ankyloglossia) by frenu-
Fig. 3. Lectin histochemistry in OFD1 kidney. A. Parietal epithelia (arrowheads) of two glomerular cysts (c) showed no reactivity with FITC-conjugated *Arachis hypogaea* lectin. Glomerular tufts are also indicated (g). Lack of staining was also noted with *Tetragonolobus lotus* lectin (not shown). B. Epithelial cells (arrowheads) lining a dilated tubule stained positively with FITC-conjugated *Arachis hypogaea* lectin (green), suggesting a distal tubule origin. All nuclei are stained red with PI. Bars are 20 µm.

Fig. 2. Renal histology of case I-2. A. Low-power view of a PKD organ showing cysts of varied diameters. B. Higher power demonstrates glomeruli with mildly dilated Bowman’s spaces as well as larger glomerular cysts. The arrows in A and B indicate a glomerular tuft attached to the wall of a cyst. C. Proxiocyes (arrowheads) in a glomerular cysts stained with WT1 antibody. D. PCNA-staining nuclei (arrowheads) in the tuft of a cystic glomerulus. E. Absence of PAX2 protein nuclear staining in a glomerular cyst. F. Representative lack of staining when first antibodies are omitted. G. A cystic distal tubule epithelium stained with antibody to PAX2; two cysts on the right were negative for PAX2 and were presumably of glomerular origin. H. High-power view of distal cyst depicted in G. In some cells PAX2 staining is clearly localized to nuclei (arrowheads). All nuclei in the panel were counterstained with methyl green, while positive immunohistochemical signals appear brown. Bars are 60 µm in A, 15 µm in B and G, and 10 µm other frames.

Lae, together with abnormal dentition. Malformations of the digits of the hands are more common than those of the feet and include syndactyly (fusion), brachydactyly (shortening), clinodactyly (curvature) and, less commonly, polydactyly (extra digits). It is notable that the dysmorphic features of OFD1 can vary within a kindred and be asymmetrical, and this has been attributed to the generation of mosaics of somatic cells due to random X chromosome inactivation early in embryogenesis [11,17]. Central nervous system disease occurs in 40% of individuals affected by OFD1 with mental retardation, hydrocephalus, and agenesia of the corpus callosum [11,15]. In this respect it is of note that mild to moderate learning difficulties were prominent in our kindred.

An association with PKD was first noted by Doege and colleagues in a member of a large OFD1 family [12]. Clearly, this single case could have been a chance association with, for example, ADPKD. With the increasing use of high-definition renal ultrasound scanning, however, it has become increasingly apparent that PKD is a common accompanying feature of OFD1 [13–16], although the incidence of PKD in a large series of OFD1 patients has yet to be reported. The kidneys may be of normal length, as in the kindred in the current report, or palpably enlarged [15]. Our kindred showed a complete concordance of PKD with the dysmorphic features of the syndrome, and additionally the family demonstrates that end-stage renal failure can occur in either late childhood or adulthood. It is intriguing to speculate that variability in the severity of the kidney disease within this kindred (e.g. severe in III-1 versus mild in her mother, II-2) might be explained by random X chromosome inactivation, discussed above. Furthermore, the occurrence of unilateral PKD as detected by ultrasound scanning of II-2 (see Table 1) might be explained by the same mechanism. Liver cysts have been previously documented in the syndrome [13] and our study suggests that multiple pancreatic cysts are another feature of OFD1, albeit an inconstant one, occurring in 1/5 affected members in our kindred. While OFD1 is the commonest of a group of eight oral-facial-digital syndromes which have overlapping dysmorphic features, PKD has only been reported in OFD1 [11].

To our knowledge there have only been two previous original reports of OFD1 renal histology published in the English literature, and both cases were not apparently part of OFD1 kindreds [13,22]. One was a female who developed renal failure in the second decade [13], while the other was an XY male neonate with massively dilated kidneys and lung hypoplasia who died hours.
Fig. 4. Dysmorphic and radiological features of the OFD1 kindred. A. Individual III-1 had marked dysmorphic features: note the irregular clefting of the tongue. B. An oral frenulum (arrow) and abnormal dentition (missing lateral incisors) in III-1. C. Shortened index finger (brachydactyly) in left hand of III-1. D. Bilateral dysmorphic toes (brachydactyly of 4th digits) of III-1. Note the pitting oedema of this teenage patient, who was treated with peritoneal dialysis. E. Ultrasound scan of kidney of III-2 detected cysts of up to 5 mm in diameter. F. Ultrasound scan of III-1 showing a polycystic pancreas.
after birth [22]. The latter case is extraordinary because the vast majority of genotypic males who carry the OFD1 mutation are thought to die in the first or second trimester of pregnancy [17]. Both previous reports noted a predominance of glomerular cysts as assessed by gross histological appearances. Our study confirms this impression and adds new histochemical insights, discussed below. Glomerulocystic kidneys have been found to occur in a variety of renal diseases including non-syndromic glomerulocystic diseases (which may be sporadic or familial), tuberous sclerosis, brachymesomelia-renal syndrome, the short rib polydactyly syndromes, Jeune asphyxiating thoracic dystrophy syndromes, Zellweger hepatorenal syndrome and familial juvenile nephronophthisis, as well as in trisomies 9, 13 and 18 [for reviews, see 23–25].

