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15 years of research on Oral-Facial-Digital syndromes: from 1 to 16 causal genes

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Abstract

Oral-facial-digital syndromes (OFDS) gather rare genetic disorders characterized by facial, oral and digital abnormalities associated with a wide range of additional features (polycystic kidney disease, cerebral malformations and several others) to delineate a growing list of OFD subtypes. The most frequent, OFD type I, is caused by a heterozygous mutation in the *OFD1* gene encoding a centrosomal protein. The wide clinical heterogeneity of OFDS suggests the involvement of other ciliary genes. For 15 years, we have aimed to identify the molecular bases of OFDS. This effort has been greatly helped by the recent development of whole exome sequencing (WES). Here, we present all our published and unpublished results for WES in 24 OFDS cases. We identified causal variants in five new genes (*C2CD3, TMEM107, INTU, KIAA0753, IFT57*) and related the clinical spectrum of four genes in other ciliopathies (*C5orf42, TMEM138, TMEM231, WDPCP*) to OFDS. Mutations were also detected in two genes previously implicated in OFDS. Functional studies revealed the involvement of centriole elongation, transition zone and intraflagellar transport defects in OFDS, thus characterizing three ciliary protein modules: the complex KIAA0753-FOPNL-OFD1, a regulator of centriole elongation; the MKS module, a major component of the transition zone; and the CPLANE complex necessary for IFT-A assembly. OFDS now appear to be a distinct subgroup of ciliopathies with wide heterogeneity, which makes the initial classification obsolete. A clinical classification restricted to the three frequent/well-delineated subtypes could be proposed, and for patients who do not fit one of these 3 main subtypes, a further classification could be based on the genotype.

Keywords

oral-facial-digital syndromes; ciliopathies

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There is no conflict of interest

Contributorship Statement

BF, DD, RHG, CAJ, LB, MM, ID, GP, BD, BGD, BR, ESG, CB, IP, AFE, AD, AD, AG, EB, DG, JA, DB, SRP, VCD, GJP, VHP, LP, PL, SS, AM, TAB, LF, CTR ascertained the family and delineated OFD syndromes. ALB, JT, LJ, EL, MAH, VC, BA, NG, JSO, TE, JSL, OR, MRL, JBW, OEB, MVN, JBR performed molecular analysis, interpretation of results in these families and characterized ciliopathy proteins. YD, JFD, JBR, ALB performed the bioinformatic analysis of the data.

All the authors participated to the writing and reviewing processes of the manuscript.

INTRODUCTION

Oral-facial-digital (OFD) syndromes are rare genetic disorders characterized by the association of abnormalities of the face (hypertelorism, low-set ears), oral cavity (lingual hamartoma, abnormal frenulae, lobulated tongue) and extremities (brachydactyly, polydactyly). OFD syndromes also comprise a broad range of additional features that initially led to the clinical delineation of 13 OFD subtypes with mainly OFDI (polycystic kidney disease, corpus callosum agenesis), OFDIV (tibial dysplasia), OFDVI (mesoaxial polydactyly, vermis hypoplasia, molar tooth sign) and OFDIX (retinopathy) (1,2). More recently, a new subtype has been described associated with microcephaly wich has designed OFDXIV by OMIM [MIM 615948]. However, the precise phenotypic description revealed new unclassified OFD subtypes, in particular with severe microcephaly (3–6). Classically, the inheritance pattern is autosomal recessive except for OFDI, which has dominant X-linked inheritance and is lethal in males. Until recently, the molecular bases of OFD syndromes were poorly known. A few years ago, the *OFD1* gene [MIM 300170] was initially described as causing the OFDI subtype (7). *OFD1* encodes a protein located in the centrosome and basal body of primary cilia, suggesting that OFD syndromes are ciliopathies.

Ciliopathies are human diseases defined by ciliary structural and/or functional defects. Cilia, microtubule-based organelles projecting from the cytoplasmic membrane of the cell body, are divided into motile and non-motile or primary cilia. The primary cilia appear to be essential in several biological processes especially during development (8) and serve a broad range of specific sensory processes using receptors and ion channels to sense photo, chemo and mechanical stimuli and allow the transduction of signalling pathways. Four structural compartments have been described: (1) the centrosome, composed of two centrioles (mother and daughter) and pericentriolar material, including the mature mother centriole, which converts to the basal body that orients and positions the cilium (9); (2) the basal body formed where the centrosome, a microtubule organizing centre, migrates to the cell surface to initiate cilium assembly; (3) the transition zone, located at the distal end of basal body and composed of Y-links connecting microtubules to the ciliary membrane and ciliary necklace; and (4) the transition fibres, that forms the ciliary gate and constitutes a diffusion barrier to regulate cytoplasmic protein entry into the ciliary compartment (10,11). The microtubules extend distally from the basal body to form the axoneme, where receptors localize on the apex and the ciliary membrane, a lipid bilayer distinct from the plasma membrane, and surround the cilium. Proteins are transported along the axoneme to permit ciliary growth, maintenance and function. This essential intraflagellar transport is composed of two modules: IFT-A for retrograde transport and IFT-B for anterograde transport, which distribute ciliary molecules to the different ciliary compartments (12).

