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ORIGINAL ARTICLE

A novel mutation in the *OFD1* (*Cxorf5*) gene may contribute to oral phenotype in patients with oral-facial-digital syndrome type 1

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BACKGROUND: Oral-facial-digital syndrome (OFDS) type 1 (OFD1) is an X-linked dominant condition associated with embryonic male lethality. It almost always affects the oral cavity, face, and digits. It is considered to be a ciliopathy caused by mutations in the *OFD1* gene. A variety of mutations have been described, and a genotype–phenotype correlation has been suggested.
OBJECTIVE AND METHODS: The proband was an 8-year-old Spanish girl with suspected OFD1. We extended the pedigree to three proband's generations, performing a thorough physical examination and screening for *OFD1* mutations in nine individuals.
RESULTS: The proband, her mother, and her sister showed oral findings consistent with OFD1. Ultrasound evaluation revealed the existence of renal cysts only in the proband's mother. The rest of the family (all male) had no relevant morphological abnormalities. A single-base deletion in exon 16 of *OFD1* (c.2183delG) leading to a frameshift was detected in the proband, her mother, and her sister.
CONCLUSION: Because all three women had a similar oral phenotype, this new mutation might be involved in the development of the OFD1 oral manifestations. In cases of OFDS, physical examination (including the oral cavity and renal function) and genetic screening of the probands and their relatives are mandatory.
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Keywords: oral-facial-digital syndrome; cleft palate; aberrant frenula; lobulated tongue; molecular screening; mutation

Introduction

The oral-facial-digital syndrome (OFDS) was first described by Mohr in 1941, when he reported a family with highly arched palate, lobate tongue with papilliform outgrowths, broad nasal root, hypertelorism, and digital anomalies. It is possible, however, that this condition was described previously, and in fact, a report of apparent Mohr syndrome can be found 100 years earlier in the literature as 'Monstrorum humanum hexadactylum' (J.B. Beckwith, Personal communication). OFDS shows a striking phenotypic heterogeneity. To date, 13 types of OFDS have been described based on clinical manifestations and inheritance patterns (Gurrieri *et al*, 2007).

Oral-facial-digital syndrome type 1 (OFD1; OFDI; OMIM 311200) was described by Papillon-Leage and Psaume in 1954. It is inherited as an X-linked dominant trait with embryonic male lethality (Wettke-Schäfer and Kantner, 1983). Type 1 accounts for the majority of OFDS cases, and its estimated frequency is approximately one case per 50 000–250 000 live births (Wahrman *et al*, 1966). It has been suggested that OFDS reflects the pleiotropic effects of a morphogenetic impairment that almost invariably affects the oral cavity, face, and digits (Gurrieri *et al*, 2007). The main oral manifestations in OFD1 include pseudo-cleft of the upper lip, cleft palate, hyperplastic frenula, aberrant frenula, lobulated or cleft tongue (bifid or trifid), lingual hamartomas, ankyloglossia, hypodontia, supernumerary teeth, and hypoplastic mandible (Lauterstein and Pruzansky, 1969; Melnick and Shields, 1975; Thauvin-Robinet *et al*, 2006; Hennekam *et al*, 2010a). The facies is remarkably characteristic, with frontal bossing, hypertelorism, dystopia cantorum, telecanthus, hypoplasia of alar cartilages, broad nasal root, low-set ears, flattened midfacial region, and microretrognathia (Macca and Franco, 2009; Hennekam *et al*, 2010a). Digital findings include brachydactyly, syndactyly, clinodactyly, polydactyly, and irregular bone mineralization (Al-Qattan and Hassanain, 1997; Hennekam *et al*, 2010b). Bilateral polycystic kidney disease is common in OFD1, although most cases are detected in adulthood (Scolari *et al*, 1997). Central nervous system malformations include agenesis of the corpus

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callosum, arachnoid cysts, cerebellar abnormalities, hydrocephalus, and porencephaly. As many as 50% of cases have mild intellectual disability (Holub *et al*, 2005; Gurrieri *et al*, 2007).

