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Oculo-Facio-Cardio-Dental Syndrome: Skewed X Chromosome Inactivation in Mother and Daughter Suggest X-linked Dominant Inheritance

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Oculo-facio-cardio-dental syndrome (OFCD) is an uncommon multiple congenital anomaly syndrome that is characterized by congenital cataracts, multiple minor facial dysmorphic features, congenital heart defects, and dental anomalies including canine radiculomegaly and oligodontia. Although most cases of OFCD are sporadic, since all reported OFCD individuals have been female, it has been suggested that OFCD is an X-linked dominant trait. Here we report two affected female patients with OFCD, a mother and daughter, who both had congenital cataracts, microphthalmia, characteristic dental anomalies, and typical facial dysmorphisms. These features were diagnostic for OFCD; thus, these cases represent the second documented instance of mother-to-daughter OFCD transmission. In addition to the clinical features typically seen in OFCD individuals, the affected daughter exhibited several additional congenital anomalies including intestinal malrotation and hypoplastic thumbs. Thus, these cases further define and expand the OFCD clinical phenotype. These two individuals also displayed a skewed pattern of X chromosome inactivation. Together, these data strongly support the hypothesis that OFCD is inherited as an X-linked dominant condition.

KEY WORDS: cataracts; dental anomalies; X chromosome; X chromosome inactivation

INTRODUCTION

Oculo-facio-cardio-dental syndrome (OFCD, OMIM no. 300166) is an uncommon syndrome with multisystemic involvement [Marashi and Gorlin, 1990; Wilkie et al., 1993; Schulze et al., 1999]. Congenital cataracts, microphthalmia, and secondary glaucoma are the most frequent ocular abnormalities. Unrelated affected patients share similar facial dysmorphic features including a long and narrow face with a high nasal bridge, separated nasal cartilages, and a pointed nasal tip. Cleft palate has also been observed in affected patients. Structural cardiac anomalies typically include atrial and/or ventricular septal defects, and mitral valve prolapse. Dental anomalies are conspicuous and were first described by Hayward [1980] before the more extensive nature of this condition was more fully elucidated. Most affected patients have hallmark dental anomalies including radiculomegaly with prolonged dental roots and widely open apices, most typically in the canine roots [Marashi and Gorlin, 1990; Wilkie et al., 1993; Schulze et al., 1999]. Additional dental anomalies include delayed eruption of both primary and permanent teeth, persistence of decidual teeth, and oligodontia. Although, in isolation, these features may be seen in a variety of other conditions, in combination these anomalies define the OFCD disease phenotype.

Twelve female patients with an unequivocal diagnosis of OFCD have been reported [Schulze et al., 1999]. Previously, based on the observation that all OFCD individuals were female, others suggested that OFCD might be an X-linked dominant trait [Gorlin et al., 1996].

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Marashi and Gorlin [1990] reported a man with mild isolated cuspid gigantism, whose sister had otherwise the typical multisystemic manifestation of OFCD; however, the absence of typical ophthalmologic and cardiac abnormalities in this male makes the diagnosis of OFCD unlikely. Only one instance of vertical transmission from mother-to-daughter has been reported [Wilkie et al., 1993].

Here we report a mother and daughter who manifest clinical features diagnostic for OFCD syndrome. To our knowledge, these patients represent the second documented instance of the vertical transmission of this condition. The daughter had additional clinical features, including hypoplastic thumbs and intestinal malrotation, that were not previously described in OFCD patients; thus, these observations further expand the phenotypic spectrum of this condition. Furthermore, DNA methylation analyses showed that both the mother and daughter displayed a skewed pattern of X chromosome inactivation. Together, these data strongly support the hypothesis that OFCD is an X-linked dominant disease.

MATERIALS AND METHODS

Human Subjects

Two female subjects with OFCD, Patient 1 and Patient 2, underwent a complete medical genetics evaluation. Follow-up clinical data for a 2 year period were available for both individuals. All available medical and dental records were reviewed. For these individuals, informed consents were obtained in accordance with the Institutional Review Board (IRB) Human Subjects Research Committee, University of Michigan Medical System.