Ciliopathies present a broad range of features (retinopathy, cerebral malformations, bone defects, deafness or renal disease …); they are thus highly genetically heterogeneous diseases, and include nephronophthisis (NPHP), Joubert (JBS), Meckel-Gruber (MKS), Bardet-Biedl (BBS) syndromes and different chondrodysplasias. Multiple allelism has been described, suggesting that human ciliopathies are genetically complex (13). More recently, mutations in six additional genes that encode ciliary proteins have been identified in one or two patients with OFDS: centrosomal proteins implicated in centriole elongation (*NEK1* [MIM 604588], *SCLT1* [MIM 611399] and *TBC1D32/C6orf170* [MIM 615867]), proteins located in the transition zone (*TMEM216* [MIM 613277] and *TCTN3* [MIM 613847]) and a protein that regulates ciliary signalling (*DDX59* [MIM 615464]). Each known gene appears to be implicated in a classified OFD subtype: *OFD1* in OFDI [MIM 311200] with polycystic kidney disease and corpus callosum agenesis, *TCTN3* in OFDIV [MIM 258860] with tibial defect, *DDX59* in OFDV [MIM 174300], *TMEM216* in OFDVI [MIM 277170] characterized by cerebellar hypoplasia with the molar tooth sign, *SCTL1* and *TBC1D32/C6orf170* in OFDIX [MIM 258865] with coloboma (7,14–22).

Using a strategy of whole exome sequencing, we identified five new causal genes in OFD syndromes and showed the implication of four additional genes previously reported in other ciliopathies, as well as their different ciliary functions. In this unique cohort, all novel genes have been published independently. This paper presents an overview of the whole series and discusses the classification of this group with the advance of molecular delineation.

PATIENTS AND METHODS

Patient cohort

We gathered an international cohort of 115 index cases affected with different OFD syndromes. In all cases with a typical OFD I phenotype, we looked for *OFD1* SNV or CNV by Sanger sequencing and targeted array-CGH, respectively (23,24). Causal *OFD1* SNV or CNV were identified in 59/115 cases. Among the 56 other index cases with atypical clinical features or negative *OFD1* sequencing (Figure 1 and Table S1), we performed whole exome sequencing (WES) in 24 index cases, including 17 sporadic cases and 7 cases from consanguineous parents. WES was limited to 24 cases because of the quality and quantity of patients' DNA and the availability of parental DNA. All of the patients presented oral abnormalities (lingual hamartoma, abnormal frenulae or lobulated tongue), facial dysmorphism and extremity abnormalities (mainly polydactyly), associated with cerebral malformations (12/14 cases), retinopathy (3/16 cases), renal abnormalities (4/14 cases) and/or cardiac malformations (9/17 cases). Six individuals were diagnosed with OFDVI because of the molar tooth sign (MTS) on brain MRI and positive diagnostic criteria, two with OFDII and one with OFDV (25). Parental DNA samples were available in 17/24 cases.

Exome Analysis

After written consent had been obtained, blood samples were collected and DNA was extracted. Three micrograms of genomic DNA per index individual was subjected to whole-exome capture and sequencing using the SureSelect Human All Exon V5 kit (Agilent). The resulting libraries were sequenced on a HiSeq 2000 (Illumina) as paired-end 76 bp reads. BAM files were aligned to a human genome reference sequence (GRCh37/hg19) using BWA (Burrows-Wheeler Aligner; v0.6.2). All aligned read data were subject to the following steps: (i) duplicate paired-end reads were removed by Picard 1.77, (ii) indel realignment and (iii) base quality score recalibration were done on the Genome Analysis Toolkit (GATK; v2.1–10). Variants with a quality score >30 and an alignment quality score >20 were annotated with SeattleSeq SNP Annotation (see Web resources). CNV were detected by XHMM software (https://www.atgu.mgh.harvard.edu/) and annotated using chromosomic coordinates of coding exonic sequences on the human genome (https://www.ncbi.nlm.nih.gov/refseq/). Rare variants present at a frequency above 1% in dbSNP 138, ExAC Browser and the NHLBI GO Exome Sequencing Project or present in 312 exomes of unaffected individuals were excluded (see Web resources). To improve our exome analysis, data were crossed with a list of known cilia-related genes from the Ciliome Database, Cildb v2.1, Syscilia (see Web resources) and transcriptomic, proteomic and bioinformatics studies of cilia to identify putative ciliary genes (26–29). First, we looked for SNV or CNV in the six known genes in OFDS (*OFD1, TCTN3, TMEM216, SCLT1, TBC1D32/C6orf170* and *DDX59*). We then focused on genes with homozygous variants in consanguineous families and with two heterozygous variants in other cases and prioritized (i) genes associated with human disease in ClinVar or HGMD databases (see Web resources), (ii) cilia-related genes and (iii) other genes (Figure 2).

Sanger sequencing

Candidate variants and parental segregation were confirmed by Sanger sequencing. The different primers are available on request. Genomic DNA was amplified by Polymerase Chain Reaction (PCR) using HotStarTaq PCR kit (Qiagen) according to the manufacturer’s protocol. PCR products were purified by the Agencourt CleanSEQ system (Beckman Coulter) and sequenced with the BigDye Terminator Cycle Sequencing kit, v3.1 (Applied Biosystems) in ABI 3730 sequencer (Applied Biosystems). Sequence data were analysed using Mutation Surveyor v4.0.9 (Softgenomics).

RESULTS

WES identified causal mutations in 14/24 cases. The first analysis of known genes implicated in OFDS identified a homozygous missense variant in the *DDX59* gene [MIM 610621] and heterozygous mutations in the *OFD1* gene [MIM 311200] in three unrelated cases (p.Tyr87Cys, p.Ala614Hisfs*15 and c.655-8A>G, predicted to affect a splice-site). In these latter cases, *OFD1* mutations were not previously detected by Sanger sequencing.

