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The oral–facial–digital syndrome type 1 (OFD1), a cause of polycystic kidney disease and associated malformations, maps to Xp22.2–Xp22.3

Sally A. Feather[1,2,*], Adrian S. Woolf[2,3], Dian Donnai[5], Sue Malcolm[1] and Robin M. Winter[4]

[1]Molecular Genetics Unit, [2]Nephrourology Unit, [3]Developmental Biology and [4]Clinical Genetics Unit, Institute of Child Health, London WC1 1EH, UK and [5]Medical Genetics Unit, St Mary's Hospital, Manchester M13 OJH, UK

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Key features of the oral–facial–digital syndrome type 1 (OFD1) include malformations of the face, oral cavity and digits. In addition, the clinical phenotype often includes mental retardation and renal functional impairment. Approximately 75% of cases of OFD1 are sporadic, and the condition occurs almost exclusively in females. In familial cases, the most likely mode of inheritance is considered to be X-linked dominant with prenatal lethality in affected males. Therefore, the *OFD1* gene product appears to have widespread importance in organogenesis and is essential for fetal survival. We have studied two kindreds in which the clinical course was dominated by polycystic kidney disease requiring dialysis and transplantation. Using polymorphic chromosome markers spaced at ~10 cM intervals along the X chromosome, we mapped the disease to a region on the short arm of the X chromosome (Xp22.2–Xp22.3) spanning 19.8 cM and flanked by crossovers with the markers *DXS996* and *DX7S105*. There was a maximum lod score of 3.32 in an 'affecteds only' analysis using a marker within the *KAL* gene ($\theta = 0.0$), thereby confirming the location of the gene for OFD1 on the X chromosome. The remainder of the X chromosome was excluded by recombinants in affected individuals. The importance of our findings includes the definitive assignment of this male-lethal disease to the X chromosome and the mapping of a further locus for a human polycystic kidney disease. Furthermore, this mapping study suggests a possible mouse model for OFD1 as the X-linked dominant *Xpl* mutant, in which polydactyly and renal cystic disease occurs, maps to the homologous region of the mouse X chromosome.

INTRODUCTION

In 1962, Gorlin and Psaume defined a syndrome characterized by malformation of the face, oral cavity and digits (1). They called this orofaciodigital dysosotosis, a disorder which subsequently has been renamed the oral–facial–digital syndrome type 1 (OFD1: OMIM 311200) (2). The incidence of OFD1 is estimated to be 1 in 250 000 live births, and it occurs in diverse racial backgrounds (3). Dysmorphic features affecting the head are described as 'remarkably characteristic' (2) and include facial asymmetry, frontal bossing, hypertelorism, micrognathia, broadened nasal bridge and facial milia. Lesions of the mouth include median pseudoclefting of the upper lip (45%), clefts of the palate (>80%) and tongue (30%), as well as lingual hamartomata and oral frenulae. Thickened alveolar ridges and abnormal dentition, including absent lateral incisors, are further characteristics of the syndrome. The digital abnormalities, which affect the hands (50–70%) more often than the feet (25%), include syndactyly, brachydactyly, clinodactyly and, more rarely, pre- or post-axial polydactyly. These clinical features overlap with those reported in the other seven oral–facial–digital syndromes (2).

The central nervous system may also be involved in as many as 40% of cases of OFD1, with reports of mental retardation, hydrocephalus, cerebellar anomalies, porencephaly and agenesis of the corpus callosum (4). In recent years, with the introduction of high definition renal ultrasound scanning, it has been realized that polycystic kidney disease is commonly associated with OFD1 (4–6). In fact, renal failure necessitating dialysis and transplantation in either childhood or adulthood can dominate the clinical course of this disease (6).