Oral-facial-digital type 1 is caused by mutations in the *Cxorf5* transcript, later named *OFD1* (MIM# 300170) (Ferrante *et al*, 2001), which comprises 23 exons encoding a 1011 amino acid protein. The gene encodes a centrosomal protein found in the primary cilia (Romio *et al*, 2003), and consequently, OFD1 has been considered a ciliopathy (Badano *et al*, 2006). It is widely expressed in metanephros, brain, tongue, and limb (Romio *et al*, 2003), which could explain the clinical expression of the syndrome. A variety of mutations have been described, and some genotype–phenotype correlations have been suggested (Thauvin-Robinet *et al*, 2006; Prattichizzo *et al*, 2008; Macca and Franco, 2009).

Diagnosis in a proband raises the level of suspicion for the proband's relatives. The objective of this study was to perform a thorough physical examination and screening for OFD1 mutations in a three-generation Spanish family with OFDS type 1, who had heterogeneous phenotypic findings.

Materials and methods

Pedigree construction

The proband was an 8-year-old girl who was referred by her pediatrician to the Special Needs Dentistry Unit at the University of Santiago de Compostela, Spain, with a diagnosis of multiple caries and abnormal dental eruption. She had a history of cleft palate, lobulated tongue, and syndactyly that had all been surgically corrected, along with agenesis of the corpus callosum and mild mental retardation. Physical examination also revealed hypertelorism, broad nasal root, sequelae arising from surgical closure of the cleft palate and bifid tongue, numerous thick fibrous bands in the lower mucobuccal fold (aberrant frenula), supernumerary maxillary deciduous canines, and absence of lower lateral incisors (Figure 1). As OFD1 was suspected, we extended the pedigree to three generations, including nine individuals (Figure 2): three affected, four unaffected, and two deceased. All participants or their legal guardians (cases II6 and III1) provided informed consent to inclusion in the study, which was approved by the Ethics Committee of the University of Santiago de Compostela.

Mutation analysis

Genomic DNA was extracted from buccal epithelial cells using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The 23 exons of the OFD1 gene were amplified as described previously (Ferrante *et al*, 2001). PCR products were separated on polyacrylamide gels (T-9%, C-5%). After confirming the success of the amplification, the PCR product was purified with a Multiscreen $PCR_{\mu}96$ Filter Plate (Millipore Corporation, Billerica, MA, USA). Sequencing reactions were carried out on both strands of the PCR product using the ABI Prism BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA), and the products