The filtering strategy extracted five homozygous variants in consanguineous families (Table 1): a frameshift in the *INTU* gene [MIM 610621], a nonsense mutation in the *C2CD3* gene [MIM 615944], *TMEM138* [MIM 614459] and *TMEM107* genes, and a synonymous variant affecting a splice site in the *IFT57* gene [MIM 606621] (6,30–33). For all these genes, Sanger sequencing and parental segregation confirmed the homozygous status in the affected cases and the heterozygous status in each parent. We also identified compound heterozygous variants in four ciliary genes (Table 1): *TMEM231* [MIM 614949], *WDPCP* [MIM 613580], *C5orf42* genes [MIM 614571] and *KIAA0753* (31,33–35). Sanger sequencing and parental segregation confirmed the compound heterozygous status in the affected cases and the heterozygous status in each parent for all genes, except for the *KIAA0753* gene. For this gene, Sanger sequencing confirmed that the nonsense variant (NM_014804.2:p.Lys631*) was maternally inherited and the intronic substitution (NM_014804.2:c.1546-3C>A) occurred *de novo* and affected a splice-site causing a truncated protein (34).

The clinical heterogeneity of OFD syndromes was confirmed with various atypical signs and the overlap between OFD subtypes. Patients with a mutated *OFD1* gene presented typical signs of the OFDI subtype (lingual hamartoma, lobulated or bifid tongue, cleft palate, renal disease and corpus callosum agenesis) associated with very rare abnormalities including cardiac malformations (case n°20), the molar tooth sign on brain MRI (case n°13) or 11 pairs of ribs (case n°12), which suggest overlapping with other subtypes. Variants in *TMEM138, TMEM107* and *C5orf42* caused OFDVI, characterized by the molar tooth sign. In unclassified OFD, *C2CD3* mutations were associated with severe microcephaly, *INTU* and *WDPCP* mutations with cardiac defects, and *IFT57* mutations with chondysplasia. *DDX59* mutations had previously been reported in OFDII and identified in this cohort in a case of OFD V (n°1). OFDV, characterized by a median cleft of the upper lip and post-axial polydactyly, overlapped with OFD II, but this was predominantly found in patients of Indian origin. Finally, variants in the *TMEM231* gene were identified in a foetal case with unclassified OFD.

We thus identified causal mutations in five new genes, in four genes previously implicated in other ciliopathies and in two genes previously known to be responsible for OFD syndromes (Figure 3).

DISCUSSION

This study presents the largest OFD cohort investigated by WES. It led to the identification of causal mutations in 58% of affected cases, thus confirming the power of WES in identifying the genetic cause in well-phenotyped cases and highly heterogeneous disorders.

Wide clinical and genetic heterogeneity of OFD syndromes

The wide clinical heterogeneity and variable modes of inheritance in OFD syndromes suggest extreme genetic heterogeneity. Exome sequencing thus appeared the obvious choice, and because OFD syndromes were suspected to be mainly recessive, we initially focused on homozygous or potential compound heterozygous mutations, and prioritized ciliary genes and truncating rare variants in the absence of OMIM genes. In cases of suspected consanguinity, the probable causal variant was expected to be located within a large stretch of a homozygous region, thereby making it easier to identify new genes. Causal variants were thus identified in five new genes, at the homozygous status (*C2CD3, INTU, IFT57, TMEM107*) or compound heterozygous status (*KIAA0753*) (6,32,33). Recently, additional *C2CD3, TMEM107* and *TMEM231* mutations confirmed the implication of these genes in OFD syndromes (Table 1) (36,37). Causal variants were also identified in six other genes previously implicated in OFD syndromes (*DDX59, OFD1*) or in other ciliopathies (*TMEM138, C5orf42, TMEM231, WDPCP*). In all these patients, the OFD phenotype was clinically heterogeneous with OFDI (*OFD1*), OFDV (*DDX59*), OFDVI (*TMEM138, TMEM107, KIAA0753, OFD1, C5orf42*) or OFDXIV (*C2CD3*), as well as unclassified OFD (*TMEM231, IFT57, INTU, WDPCP*), with cerebellar hypoplasia, severe microcephaly, chondrodysplasia or cardiopathy. These results demonstrate the wide clinical and genetic heterogeneity of OFD syndromes, with, to date, 16 different genes. However, except for *OFD1*, few mutations have been reported in the other OFD genes because OFD syndromes remain rare with wide genetic heterogeneity and because some mutations are found in specific ethnic groups (figure 4).

Frequent clinical and genetic allelism between OFD and ciliopathies

The progressive identification of the molecular bases has highlighted the involvement of the primary cilia in OFD syndromes and confirmed the clinical and genetic overlap between OFD and other ciliopathies (38). Indeed, *OFD1*, which is responsible for OFDI syndrome, was also reported in JBS and severe retinitis pigmentosa (39–42). *TMEM216*, initially implicated in JBS and MKS, also caused OFDVI (43). Moreover, we identified OFD mutations in the *TMEM107* gene which also cause JBS (30,33), as well as in four other genes previously implicated in other ciliopathies (*TMEM138, C5orf42, TMEM231, WDPCP*) (table 1). To date, allelism with other ciliopathies affects nine of the 15 OFD genes. The most frequent allelism concerns OFDVI and JBS (*TMEM216, TMEM138, TMEM231, TMEM107, OFD1*, and *C5orf42*) (30,31,33,35,43,44). *TMEM231, TMEM107* and *C5orf42* genes also cause MKS (30,45,46), thus confirming the clear allelism between OFDVI, MKS and JBS syndromes with variable phenotypic expression. *INTU* and *WDPCP* mutations are also reported in NPHP and BBS, respectively, but the allelism between OFD and BBS remains uncertain because of the absence of clinical data in the reported cases (33,47). Recently, *C2CD3* mutations have also been reported in two foetuses with skeletal dysplasia, suggesting a short rib-polydactyly (SRP) syndrome (48).