Molecular Genetic Analyses

DNA was extracted from peripheral blood lymphocytes as previously described [Allen et al., 1992]. To assess X chromosome methylation status and infer patterns of X chromosome inactivation, 150 ng of DNA was digested to completion overnight with either HpaII or RsaI restriction endonuclease, and digested DNA was amplified by polymerase chain reaction (PCR) as previously described [Allen et al., 1992]. HUMARA locus primers (primer pair: 5′-GCT GTG AAG GTT GCT GTT CCT CAT-3′ and 5′-TCC AGA ATC TGT TCC AGA GCG TGC-3′) were used to amplify DNA fragments. PCR products were electrophoresed on 7% polyacrylamide gels; PCR amplified alleles were scored from gels after visualization using silver stain.

RESULTS

Clinical Reports

Patient 1. The patient was born full term to a 25-year-old primigravida woman by primary cesarean section after an unremarkable pregnancy; her birth weight was 3,954 g (95th centile), length was 51 cm (50th centile), and head circumference was 35 cm (50th centile). Dense bilateral cataracts and severe microphthalmia with right-sided posterior embryotoxon were noted at birth as were a linear cleft of the soft palate and bilateral hypoplastic thumbs. An echocardiogram showed a large ostium secundum atrial septal defect (ASD), a small patent ductus arteriosus (PDA), and a thickened pulmonary valve. Bilateral cataract surgery was complicated by repeated right-sided retinal bleeding requiring additional laser surgeries. Family history showed that the patient's mother had a similar constellation of medical problems (see Patient 2). The patient's maternal aunt and maternal grandmother did not have the history of dental, ocular, or cardiac abnormalities. At 5 ½ months, the patient was hospitalized for failure to thrive with an admission weight below the 5th centile. Gastroenterological radiographic evaluations demonstrated partial intestinal malrotation with intermittent volvulus and gastroesophageal reflux; laparoscopic surgery showed multiple peritoneal bands. Cystoscopic evaluation demonstrated bilateral grade II vesicouretheral reflux. Laboratory evaluation demonstrated a normal female 46,XX karyotype at 550 band resolution; fluorescent in situ hybridization (FISH) analysis for a deletion of chromosome 22q11.2 was normal. Head MRI analyses and a plasma 7-dehydrocholesterol level were also normal.

Examination at the age of 8 months showed normal growth parameters (90th centile for head circumference, 80th centile for length, and 30th centile for weight). Facial dysmorphic features included right-sided microphthalmia, a long narrow face, high nasal bridge, and a bulbous nasal tip with separated nasal tip cartilages. Ears were cupped with thickened helixes. A midline cleft soft palate was present; primary teeth were absent. Examination showed long fingers and toes, hypoplastic thumbs, prominent fifth digit clinodactyly, and cutaneous 2/3 toe syndactyly. Neurologic examination was notable for an inability to sit independently. A repeat examination at 22 months (Fig. 1) was essentially unchanged with the exception of the eruption of two upper incisors at 18 months and repair of the cleft palate. A review of psychomotor development showed that she achieved independent sitting at 10 months and walked at 21 months. She spoke only a few words at 22 months.

Patient 2. The patient is a 26-year-old woman and the mother of Patient 1. Patient 2 had congenital cataracts requiring bilateral surgery with removal of cataracts at the age of 6 and 12 months, respectively. She subsequently developed secondary right-sided glaucoma that resulted in profound visual loss at the age of 16 years. She had a longstanding heart murmur, but had not been diagnosed with a structural heart defect. She reported normal psychosocial and intellectual development, and graduated from a high school. She did not have a history of pregnancy losses. Review of her dental records showed that she had delayed eruption of her teeth at 16 months. As shown in Figure 2A, she had delayed shedding of her primary teeth at 14 years. Primary teeth were extracted between 11 years and 14 years to facilitate eruption of the secondary teeth. As shown in Figure 2B, at the age of 19 years, her permanent teeth demonstrated canine radiculomegaly in