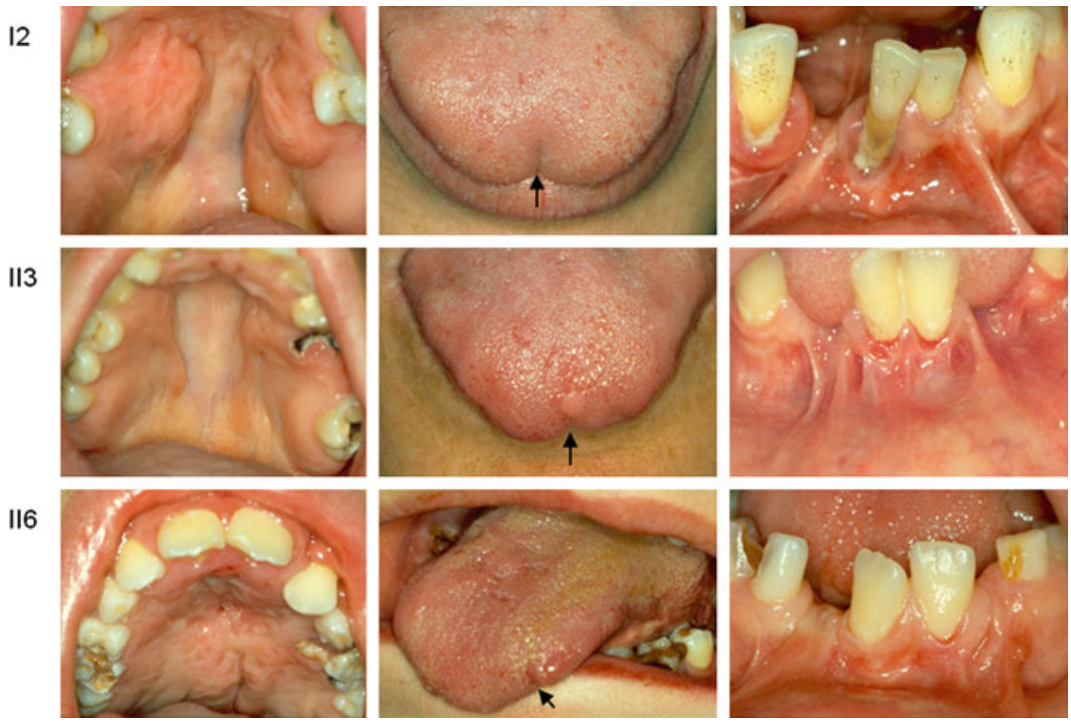

Figure 1 Oral findings. I2 (proband's mother, 43 years old) and II3 (proband's sister, 23 years old) had a pseudocleft palate, bifid tongue, aberrant frenula, and absence of the lower lateral incisors. II6 (proband, 8 years old) had a cleft palate and lobulated tongue that had been surgically corrected, along with aberrant frenula and absence of the lower lateral incisors

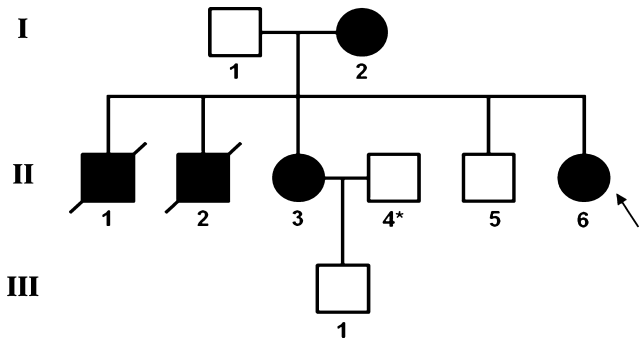

Figure 2 Family pedigree. Peripheral blood samples were not available for deceased members and those marked with an asterisk. The proband is indicated with an arrow. □ Unaffected male, ● Affected female, ■ Deceased affected male

were purified with the Montage®SEQ96 Sequencing Reaction Cleanup kit (Millipore). Electrophoresis was performed on an ABI PRISM 3730xl™ Genetic Analyzer (Applied Biosystems). SeqScape v.2.5 (Applied Biosystems) software was set up to automatically detect the presence of mutations by comparison with a reference sequence (GeneBank NM_003611), and in addition, all electropherograms were visually inspected. Finally, sequence variant descriptions were analyzed with the Mutalyzer 2.0 *β*-4 program (http://www.LOVD.nl/mutalyzer) (Wildeman *et al*, 2008), and the software was used to predict the affected protein from the variant coding region.

Results

Clinical diagnosis

The proband (II6) had morphological characteristics consistent with OFD1 (Thauvin-Robinet *et al*, 2006; Gurrieri *et al*, 2007). Orofacial examination of the proband's mother (I2) revealed pseudocleft palate, bifid tongue, aberrant frenula, and absence of the lower lateral incisors (Figure 1), as well as one nostril smaller than the other; she had a history of two spontaneous male miscarriages with severe malformations (II1 and II2) before conceiving the proband (21 and 17 years earlier). Examination of the proband's sister (II3) revealed a slight pseudocleft palate and bifid tongue, aberrant frenula, and absence of the lower lateral incisors (Figure 1). Ultrasound evaluation of the kidneys in the three affected women (I2, II3 and II6) revealed the existence of small, bilateral renal cysts only in the proband's mother (I2). The rest of the family (all male) had no relevant morphological abnormalities.