Characterization of three ciliopathy protein complexes and cilia disturbance in OFD syndromes

The clinical description of different subtypes suggested that the causal proteins could be assembled in different functional modules. Because the 15 genes encode for proteins located in different compartments of primary cilia, new ciliary functions were suspected of being implicated in OFD syndromes (Table 2). Different functional studies have revealed two new ciliary complexes, CPLANE and KIAA0753-OFD1-FOPNL, and better characterized the transition zone and MKS module.

At the centrosomal level, the positive regulator C2CD3 was found to be an antagonist of OFD1, a negative regulator of centriole elongation (6). KIAA0753 or OFIP (OFD1 and FOR20 Interacting Protein) forms a ternary complex with OFD1 and FOPNL (also known as FOR20) to initiate ciliogenesis and control centriole length (34). When KIAA0753 is necessary to recruit OFD1 and FOPNL in centriole and pericentriolar satellites and to stabilize microtubule organization in the centrosome, C2CD3 was thought to be associated with the KIAA0753-OFD1-FOPNL complex probably via OFD1 protein. Knockdown of OFD1, C2CD3 or KIAA0753 induces hyperelongated (OFD1, KIAA0753) or shortened centrioles with the absence of subdistal appendages (C2CD3). These centriole defects affect membrane anchoring with the absence of cilia or greatly decreased cilium length. All these proteins control centriole elongation as do other centrosomal complexes, consisting of subunits with antagonist functions in ciliogenesis.

At the basal body level, a new protein complex, CPLANE (Ciliogenesis and Planar polarity Effectors) formed by FUZ, RSG1 and the three OFD proteins INTU, WDPCP and C5orf42, was characterized (33). C5orf42 initially recruits CPLANE components in the hierarchical assembly of this complex. CPLANE complex binds extensively with the IFT-A complex involved in retrograde intraflagellar transport, which is crucial for the recruitment of peripheral IFT-A proteins (IFT144, IFT43, IFT121 and IFT139) and their cytosolic pre-assembly. CPLANE defects affect intraflagellar transport and induce shortened cilia. Thus *RSG1* and *FUZ* genes are good candidates for OFD syndrome, but so far, Sanger sequencing of a local cohort negative for known OFD genes has not revealed any mutations in these genes.

At the transition zone (TZ), two functional modules, MKS and NPHP, interact to regulate ciliogenesis, the assembly of membrane-microtubule Y-link connectors, diffusion barrier formation, and the entry of IFT particles into the cilia (30,31,49). The NPHP module consists of two subunits (NPHP1-4) and the MKS module of twelve subunits (RPGRIP1L, TMEM107, TMEM216, B9D1, B9D2, MKS1, TMEM17, TMEM231, TMEM218, TMEM237, TMEM67 and CC2D2A), some of which are now known to be involved in OFD syndromes (TMEM231, TMEM216). It has been reported that TMEM107 occupies a new intermediate layer in the hierarchical assembly of the MKS module and is necessary to recruit TZ-proteins MKS1, TMEM17, TMEM237 and the new OFD protein TMEM231 (30). In *C. elegans*, CEP290 is required for the TZ localization of the MKS protein module and of other TZ-proteins, such as TMEM138, involved in OFD syndrome (31).

The new *IFT57* gene encodes a peripheral subunit of the IFT-B complex, which consists of 14 members. It is believed that the IFT-B complex has been highly conserved during evolution and has an essential function in the formation and maintenance of primary cilia. Only five subunits are involved in ciliopathies (IFT27, IFT80, IFT81, IFT88, IFT172) (50). *IFT57* mutations induce staining of IFT57 in the basal body in OFD patients' fibroblasts, whereas IFT57 was observed in the whole cilia in controls. Likewise, the *IFT57* mutation affects the SHH pathway, thus confirming the involvement of IFT57 in ciliary transport and signalling transduction (32).

Most of the genes involved in the same ciliopathy encode for subunits of the same protein complex and usually affect one ciliary function. In contrast, OFD syndromes implicate several protein complexes with various localizations and various ciliary functions, from centriole elongation to intraflagellar transport, thus illustrating the wide genetic heterogeneity. However, we noted a correlation between the genotype and the phenotype. Mutations in TZ-genes mainly caused OFDVI, CPLANE-gene mutations caused unclassified OFD with cardiac malformations and mutations in genes coding for centrosomal proteins were implicated in various subtypes (OFDI, IX, XIV or unclassified) but with a clinical continuum between C2CD3, KIAA0753 and OFD1, sometimes including the molar tooth sign on brain MRI or renal abnormalities.

