



UNIVERSITY OF LEEDS

This is a repository copy of *Fifteen years of research on oral–facial–digital syndromes: from 1 to 16 causal genes*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/130748/>

Version: Accepted Version

Article:

Bruel, A-L, Franco, B, Duffourd, Y et al. (49 more authors) (2017) Fifteen years of research on oral–facial–digital syndromes: from 1 to 16 causal genes. *Journal of Medical Genetics*, 54 (6). pp. 371-380. ISSN 0022-2593

<https://doi.org/10.1136/jmedgenet-2016-104436>

© 2017, The Authors. This is an author produced version of a paper published in *Journal of Medical Genetics*. Uploaded in accordance with the publisher's self-archiving policy.

Reuse

Items deposited in White Rose Research Online are protected by copyright, with all rights reserved unless indicated otherwise. They may be downloaded and/or printed for private study, or other acts as permitted by national copyright laws. The publisher or other rights holders may allow further reproduction and re-use of the full text version. This is indicated by the licence information on the White Rose Research Online record for the item.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>

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

Ange-Line Bruel^{1,2}, Brunella Franco^{3,4}, Yannis Duffourd^{1,2}, Julien Thevenon^{1,2,5}, Laurence Jego^{1,2}, Estelle Lopez², Jean-François Deleuze⁶, Diane Doummar⁷, Rachel H. Giles⁸, Colin A. Johnson⁹, Martijn A. Huynen¹⁰, Véronique Chevrier^{11,12,13,14}, Lydie Burglen⁷, Manuela Morleo^{2,3}, Isabelle Desguerres¹⁵, Geneviève Pierquin¹⁶, Bérénice Doray¹⁷, Brigitte Gilbert-Dussardier¹⁸, Bruno Reversade¹⁹, Elisabeth Steichen-Gersdorf²⁰, Clarisse Baumann²¹, Inusha Panigrahi²², Anne Fargeot-Espaliat²³, Anne Dieux²⁴, Albert David²⁵, Alice Goldenberg²⁶, Ernie Bongers²⁷, Dominique Gaillard²⁸, Jesús Argente²⁹, Bernard Aral³⁰, Nadège Gigot^{1,2,30}, Judith St-Onge^{1,2}, Daniel Birnbaum^{11,12,13,14}, Shubha R. Phadke³¹, Valérie Cormier-Daire^{3,32,33}, Thibaut Eguether³⁴, Gregory J. Pazour³⁴, Vicente Herranz-Pérez^{35,36}, Jaclyn S. Lee³⁷, Laurent Pasquier³⁸, Philippe Loget³⁹, Sophie Saunier^{40,41}, André Mégarbané⁴⁵, Olivier Rosnet^{11,12,13,14}, Michel R. Leroux⁴², John B. Wallingford⁴³, Oliver E. Blacque⁴⁴, Maxence V. Nachury³⁷, Tania Attie-Bitach^{32,33,41}, Jean-Baptiste Rivière^{1,2,30}, Laurence Faivre^{1,2,5}, Christel Thauvin-Robinet^{1,2,5}

Affiliations

1. FHU-TRANSLAD, Université de Bourgogne/CHU Dijon, France
2. Équipe EA42271 GAD, Université de Bourgogne, Dijon, France
3. Department of Translational Medicine, Medical Genetics Federico II University of Naples, Italy
4. Telethon Institute of Genetics and Medicine-TIGEM, Naples, Italy
5. Centre de Référence maladies rares « Anomalies du Développement et syndrome malformatifs » de l'Est et Centre de Génétique, Hôpital d'Enfants, CHU, Dijon, France
6. Centre National de Génotypage, Evry, France
7. APHP, hôpital TROUSSEAU, Centre de référence des malformations et maladies congénitales du cervelet et département de génétique, Paris, France
8. Department of Nephrology and Hypertension, University Medical Center Utrecht, Utrecht, The Netherlands
9. Section of Ophthalmology and Neurosciences, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, LS9 7TF, UK
10. Centre for Molecular and Biomolecular Informatics, Radboud Institute for Molecular Life Sciences, Radboud university medical center, Geert Grooteplein 26-28, 6525 GA Nijmegen, Netherlands
11. Centre de Recherche en Cancérologie de Marseille, INSERM UMR1068, F-13009 Marseille, France
12. Institut Paoli-Calmettes, F-13009 Marseille, France
13. CNRS U7258, F-13009 Marseille, France
14. Aix-Marseille Université, F-13007 Marseille, France
15. Service de neurométabolisme, Hôpital Necker-Enfants Malades, CHU, Paris, France
16. Service de Génétique, CHU, Liège, Belgium
17. Service de Génétique Médicale, Hôpital de Hautepierre, CHU, Strasbourg, France
18. Centre de Référence Maladies Rares « Anomalies du Développement et Syndromes malformatifs » de l'Ouest, Service de Génétique Médicale, CHU de Poitiers, EA 3808, Université de Poitiers, France