Mutation analysis

A single-base deletion (c.2183delG) in exon 16 of the OFD1 gene leading to a frameshift was identified. Mutation nomenclature numbering is based on the GenBank accession number_NM003611, with +1 as the A of the ATG initiation codon. The variant c.2183delG was present in the proband (II6), her mother (I2), and her sister (II3) (Figure 3). The predicted protein based on analysis with Mutalyzer 2.0 *β*-4 software is

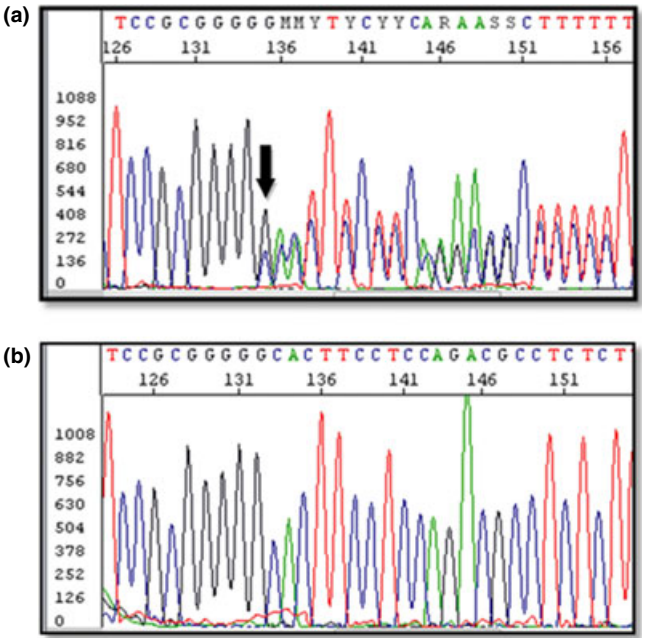

Figure 3 Sequence analysis of PCR products of exon 16. (**a**) Direct sequencing of the PCR product in an affected individual. (**b**) Wild type

p.(Gly728Alafs*89). As expected, the other members of the family did not carry the mutation.

Discussion

Clinical findings

The diagnosis of OFD1 is usually established at birth on the basis of characteristic oral, facial, and digital abnormalities (Toriello and Franco, 2010). In spite of the severe phenotypic findings observed in the proband, a suspected diagnosis OFDS had not been suggested until she was referred for oral evaluation at 8 years of age. In this case, the proband was the first affected family member. A pedigree was therefore constructed to rule out the possibility that other relatives might be undiagnosed owing to incomplete penetrance or variable expressivity.

It has been suggested that neither the oral nor the facial features of OFDS can be used to reliably distinguish X-linked from autosomal recessive variants (Siebert, 2008). Oral abnormalities including pseudocleft palate, bifid tongue, aberrant frenula, and absence of the lower lateral incisors were the most relevant findings in both the proband's sister and her mother. In a worldwide cohort of 120 individuals clinically diagnosed with OFD1, cleft palate/high-arched palate was present in 23.5%, tongue anomalies in 90.1%, aberrant frenula in 65.4%, and abnormal teeth in 42% (Prattichizzo *et al*, 2008). None of these abnormalities is specific to OFDS, and accessory frenula, for instance, may be more suggestive of Pallister–Hall syndrome (Hennekam *et al*, 2010b).

The proband's mother had renal cystic disease. Renal impairment can be present at birth but usually develops later on, occurring in 63% of patients older than

18 years (Macca and Franco, 2009). Some reports describe renal disease as completely dominating the clinical course of the syndrome (Feather *et al*, 1997), indicating that morphological assessment and biochemical monitoring of renal function are necessary in OFD1 patients (Prattichizzo *et al*, 2008). The proband's mother also had a history of two male miscarriages. Male lethality usually occurs in the first or second trimester of pregnancy (Wettke-Schäfer and Kantner, 1983), and only a few exceptional male cases of OFD1 have been reported to date (Wahrman *et al*, 1966).