Approximately 75% of cases of OFD1 are sporadic. The condition occurs almost exclusively in females. In familial cases, the most likely mode of inheritance is considered to be X-linked dominant with prenatal lethality in affected males (7,8). This hypothesis is supported by the high incidence of miscarriage of male fetuses and the ratio of 2:1 females to males in reported sibships. Phenotypic variability is often found in affected females within a family, and this might be explained by different degrees of somatic mosaicism resulting from random X chromosome inactivation. A case of a liveborn OFD1 male (XY) with massively enlarged polycystic kidneys has been reported, but death occurred within 4 h due to pulmonary hypoplasia (9). Furthermore, a case of Klinefelter syndrome (XXY) has been reported with OFD1, with a phenotype very similar to that of affected females (10). There have been no genetic studies to date which definitively link the syndrome to the X chromosome. An alternative hypothesis would be that OFD1 is a sex-limited

*To whom correspondence should be addressed. Tel: + 44 171 242 9789 ext. 2222; Fax: +44 171 831 0488; Email: sfeather@hgmp.mrc.ac.uk

autosomal dominant disease. In this context, an early report of insertion of an extra segment in chromosome 1 (11) has yet to be substantiated by modern cytogenetic techniques. With regard to the other oral–facial–digital syndromes, most are autosomal recessive but it has been suggested that type VIII is X-linked recessive (2).

In the current study of two OFD1 families with pronounced polycystic kidney disease, we map the locus for the syndrome to a 19.8 cM region on the short arm of the X chromosome between the markers *DXS996* and *DXS7105*. The importance of our new findings include the definitive assignment of this male-lethal disease to the X chromosome as well as the mapping of a further locus for human polycystic kidney disease.

Table 1. Two point linkage analysis for nine markers in order from *DXS996* to *DXS7105*

Marker	Recombination fractions (θ)						
	0.0	0.01	0.05	0.1	0.2	0.3	0.4
DXS996	–∞	0.16	0.68	0.76	0.62	0.39	0.19
	–∞	0.24	0.30	0.41	0.36	0.25	0.14
KAL	–∞	1.90	2.34	2.29	1.85	1.25	0.60
	3.32	3.25	2.97	2.61	1.89	1.19	0.54
DXS1223	**2.67**	2.62	2.43	2.18	1.66	1.11	0.54
	2.32	2.27	2.09	1.86	1.40	0.94	0.47
DXS8051	1.83	1.81	1.73	1.61	1.32	0.96	0.51
	2.24	2.20	2.03	1.82	1.40	0.96	0.49
DXS7108	–∞	–0.30	0.51	0.82	0.92	0.71	0.37
	2.13	2.11	2.02	1.83	1.37	0.86	0.37
DXS8022	**2.52**	2.50	2.41	2.24	1.79	1.23	0.59
	2.84	2.79	2.60	2.35	1.80	1.19	0.55
DXS987	1.05	1.06	1.08	1.06	0.94	0.71	0.40
	1.49	1.48	1.40	1.29	1.02	0.71	0.37
DXS8036	1.76	1.74	1.67	1.54	1.19	0.75	0.29
	2.32	2.27	2.10	1.87	1.38	0.84	0.32
DXS7105	–∞	0.20	0.78	0.93	0.87	0.62	0.28
	–∞	–0.00	0.58	0.72	0.68	0.47	0.20

For each marker, the upper line of figures indicates the two-point lod score for all family members using 100% penetrance, and the lower line indicates the score for affected females and unaffected males only. The maximum lod scores are highlighted in bold in each case.

RESULTS

A G-banded karyotype performed on an affected individual in each family showed no gross chromosomal aberrations.

Two-point linkage analysis of the two kindreds (see Fig. 1) using 100% penetrance gave a maximum two-point lod score of 2.67 at θ = 0.0 with the marker *DXS1223* and a slightly lower lod score (2.52 at θ = 0.0) with the more proximal marker *DXS8022* (see Table 1). An 'affecteds only' analysis (12) gave a maximum lod of 3.32 at θ = 0.0 with *KAL* and a lod of 2.84 at θ = 0.0 with *DXS8022*. The higher lod score in the 'affecteds only' analysis was due to the elimination of individual II-6 in family 1 from the analysis. Although apparently phenotypically normal, she could represent a case of incomplete penetrance, as could individual II-5 from family 2, as has been described in other families (13). An 'affecteds only' multipoint analysis gave a maximum lod score of 3.56 at θ = 0 at *KAL* and but also a lod score of 3.56 between *DXS987* and *DXS8036*. Other markers on the X chromosome (*DXS6807*, *DXS989*, *DXS1068*, *DXS6810*, *DXS1003*, *DXS6800*, *DXS6789*, *DXS6799*, *DXS6797*, *DXS6804*, *DXS1001*, *DXS1047*, *GATA31E08* and *DXS1193*) gave consistently negative lod scores due to recombinations in affected individuals.The critical region that is most likely to contain the gene is between markers *DXS996* and *DXS7105* and spans 19.8 cM.