OFD syndromes: a distinct subgroup of ciliopathies and phenotype-genotype correlations

OFD syndromes were initially classified as 13 clinical subtypes depending on the additional clinical features (polycystic kidney disease, corpus callosum agenesis, tibial dysplasia, retinopathy…). While numerous cases of OFDI, OFDIV and OFDVI syndromes have been reported, only anecdotal or single cases of some other subtypes have been published. This initial classification now appears to be obsolete given the wide clinical and molecular heterogeneity, with different overlapping ciliopathies such as JBS, MKS, BBS, SRP and NPHP. When *OFD1* mutations induce OFDI or OFDVI subtypes, the OFDVI subtype appears to be linked to different genes also implicated in JBS and MKS. Considering the clinical and molecular data, the OFD classification could be reduced to three main subtypes and several additional anecdotal cases (Table 3). Indeed, while a fine clinical description of the disease remains important for reverse phenotyping, prognosis and genetic counselling, a detailed classification appears to be extremely complex and of little use in such diseases with high clinical and genetic heterogeneity. Indeed, this high genetic heterogeneity leads to the use of WES for the molecular diagnosis of patients with OFD syndromes.

The high efficiency of the WES strategy in highly heterogeneous diseases

Despite the high clinical and genetic heterogeneity of these diseases, the solo WES strategy was very successful and led to the identification of five new genes responsible for OFD (*C2CD3, KIAA0753/OFIP, IFT57, INTU, TMEM107*). It also confirmed that OFD, BBS, JBS, MKS and SRP are allelic disorders and extended the clinical spectrum of *TMEM138, TMEM231, C5orf42, C2CD3* and *WDPCP* genes, thus increasing to 16 the number of genes known to be responsible for OFDS (Figures 3 and 4). This was possible thanks to a large 15-year international cohort and to knowing the probable mode of inheritance and the functions of candidate proteins. However, 42% of affected cases remained negative, raising questions about the choice of strategy. Indeed, the hypothesis of autosomal recessive inheritance and the limited availability of parental DNA at the beginning of the study led us to preferentially use the solo WES strategy, which is known to be less effective for the identification of sporadic mutations. In these negative patients, a trio WES strategy or whole genome sequencing (WGS) could now be considered to look for non-exonic variants. In these negative cases, genetic counselling remains difficult because an autosomal recessive mode of inheritance could be excluded.

In conclusion, this solo WES strategy in 24 OFDS cases identified five new genes responsible for OFD (*C2CD3, KIAA0753/OFIP, IFT57, INTU, TMEM107*), confirmed that OFD, BBS, JBS, MKS and SRP are allelic disorders and extended the clinical spectrum of *TMEM138, TMEM231, C5orf42, C2CD3* and *WDPCP* genes, thus increasing to 16 the number of genes known to be responsible for OFDS (Figures 3 and 4). Negative patients explored by secondary WES or WGS analysis with the trio strategy could extend these results to additional new genes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Clinical pictures, X-rays and brain MRI of OFD cases

Case 3a (K), case 3b (L), case 4 (B, N, V), case 5 (κ), case 6a (A, R, S, T, U), case 6b (F, Y, Z, α, β), case 7 (G), case 8 (E, L, Q, ε, ζ, ι, κ), case 10 (J, Y), case 11 (O), case 17 (D, I, K), case 19 (E), case 22 (ε), case 25 (λ, μ), case 26b (D, X, ζ), case 27 (P, ν, ο, π) case 28b (Q, ρ, ς), case 29 (υ, φ) with facial dysmorphism (A-D) including low-set ears, median pseudo-cleft of upper lip (F), missing incisors (A) or severe microcephaly (B), abnormal frenulae (E), cleft palate (I), lobulated tongue or hamartoma (G, H, J), pre and postaxial polydactyly of hands and feet (R, S, V, W, ε, ζ, ι, κ-υ), broad duplicated and/or deviated hallux (T, U, V, ε, ζ, η, θ, μ, ν, υ), Y-sharped metacarpal abnormality (κ, π), hypothalamic hamartoma (P), cerebellar hypoplasia (Q), brain MRI with MTS (K-O).