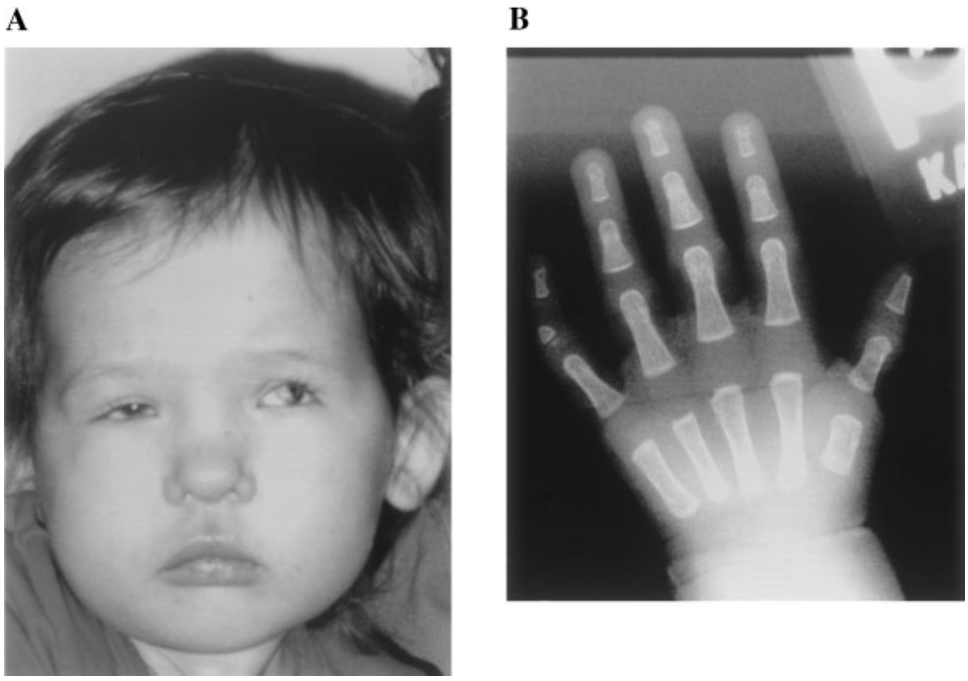

Fig. 1. Clinical features of Patient 1. **A**: Photograph of Patient 1 at 22 months demonstrates right-sided microphthalmia, enlargement of the left eye secondary to glaucoma, long narrow facies, and a wide nasal tip. **B**: Radiograph of the right hand of Patient 1 at 6 months shows hypoplasia of the first metacarpal and thumb, and marked fifth digit clinodactyly.

all four quadrants with markedly open apices. Increased root length variability was also observed.

Examination at the age of 26 years revealed normal height, weight, and head circumference, corresponding to the 50th centile for an adult woman. Visual acuity was 20/100 on the left-side and light perception on the right-side. As shown in Figure 3, physical features included a long face, prominent supraorbital ridges, deeply set eyes, flattening of the zygomatic region, a high nasal root, and a broad nasal tip with the separation of nasal cartilages. Grade II malocclusion with a deep overbite, a relatively short chin, and a highly arched palate were also observed. She had a thickened maxillary frenulum with a 4-mm diastema between the upper incisors. She had long fingers and toes, normal thumbs, and cutaneous 2/3 toe syndactyly. Although, she had an audible systolic cardiac murmur, she declined further medical evaluation.

X Chromosome Inactivation Analyses

DNA methylation studies were performed to determine whether OFCD individuals had a skewed pattern of X chromosome inactivation. For this purpose, primers for the polymorphic HUMARA gene locus were used to perform PCR amplifications with DNA digested with

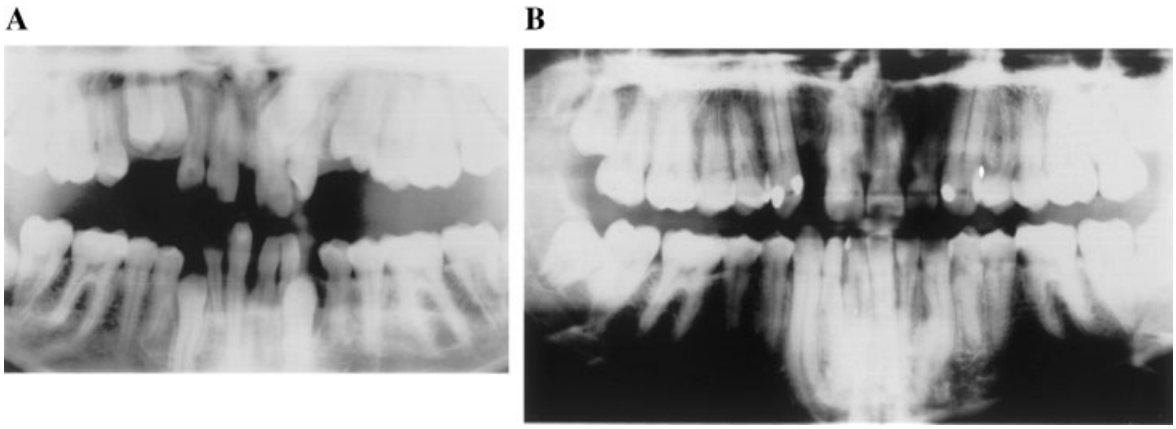

Fig. 2. Panorex radiographs of Patient 2 at the age of 14 (**A**) and 19 years (**B**), respectively. A: Panorex study demonstrating delayed shedding of primary teeth at 14 years. B: Panorex study showing canine radiculomegaly in all four quadrants. Markedly open root apices and increased root length variability are also illustrated.