Efforts to classify OFDS into distinct types have met criticism based on reported individuals or family members with different phenotypic findings. As in the present case, affected family members may not exhibit defects of all three areas: oral region, face, and digits (Siebert, 2008). Clinical variability is often seen in affected women even within the same family (Toriello, 1988), possibly because of the different degrees of somatic mosaicism (Thauvin-Robinet *et al*, 2006). Although the findings described for OFD1 overlap with those observed in other OFDS types, it has been suggested that OFD1 can be distinguished because of its X-linked dominant inheritance with male lethality and the presence of cystic kidneys (Gurrieri *et al*, 2007). Consequently, the clinical findings in the proband, the pedigree analysis, and the molecular analysis of the OFD1 gene led to a diagnosis of OFD1.

Mutation analysis

To date, 99 mutations have been described in the OFD1 gene, the majority of which have been identified in exons 3 (14%), 8 (14%), 9 (10%), 12 (6%), 13 (10%), and 16 (7%), which may represent mutational hotspots (Macca and Franco, 2009). Intriguingly, no mutations or polymorphisms were identified beyond exon 16 (Macca and Franco, 2009). The mutation in exon 16 identified in this study is the hundredth to be reported. This is a deletion mutation causing a frameshift. Previously, eight frameshift mutations and one splice site mutation have been described in exon 16 and intron 16, respectively (Macca and Franco, 2009). As with many of the other mutations that have been described, it is predicted to cause premature truncation of the protein that would probably lead to loss of function (Gurrieri *et al*, 2007; Prattichizzo *et al*, 2008).

In more than 20% of patients, bi-directional DNA sequencing of the exons and intron–exon boundaries of the OFD1 gene remains negative, but in 23% of these cases, a genomic deletion of the OFD1 transcript has been identified through a combination of quantitative multiplex PCR of short fluorescent fragments and relative quantification by real-time PCR (qPCR) (Thauvin-Robinet *et al*, 2008). However, only 85% of cases are explained by identifiable deletions and mutations, while the remaining cases are not identified using currently available techniques (Macca and Franco, 2009). Recently, Thauvin-Robinet *et al* (2009), in an effort to identify new regions containing candidate genes that could be implicated in OFDS, performed high-resolution array-CGH in a series of OFDS patients, but without success. Furthermore, genomic deletions of the OFD1 transcript have been identified in cases in which direct sequencing of the exons was negative because of the presence of the wild-type allele (Morisawa *et al*, 2004; Thauvin-Robinet *et al*, 2009).

When analyzing genotype–phenotype correlations, mental retardation was more frequently associated with mutations in exons 3, 8, 9, 13, and 16 (Thauvin-Robinet *et al*, 2006). Polycystic kidney disease appears to be correlated with splice mutations (Thauvin-Robinet *et al*, 2006), mainly located in exons 9 and 12 (Prattichizzo *et al*, 2008). High-arched/cleft palate was found most frequently with missense and splice site mutations (Prattichizzo *et al*, 2008). Cleft lip was more often associated with mutations in exon 3 (Prattichizzo *et al*, 2008). Tongue anomalies were more often detected in patients showing a mutation in exon 12, and tooth abnormalities more frequently associated with mutations in coiled-coil domains (Toriello and Franco, 2010).

Extensive phenotypic variability has been detected even among female members of the same family, probably due to X inactivation (Thauvin-Robinet *et al*, 2006; Macca and Franco, 2009). Another possible explanation for the inter- and intrafamilial variability observed in OFD1 patients could be ascribed to modifier genes (Prattichizzo *et al*, 2008). To our knowledge, in the literature, no correlation has been found between OFD1 clinical features and frameshift compared with other mutations. Moreover, mutations located in exon 16 have not been related to any specific phenotype to date. In the present paper, the three women with OFD1 carried the c.2183delG variant and had a similar oral phenotype, suggesting that the deletion could be implicated in the development of the oral manifestations of the syndrome.

Further molecular studies will be required before adequate genetic counseling can be offered to all family members of affected individuals. Nevertheless, it is of critical importance to offer a thorough physical examination and molecular screening of OFD1 to the probands and their relatives, along with monitoring of renal function.

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Competing interests

The authors declare that they have no competing interests.

Author contributions

PD and AC, designed the study and revised the final version of the manuscript. VA-I and IT, performed the molecular analysis. JL and JFF, performed dental treatment to proband and proband's family. JS, drafted the paper.

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