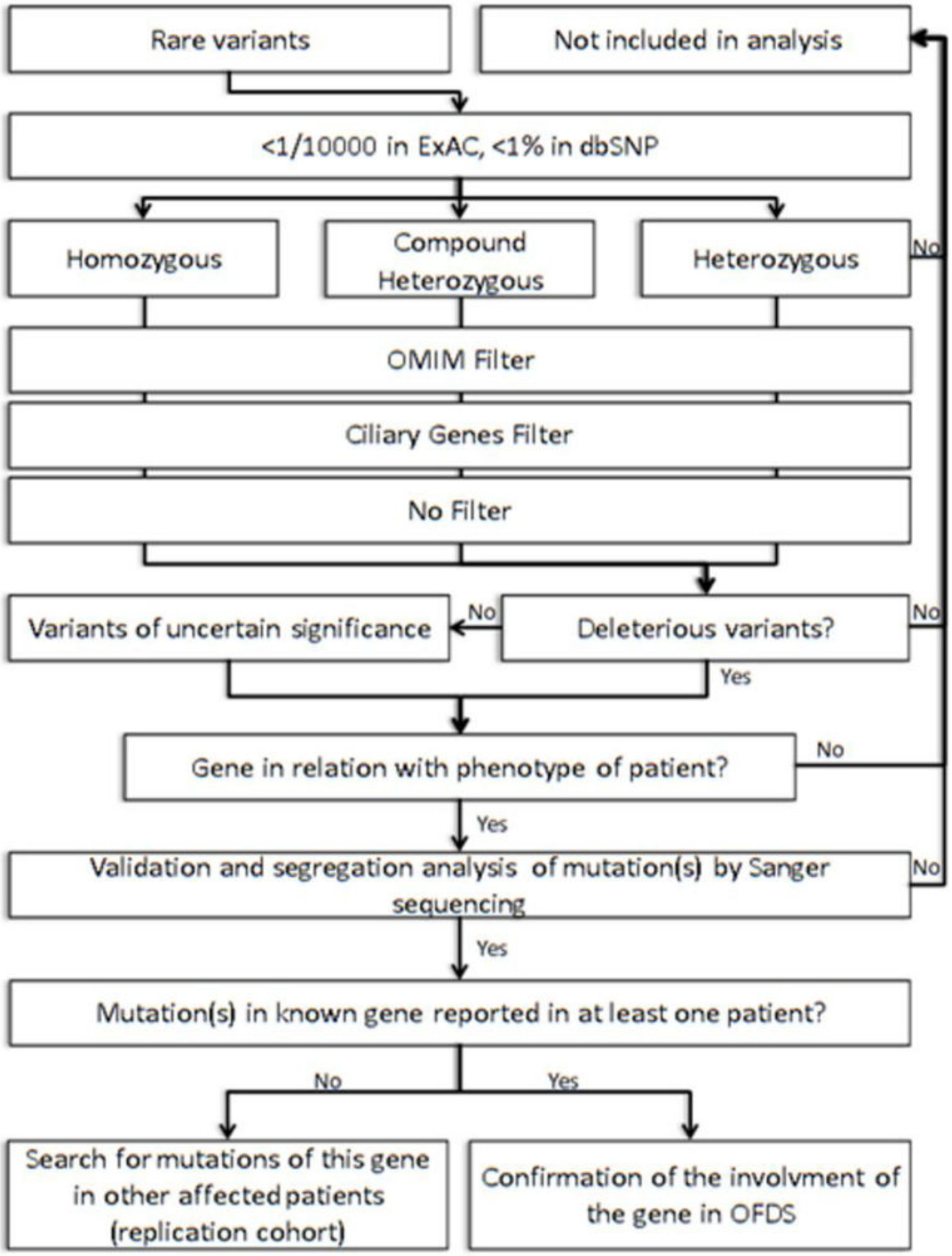

Figure 2.
Strategy for exome analysis

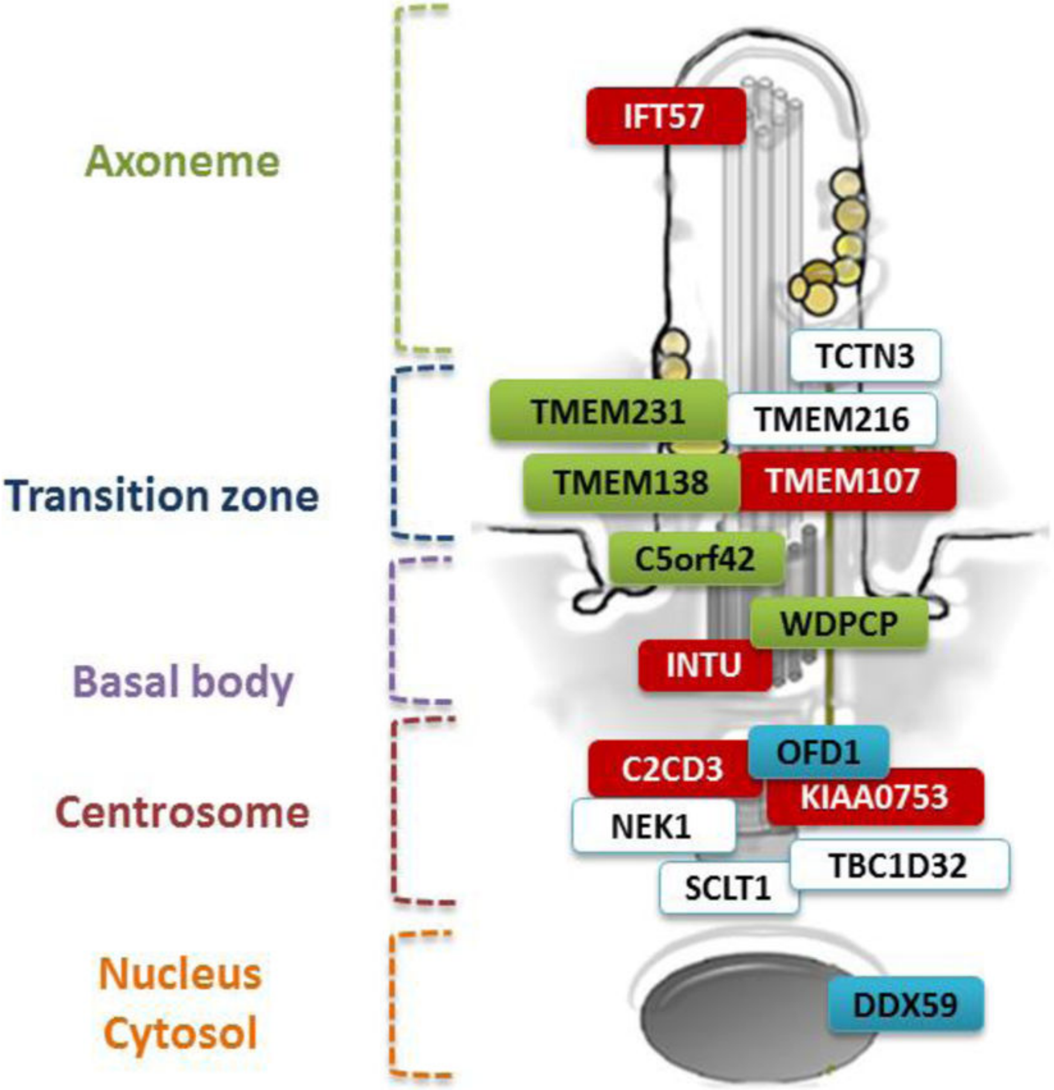

Figure 3. Localization of proteins encoded by the 16 OFD genes in primary cilia

5 new OFD genes (in red), 4 genes previously implicated in other ciliopathies (in green), 7 genes previously reported in OFD - 2 with presented mutations (blue) and 5 others (white).