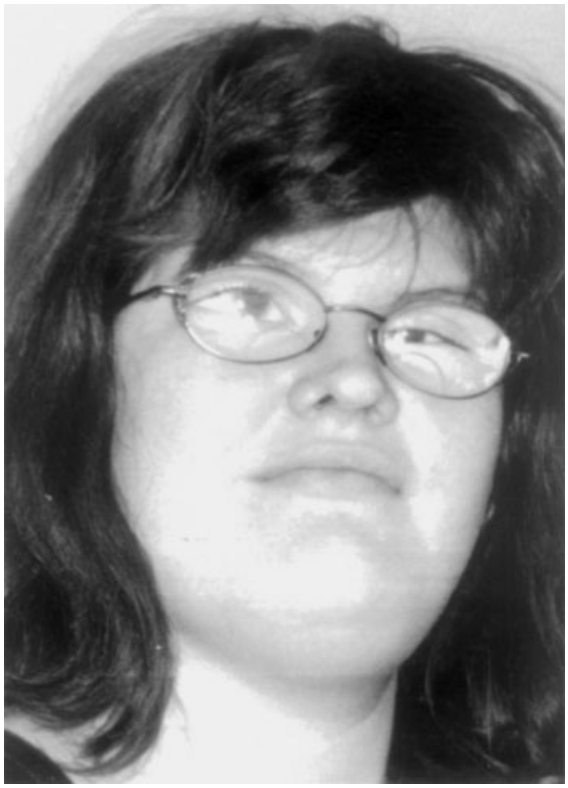

Fig. 3. Clinical features of Patient 2. Photograph of Patient 2 at the age of 26 years demonstrates deeply set eyes, a long face, and a broad nasal tip. As a consequence of glaucoma, the globe of the right eye is larger than the left.

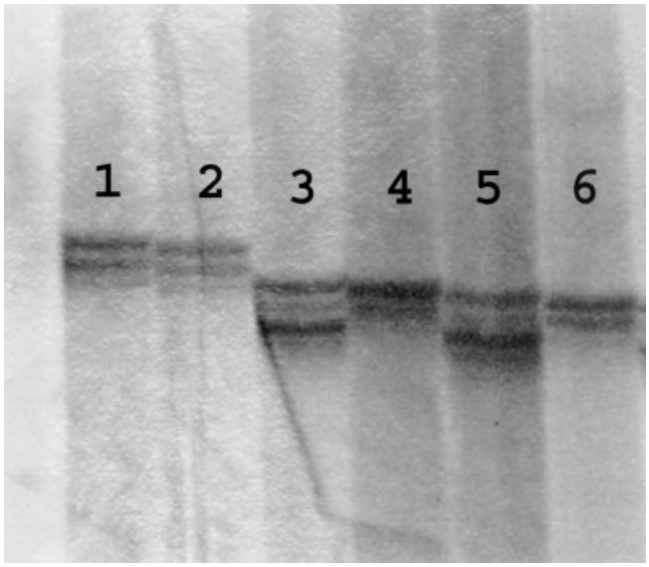

Fig. 4. Methylation PCR analyses indicate that OFCD individuals exhibit a skewed pattern of X chromosome inactivation. Photograph of a silver-stained gel shows PCR products amplified from DNA digested with either RsaI (**lanes 1**, **3**, and **5**) or HpaII (**lanes 2**, **4**, and **6**) restriction endonucleases. DNA was derived from either an unaffected female (lanes 1 and 2); or OFCD individuals, Patient 1 (lanes 3 and 4), and Patient 2 (lanes 5 and 6). Restriction digests and PCR amplifications were performed as described in "Materials and Methods."