DISCUSSION

In this study, we have established linkage of OFD1 to the short arm of the X chromosome. This result is consistent with OFD1 being an X-linked dominant disorder with lethality in hemizygous males. Linkage studies of other families with OFD1 are now required to test whether this syndrome is genetically homogeneous and to narrow down the critical region. The OFD1 critical region defined by our linkage study is a 19.8 cM interval (14).

This interval, between *DXS996* and *DXS7105*, is shared by affected individuals in family 1. This is the only region on the X chromosome shared by affected individuals in this family. Affected individuals in family 2 also share this region, but the limits are wider than those defined by family 1. Individual II-6 in family 1 would define the telomeric limit of this region with a recombination at marker *DXS7108*. This female superficially has none of the features of her sisters, II-2 and II-4, but has a history of miscarriage and has three normal sons all of whom have inherited the unaffected haplotype from individual I-1 for this region. These findings and the highly variable phenotypic expression of OFD1 in females noted both in our kindreds and in others in the literature (13) suggest that it is not possible to be certain about the unaffected status of females. The two-point analysis was therefore repeated using unaffected males and affected females only. There is also a possible crossover within the critical region in individual II-5 in family 2. However, as this individual is 'unaffected' and the haplotypes in her affected mother have to be inferred, the data cannot be reliably used to narrow down the candidate region.

The prior evidence that the gene might be X-linked (a lack of unaffected males and a 2:1 ratio in the offspring of affected females), combined with the significant lod scores obtained in this study, makes it highly likely that the gene is localized to distal Xp. This region contains genes already implicated in known diseases such as steroid sulphatase deficiency and amelogenesis imperfecta (14).The biology of these gene products, together with the phenotypes of established human mutations, must make them extremely unlikely candidates for the *OFD1* gene.

KAL, a gene mutated in X-linked Kallmann's syndrome, is also located in this region, and encodes a secreted molecule thought to be important in signalling between cells (15). Affected males have infertility due to hypogonadotrophic hypogonadism, anosmia due to failure of development of the olfactory bulbs and variable renal agenesis. *KAL* mutations in this syndrome are mainly predicted to cause loss of function (16). The external features of Kallmann's syndrome are clearly different from those of OFD1. However, the expression patterns of KAL in human and chick development, i.e. the kidney, the limb bud and central nervous system, would be consistent with the clinical features of