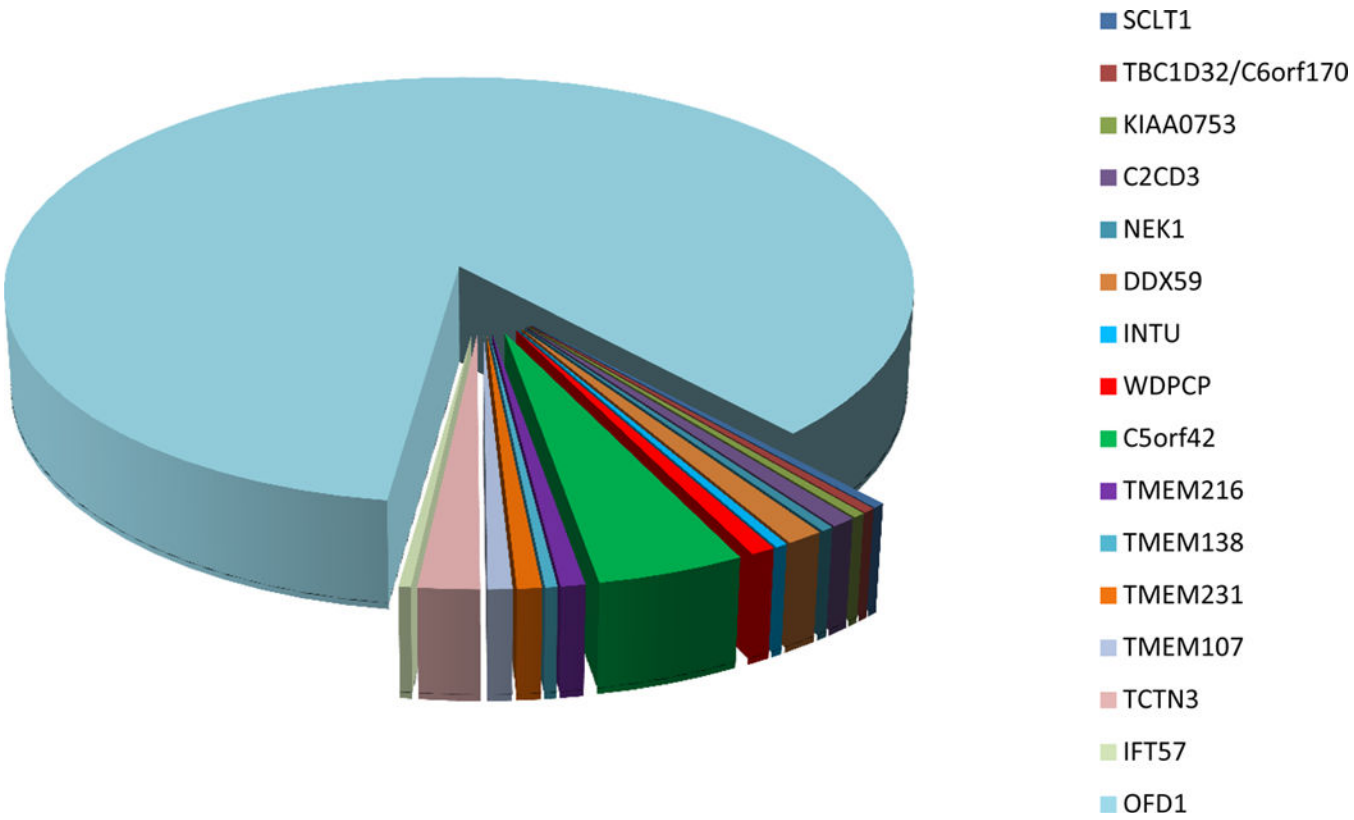

Figure 4.

Distribution of mutated genes in genotyped OFD cases reported in this study and in the literature.