specific restriction endonucleases (see "Materials and Methods"). Unfortunately, the father of Patient 1 was not available for analysis. In most females, X chromosome inactivation is random and polymorphic X chromosomal alleles are represented equally well in DNA digested with restriction enzymes that are sensitive or insensitive to DNA methylation [Allen et al., 1992]. For example, using control female DNA and HUMARA-specific primers, a similar pattern of PCR products was obtained from DNA digested with restriction enzymes that are either sensitive (HpaII) or insensitive (RsaI) to DNA methylation (Fig. 4; compare lanes 1 and 2). In contrast to a random pattern of X chromosome methylation (and X chromosome inactivation), both OFCD subjects demonstrated a markedly different pattern. As shown in Figure 4, when digested with RsaI, an enzyme insensitive to DNA methylation, DNA derived from OFCD patients yielded two major PCR products for both Patient 1-(lane 3) and Patient 2-derived (lane 5) DNA. These data showed that, like the control female, Patient 1 and Patient 2 were polymorphic for the HUMARA locus. However, DNA derived from OFCD individuals showed a sensitivity to DNA methylation status. In marked contrast to RsaI digested OFCD-derived DNA, HpaII digested DNA derived from Patient 1 and Patient 2 yielded only one major HUMARA PCR product (Fig. 4; compare lanes 3 and 4, and lanes 5 and 6, respectively). These data indicated that the DNA of both Patient 1 and Patient 2 contained one HUMARA allele that was selectively sensitive to DNA methylation. Thus, these data showed that both OFCD individuals displayed a skewed pattern of X chromosome methylation and a non-random pattern of X inactivation.

DISCUSSION

We report two individuals, a daughter (Patient 1) and her mother (Patient 2), who have clinical features diagnostic for OFCD syndrome. As shown in Table I, the constellation of congenital anomalies and clinical features demonstrated by these patients are strikingly similar to those described in other OFCD individuals. Mother and daughter both had congenital cataracts, a cardinal feature of OFCD [Marashi and Gorlin, 1990; Wilkie et al., 1993; Schulze et al., 1999]; both also had secondary glaucoma, and Patient 1 had microphthalmia and posterior embryotoxon. Both individuals had minor facial dysmorphic features characteristically seen in OFCD (Figs. 1 and 3); these features included a relatively long and narrow face, deeply set eyes, a prominent nasal bridge, and a broadened nasal tip with separated nasal cartilages. In addition, Patient 1 had a cleft soft palate and her mother had a highly arched palate. Like other OFCD individuals, Patient 2 had cardinal OFCD dental anomalies including dental radiculomegaly, variable root length with widely opened apices, persistent primary teeth, and delayed dental eruption (Fig. 2A,B). Although Patient 1 was too young to manifest many of the characteristic dental anomalies, she had delayed dental eruption and apparent hypodontia. In addition, she had an ASD, a PDA, and a dysplastic pulmonary

TABLE I. Summary of Clinical Findings in OFCD Syndrome Patients

	OFCD patients[a]													
Cardinal clinical features	Pt1	Pt2	A	B	C	D	E	F	G	H	I	J	K	L
Ophthalmologic features														
Congenital cataracts	+	+	+	+	+	+	−	−	+	+	+	+	+	+
Microphthalmia	+	−	+	+	−	−	−	−	+	+	−	+	+	+
Facial features														
Narrow face	+	+	+	−	−	+	−	−	+	+	+	−	+	+
High nasal bridge	+	+	+	+	−	−	−	−	+	+	−	+	+	+
Separated nasal cartilages	+	+	+	+	−	+	−	−	+	+	+	+	+	+
Palatal cleft	+	−	−	−	−	+	−	−	−	+	+	+	+	+
Cardiac features														
Septal heart defect	+	ND	+	+	+	−	−	−	+	+	−	+	−	+
Dental features														
Radiculomegaly	ND	+	+	−	+	+	+	+	+	+	+	+	+	+
Delayed eruption	+	+	+	+	+	+	−	−	+	+	−	+	+	+
Malocclusion	ND	+	+	+	+	+	−	+	+	+	+	+	+	+
Less common features														
Developmental delay	+	−	−	+	−	−	−	−	−	−	+	+	−	−
Intestinal malrotation	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Emesis/feeding problems	+	−	−	+	−	−	−	−	−	−	−	+	−	−
Musculoskeletal anomalies[b]	+[b] (1)	−	−	+[b] (2)	−	−	−	+[b] (2)	+[b] (3–5)	+[b] (5, 6)	−	−	−	+[b] (5, 7)

A plus (+) or minus (−) sign denotes the presence or absence of a particular physical feature, respectively; "ND" denotes that the presence or absence of a feature was not determined.