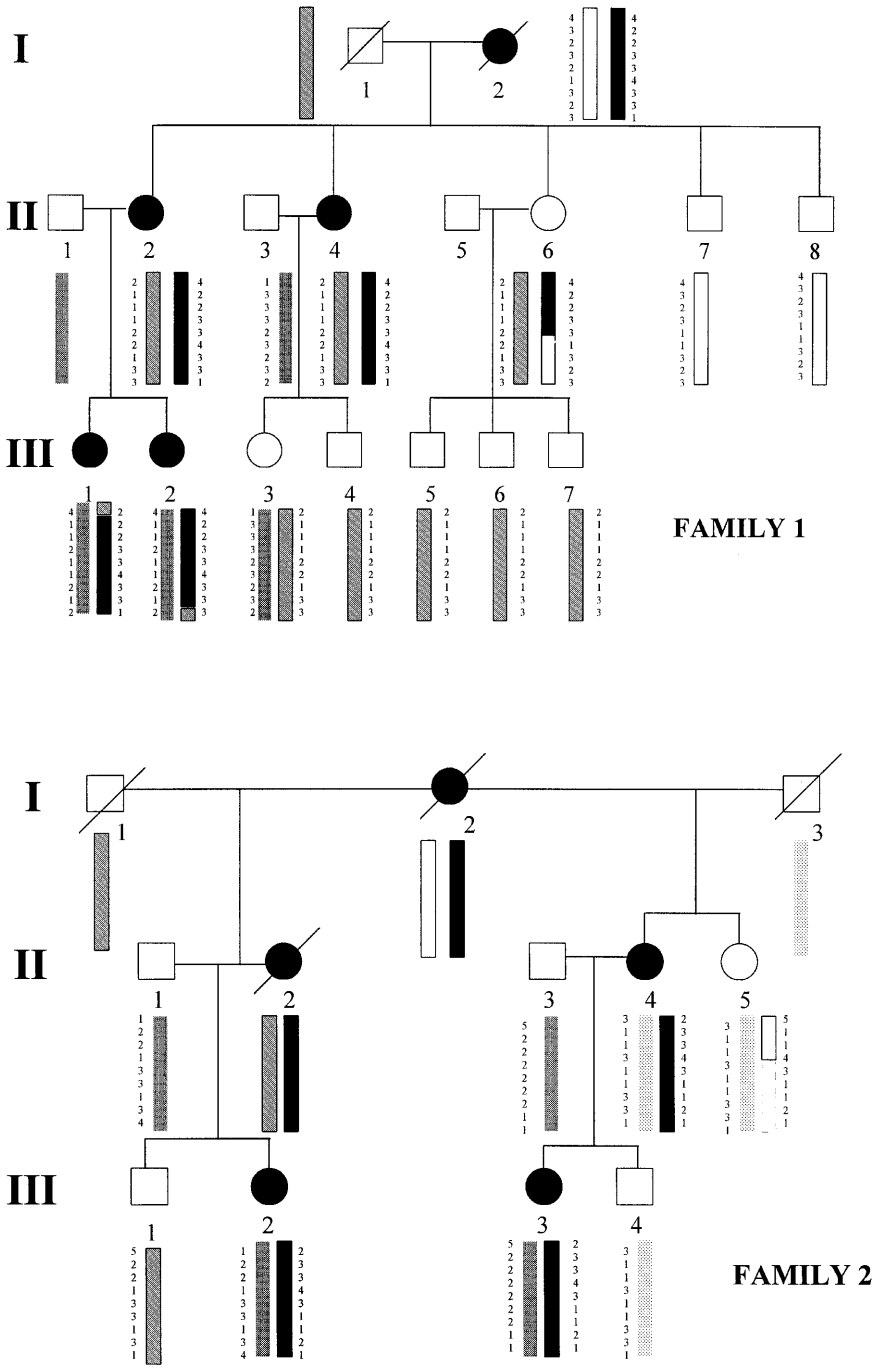

Figure 1. Family trees used in the analysis. The shaded circles represent affected individuals. The order of markers displayed is *DXS996*, *KAL*, *DXS1223*, *DXS8051*, *DXS7108*, *DXS8022*, *DXS987*, *DXS8036* and *DXS7105*. The bars indicate the most likely haplotypes. The affected haplotype is indicated by the solid black bars and the unaffected haplotype by the solid white bars. In family 1, the haplotype of I-1 is indicated by the diagonal shaded bars. In family 2, the haplotype of I-1 is indicated by diagonal shaded bars and I-3 by light grey bars. In family 2, the haplotype of II-5, which could either be affected or unaffected, is indicated by a dotted outline. In both families, additional male haplotypes that have been introduced are indicated by the dark grey bars.

OFD1 (17,18). It is conceivable that different mutations in *KAL*, causing, for example, a gain in function, might generate an OFD1 phenotype.