Although glomerular cysts can also occur in ADPKD, the histology is usually dominated by cysts of tubular origin [23–24].

In our OFD1 patient (I-2) in which the kidneys were examined by histology, the majority of renal cysts failed to stain with lectins which bind to proximal and distal tubules, and some contained tufts with capillary loops surrounded by cells staining for WT1, a protein expressed by podocytes in the postnatal kidney [18]. These data demonstrate that these cysts are of glomerular origin. However, a minority of smaller cysts stained with *Arachis hypogaea* lectin, indicating an origin in distal tubules. This observation is important because it suggests that the (as yet unknown) OFD1 gene product plays a biological role in distal as well as glomerular epithelia. We have previously reported that <1% of nuclei in normal postnatal glomeruli or tubules are PCNA positive [18], yet we detected proliferation in approximately 50% of cells within glomerular tufts attached to OFD1 cyst walls. Deregulated proliferation might therefore be important in the genesis of OFD1 renal cysts, and it is notable that epithelial hyperproliferation has been implicated in a variety of human and animal models of PKD [26]. We previously reported that the PAX2 transcription factor protein is barely detectable by immunohistochemistry in mature human kidneys [18], but in this study we found that PAX2 was strongly expressed by epithelia lining distal tubule OFD1 cysts. PAX2 is highly expressed by proliferating distal tubule precursor cells in human fetal kidneys, in cysts of human dysplastic kidneys [18] and in Wilms’ tumours [27]; furthermore, overexpression of PAX2 causes renal cysts in transgenic mice [28]. Conversely, we found that PAX2 was not expressed in OFD1 glomeruli, suggesting that other factors must drive proliferation in those structures. In future, it would be interesting to examine histology of other OFD1 patients who have PKD but are not in severe renal failure.

Approximately 75% of OFD1 cases are sporadic and these occur almost exclusively in females [10–12,17]. The remaining cases are familial and these too are essentially limited to females. The most likely form of inheritance has been considered to be X-linked dominant with prenatal death of males carrying a single, mutated, OFD1 gene [12,17]. The cause of death in utero is currently unknown but affected fetuses usually spontaneously abort in the first or second trimester. At the time of writing, the specific mutation is undefined but, based on the clinical phenotype of syndrome, the wild-type gene is likely to code for a protein which affects the development of the face, mouth, digits, and central nervous system as well as the biology of epithelia in the kidney, pancreas, and liver. Moreover, the OFD1 gene appears to be essential for life in utero as assessed by the in utero death of males carrying the mutated gene.

These observations have important implications for genetic counselling of PKD patients with OFD1. In an established kindred with OFD1, an affected female will transmit the mutation to 50% of her female progeny and these heterozygotes will exhibit the clinical syndrome. Essentially all live-born boys will be normal because males who harbor a mutated OFD1 gene would be expected to die in utero [11,12,17]. In addition 50% of female siblings of an index case will carry the mutation, while all living brothers will be unaffected. The probability that an individual with sporadic OFD1 will produce affected offspring is currently unknown, but we suggest such patients should be counselled as in familial cases. This outlook is clearly very different from the ADPKDs in which mutations are also inherited in a dominant manner but male and female offspring are affected in equal measure because of the autosomal localization of the PKD1 and PKD2 genes [1,2]. Finally, multiple renal cysts also occur in tuberous sclerosis and von Hippel-Lindau disease; both entities can be inherited in an autosomal dominant manner but each has well-described clinical features which allow discrimination from both OFD1 and the ADPKDs [3–6].

In summary, clinically significant PKD can occur in OFD1. Although the renal disease superficially resembles ADPKD with macroscopic cysts and dominant inheritance, OFD1 PKD kidneys are usually of normal size and contour, the renal histology shows a predominance of glomerular cysts, and the specific inheritance pattern is X-linked with practically all affected males dying before birth. The recognition of the accompanying dysmorphic features is the key to a diagnosis of OFD1 in a female child or adult who presents with PKD. In particular, the diagnosis should be suspected in PKD kindreds in which only females are affected. Finally, we recommend that all children with the dysmorphic features of OFD1 are followed up with renal ultrasound scans and simple measures of renal function (e.g. blood pressure and plasma creatinine) in order to detect PKD and its clinical consequences.

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Note added in proof

We have recently mapped OFD1 to the short arm of the X chromosome [29].

References


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