[a]A and B refer to the mother and daughter reported by Wilkie et al. [1993], respectively. C refers to the case reported by Hayward [1980]; D and E refer to the cases reported by Marashi and Gorlin [1990]; F refers to the case reported by Wilkie and Chambers [1990]; G and H refer to the cases reported by Aalfs et al. [1996]; I refers to the case reported by Obwegeser and Gorlin [1997]; and J, K, and L refer to the cases reported by Schulze et al. [1999].

[b]Individuals with musculoskeletal anomalies are indicated by the presence of a plus (+) sign; the number below the plus sign indicates the precise anomaly reported including: (1) Hypoplastic thumbs, (2) short stature, (3) short metacarpals, (4) cervical spine anomalies, (5) limb length asymmetry, (6) rib hypoplasia, and (7) radioulnar synostosis.

valve, congenital heart defects typically observed in OFCD [Schulze et al., 1999]; Patient 2 had a heart murmur but declined definitive diagnostic studies. These are cardinal features of OFCD seen in every subject with this diagnosis (Table I); their presence in both affected individuals unequivocally establishes the diagnosis of OFCD in both patients.

In addition to cardinal findings for OFCD, Patient 1 also had two novel clinical findings, hypoplastic thumbs and intestinal malrotation, heretofore not observed in OFCD patients (Table I). Seemingly novel diagnostic features have been reported previously in OFCD patients; in comparing previously reported cases, Schulze et al. [1999] noted that several OFCD patients had apparently novel diagnostic features including radioulnar synostosis [Schulze et al., 1999], short metacarpals [Aalfs et al., 1996], cervical spine anomalies [Aalfs et al., 1996], rib hypoplasia [Aalfs et al., 1996], and limb length asymmetry [Aalfs et al., 1996, Schulze et al., 1999]. To our knowledge, hypoplastic thumbs have not been observed previously in OFCD patients. Based on the observed frequency of these skeletal anomalies, it is reasonable to hypothesize that additional skeletal anomalies might comprise the complete OFCD disease phenotype.

Patient 1 also experienced a psychomotor delay, while her mother (Patient 2) had normal development and intelligence. Similarly, Wilkie et al. [1993] reported a mother and daughter diagnosed with OFCD, and the daughter had mild mental retardation not present in her mother. Even though this may suggest genetic anticipation and a more severe and complex phenotype in successive generations, two unrelated OFCD cases with mental retardation born to clinically unaffected mothers have also been described [Obwegeser and Gorlin, 1997; Schulze et al., 1999]. Patient 1 also had feeding difficulties with failure to thrive, and documented intestinal malrotation. Feeding difficulties with frequent vomiting were also noted in a case reported by Schulze et al. [1999] and in another case reported by Wilkie et al. [1993]. It is unclear whether those two patients had similar structural gastrointestinal changes. However, in light of these findings, it seems reasonable to recommend that OFCD patients with feeding difficulties be evaluated for gastointestinal anomalies to exclude intestinal malrotation and/or other gastrointestinal malformations.

Our data also show that, in the described OFCD kindred, two OFCD individuals demonstrate a skewed pattern of X chromosome inactivation. Similar patterns of non-random lyonization have been documented in other X-linked diseases including incontinentia pigmenti (IP; OMIM no. 308300) and focal dermal hypoplasia (DHOF; OMIM no. 305600), X-linked dominant conditions with male hemizygote lethality [Wieacker et al., 1985; Gorski, 1991; Parrish et al., 1996]. Although there has not been a reported increase of fetal loss among OFCD women, the absence of any credible male OFCD cases suggests that, like IP and DHOF, OFCD is an X-linked dominant and male hemizygote lethal disorder. There is no indication of male spontaneous miscarriages in this family or in other families reported thus far. In OFCD, it is possible that male lethality might occur very early in embryogenesis before the pregnancy is recognized. Since the disease phenotype affects several different systems, it is reasonable to hypothesize that the gene causing OFCD plays multiple critical roles during embryonic development.

In summary, we report a second instance of a vertical transmission of OFCD from a mother-to-daughter. In addition to the clinical features typically seen in OFCD individuals, the affected daughter exhibited several additional congenital anomalies including intestinal malrotation and hypoplastic thumbs. Thus, these cases further define and expand the OFCD clinical phenotype. Furthermore, molecular genetic studies demonstrate that both OFCD individuals display a non-random pattern of X chromosome methylation and a skewed pattern of X chromosome inactivation. Taken together, these data strongly support the hypothesis that OFCD is an X-linked dominant disease.

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