Genes in the critical region which are not yet implicated in human genetic diseases are the voltage-gated chloride channel 4 (*CLCN4*) (19) and *Xenopus laevis*-like apical protein (*APXL*) (20). We suggest that both of these are candidates for the OFD1 gene. CLCN4 is a voltage-gated chloride channel which is highly conserved in evolution and which is known to be expressed in skeletal muscle, brain and heart (19) and, of note, mutations in another chloride channel gene *CLCN5* cause renal diseases; X-linked recessive nephrolithiasis and hypophosphataemic rickets (21). APXL, *Xenopus laevis*-like apical protein, is implicated in amiloride-sensitive sodium channel activity (20),

which is of interest as the protein encoded by the *PKD2* gene, a gene mutated in autosomal dominant polycystic kidney disease, has homology on database searching with voltage-gated sodium and calcium channels (22). APXL is expressed in melanoma cells, brain, placenta, lung, kidney and pancreas. The *APXL* gene has been excluded as a candidate for optic atrophy which maps to this region (20). Further studies are underway to examine these and other genes for mutations in OFD1 cases.

The mouse mutant with X-linked dominant polydactyly *(Xpl)* maps to the region of the mouse X chromosome that is homologous with the region defined in this study for OFD1 (23). Urogenital malformations are known to occur in this mouse, and include hydroureter, hydronephrosis and cystic or absent kidneys (23). The renal cysts in the *Xpl* mouse deserve further examination to determine whether they are glomerulocystic, the polycystic renal disease associated with OFD1 (24), as this mutant should be considered to be a good candidate for the mouse model of OFD1.

MATERIALS AND METHODS

Family descriptions

Two families with OFD1 were used for linkage analysis. Family 1 (Fig. 1) consisted of five affected females (I-2, II-2, II-4, III-1 and III-2) all with classical features of OFD1 and also polycystic kidney disease as assessed by ultrasound scanning. There was a strong family history of miscarriage. Two cases required treatment with dialysis (I-2 and III-2). The full clinical details, radiology and renal histology of this kindred are described elsewhere (6). I-2 died of a myocardial infarction after renal transplantion, and samples of kidney tissue taken at autopsy were used as the source of DNA (see DNA studies, below). The other relatives were clinically unaffected; they had no dysmorphic features, and renal ultrasound scans in individuals II-6, II-7, II-8 and III-3 were normal. Individual II-6 (kindred 1) has had a miscarriage. Family 2 (Fig. 1) examined in this study has been described previously (5) and contains five affected females, three of whom were alive.

DNA studies

DNA was extracted from peripheral blood lymphocytes by standard techniques and from the pathological specimen by first dissolving the paraffin wax with xylene (Merck Ltd, Poole, UK) (25). DNA was amplified using the polymerase chain reaction (PCR) using primers flanking microsatellite polymorphisms. The first set of microsatellite markers which were used spanned the X chromosome at an average spacing of 10–20 cM (Research Genetics, Huntsville, USA). Additional markers for finer mapping were selected from the Genethon map (www.genethon.fr) (26). The PCR reaction mix consisted of 250 ng of genomic DNA, 50 pmol of each primer, 1.5 M Tris (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl, 0.2 mM dGTP, dATP, dTTP and 0.02 mM dCTP, 1 µl [^{32}P]dCTP (3000 Ci/mmol)/ml of reaction mix and 1 U *Taq* polymerase [Bioline (UK) Ltd, London, UK] in a final volume of 25 µl. Conditions for thermal cycling consisted of denaturation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final extension step of 72°C for 10 min. The products were separated electrophoretically on 6% acrylamide–7 M urea denaturing gels at 65 W for 2–3 h. Dried gels were exposed to X-ray film (X-Omat, Kodak, Rochester, USA) for 12–24 h.

Linkage studies

Genotypes were recorded manually. A gene frequency of 1 in 500 000 was used with an X-linked dominant mode of inheritance. The disease was assumed to be 100% penetrant in males in view of the lethality in hemizygous males reported in the literature and the incidence of miscarriage in the kindreds used in this study. As stated in the Results, the analysis was performed with and without 'unaffected females' in view of the highly variable phenotype (13). Marker allele frequencies were obtained from the Genome Database (www.gdb.org). The genetic distances between markers were obtained from the Genethon map. Two-point and multipoint linkage analysis were performed using the MLINK and LINKMAP programme of the LINKAGE package (27).

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