Table 1

OFD genes identified by whole-exome sequencing or targeted gene sequencing

Case	Gene	Ciliary gene	OMIM	Mutation		Inheritance	EVS	ExAC	cDNA tests
				c. position	p. position				
Cohort analyzed by exome									
1	*DDX59*	NA	Oral-facial-digital syndrome V [174300]	c.754G>A	p.Gly252Arg	Maternal	–	–	–
				c.754G>A	p.Gly252Arg	Paternal			
2	*TMEM138*	+	Joubert syndrome 16 [614465]	c.352A>T	p.Met118Leu	NA	–	–	–
				c.352A>T	p.Met118Leu	NA			
3a/b	*TMEM107*	+	–	c.134A>G	p.Glu45Gly	Maternal	–	–	–
				c.134A>G	p.Glu45Gly	Paternal			
4	*C2CD3*	+	Oral-facial-digital syndrome XIV [615948]	c.184C>T	p.Arg62*	Maternal	–	–	–
				c.184C>T	p.Arg62*	Paternal			
5	*INTU*	+	–	c.396delT	p.Asn132Lysfs*11	NA	–	–	–
				c.396delT	p.Asn132Lysfs*11	NA			
6a	*IFT57*	+	–	c.777G>A	p.Lys259Lys	NA	–	–	Splice defect
				c.777G>A	p.Lys259Lys	NA			
7	*C5orf42*	+	Joubert syndrome 17 [614615]	3557delA	Lys1186Argfs*22	NA	–	–	–
				c.3577C>T	p.Arg1193Cys	NA	–	–	
8	*C5orf42*	+	Joubert syndrome 17 [614615]	c.3290-2A>G	–	Maternal	–	–	–
				c.493delA	p.Ile165Tyrfs*17	Paternal	1/6155	–	
9	*TMEM231*	+	Joubert syndrome 20 [614970]	c.656C>T	p.Pro219Leu	Maternal	–	–	–
			Meckel syndrome 11 [615397]	c.532C>G	p.Pro178Ala	Paternal	–	–	
10	*WDPCP*	+	Bardet-Biedl syndrome 15 [209900]	c.160G>A	p.Asp54Asn	Paternal	1/11827	7/119586	–
				c.526 527delTT	Leu176Ilefs*21	Maternal	–	–	
11	*KIAA0753*	+	–	c.1546-3C>A	–	de novo	–	–	Splice defect
				c.1891A>T	p.Lys631*	Maternal	–	–	–
12	*OFD1*	+	Oral-facial-digital syndrome I [3111200]Joubert syndrome 10 [300804]Simpson-Golabi-Behmel syndrome 2 [300209] Retinitis pigmentosa 23 [300424]	c.260A>G	p.Tyr87Cys	de novo	–	–	–
13	*OFD1*	+		c.1840delG	p.Ala614Hisfs*15	de novo	–	–	–
20	*OFD1*	+		c.655-8A>G	–	de novo	–	–	–

Case	**Gene**	**Ciliary gene**	**OMIM**	**Mutation**		**Inheritance**	**EVS**	**ExAC**	**cDNA tests**
				c. position	**p. position**				
Replication cohort									
25	*C5orf42*	+	Joubert syndrome 17 [614615]	c.3550C>T	p.Arg1184Cys	Paternal	–	–	–
				c.9121C>T	p.Gln3041*	Maternal	–	–	
26a/b	*C5orf42*	+	Joubert syndrome 17 [614615]	c.3150-1G>T	–	Maternal	–	–	Splice defect
				c.3150-1G>T	–	Paternal	–	–	Splice defect
27	*C5orf42*	+	Joubert syndrome 17 [614615]	c.2377C>T	p.Gln793*	Paternal	–	2/22038	–
				c.8509G>T	p.Val2837Leu	Maternal	–	–	
28b	*C5orf42*	+	Joubert syndrome 17 [614615]	c.493delA	p.Ile165Tyrfs*17	Paternal	–	–	–
				c.3380C>T	p.Ser1127Leu	Maternal	–	–	
29	*C2CD3*	+	–	c.3085T>C	p.Cys1029Gly	NA	–	–	–
				c.3911-2A>T	–		6/ 12978	31/120818	Splice defect

NA: Not Available

Table 2

ell as localization and function of OFD proteins

Functional protein complex	Protein function	OFD subtype	Pre-axial polydactyly	Post-axial polydactyly	Retinopathy	Renal anomaly	Cerebral malformation	MTS	Tibial dysplasia	Reference
OFD1-KIAA0753-FOPNL	Negative regulator of centriole elongation	OFDI	x			x	x			Ferrante et al., 2001
–	Positive regulator of centriole elongation	OFDXIV		x	x		x			Thauvin-Robinet et al., 2014
OFD1-KIAA0753-FOPNL	Recruitment of OFD1 at centriole	OFD VI		x			x	x		Chevrier et al., 2015
–	Unknown, ciliogenesis	OFDIX					x			Adly et al.,2013
–	Unknown	OFDIX		x			x			Adly et al.,2013
–	Regulation of ciliary signalling	OFDV		x						Present study
CPLANE	IFT-A pre-assembly	OFDII?		x		x				Toriyama et al., 2016
CPLANE	IFT-A pre-assembly	–		x						Toriyama et al., 2016
CPLANE	IFT-A pre-assembly	OFDVI	x	x			x	x		Lopes et al., 2014
–	Regulation of ciliary signalling	OFDIV	x	x		x	x		x	Thomas et al., 2012
MKS	Ciliary gate formation	OFDVI	x	x			x			Valente et al., 2012
MKS	Ciliary gate formation	OFDVI?		x			x			Li et al., 2016
MKS	Ciliary gate formation	OFDVI		x	x		x	x		Lambacher et al., 2015
–	Vesicular transport	OFDVI					x	x		Li et al., 2016
IFT-B	Intraflagellar transport	–		x						Thevenon et al., 2016

: Transition fibers

Table 3

Novel classification of OFDS based on the association between clinical and molecular features

OFD subtype	Clinical data	Genes
OFDI	Polycystic kidney disease, Corpus callosum agenesis	*OFD1*
OFDIV	Tibial dysplasia	*TCTN3*
OFDVI	Molar tooth sign	*TMEM216, TMEM231, TMEM138, C5orf42, TMEM107, KIAA0753*
Classification based on the genotype for other patients	Median cleft of the upper lip	*DDX59, NEK1*
	Cardiac defects	*INTU, WDPCP*
	Retinopathy	*SCLT1, TBC1 D32/C7orf170*
	Severe microcephaly	*C2CD3*
	Chondrodysplasia	*IFT57*