19. Laboratory of Human Embryology and Genetics, Institute of Medical Biology, Singapore
20. Department of Pediatrics I, Innsbruck Medical University, Innsbruck, Austria
21. Département de Génétique, Unité Fonctionnelle de Génétique Clinique, Hôpital Robert Debré, CHU, Paris, France
22. Genetic-Metabolic Unit, Department of Pediatrics, Advanced Pediatric Centre, Pigmmer, Chandigarh, India
23. Pédiatrie Neonatologie, Centre Hospitalier Général, Brive-la-Gaillarde, France
24. Centre de Référence CLAD NdF, Service de Génétique Clinique, Hôpital Jeanne de Flandre, CHRU, Lille, France
25. Service de Génétique Médicale, Unité de Génétique Clinique, Hôpital Mère-Enfant, CHU, Nantes, France
26. Service de Génétique, CHU de Rouen, Centre Normand de Génomique Médicale et Médecine Personnalisée, Rouen, France
27. Department of Human Genetics, Radboud University, Nijmegen, The Netherlands
28. Service de Génétique, Hôpital Maison Blanche, CHRU, Reims, France
29. Department of Pediatrics & Pediatric Endocrinology, Hospital Infantil Universitario Niño Jesús. Departement of Pediatrics, Universidad Autónoma de Madrid. CIBEROBN de fisiopatología de la obesidad y nutrición. Instituto de Salud Carlos III. Madrid, Spain.
30. Laboratoire de Génétique Moléculaire, PTB, CHU, Dijon, France
31. Department of Medical Genetics, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India
32. INSERM UMR1163, Université de Paris-Descartes-Sorbonne Paris Cité, Institut IMAGINE, Hôpital Necker-Enfants Malades, Paris, France
33. Service de génétique médicale, Hôpital Universitaire Necker-Enfants Malades, AP-HP, Institut Imagine, Paris, France
34. Program in Molecular Medicine, University of Massachusetts Medical School, Worcester, Massachusetts, USA
35. Laboratorio de Neurobiología Comparada, Instituto Cavanilles, Universitat de València, CIBERNED, Spain
36. Unidad mixta de Esclerosis múltiple y neuroregeneración, IIS Hospital La Fe-UEVEG, Valencia, Spain
37. Department of Molecular and Cellular Physiology, Stanford University School of Medicine, Stanford, CA, USA
38. Centre de Référence Maladies Rares « Anomalies du Développement et Syndromes malformatifs » de l'Ouest, Unité Fonctionnelle de Génétique Médicale, CHU Rennes, France
39. Laboratoire d'Anatomie-Pathologie, CHU Rennes, France
40. INSERM U983, Institut IMAGINE, Hôpital Necker-Enfants Malades, Paris, France
41. Département de Génétique, Hôpital Necker-Enfants Malades, AP-HP, Paris, France

42. Department of Molecular Biology and Biochemistry and Centre for Cell Biology, Development and Disease, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada
43. Department of Molecular Biosciences, Center for Systems and Synthetic Biology, and Institute for Cellular and Molecular Biology, University of Texas at Austin
44. School of Biomolecular and Biomedical Science, University College Dublin, Belfield, Dublin 4, Ireland
45. Institut Jérôme Lejeune, Paris, France

There is no conflict of interest

Keywords: oral-facial-digital syndromes, ciliopathies

Corresponding author:

Pr Christel Thauvin-Robinet, MD-PhD

Centre de Génétique, Hôpital d'Enfants

10 Bd du Maréchal de Lattre de Tassigny

21034 Dijon cedex

France

tel: 33 3 80 29 53 13

fax: 33 3 80 29 32 66

e-mail: christel.thauvin@chu-dijon.fr

Dr Ange-Line Bruel, PhD

Genetics of Developmental Disorders

Bât B3, Université de Bourgogne-Franche Comté

15 boulevard Maréchal Delattre de Tassigny

21070 Dijon

France

tel: 33 3 80 39 32 38

fax: 33 3 80 29 32 66

e-mail: ange-line.brue@u-bourgogne.fr

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 other) to delineate a growing list of OFDS 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.

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 which has designated 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, *SCLT1* 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 chromosomal 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 chondyplasia. *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 fetuses 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.

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.

Funding

There are no funders to report for this submission

Acknowledgements

We thank the patients and their families for their participation. We thank the Integragen society, CNG and IBGMC for the exome analyses and the Ferdinand Cabanne Biological Resources Centre (CRBFC) for access to the biobank. This work was supported by grants from the GIS-Institut des Maladies Rares (HTS), the French Foundation for Rare Diseases, the French Ministry of Health (PHRC national 2010-A01014-35 and 2013), and the Regional Council of Burgundy. We also thank the NHLBI GO Exome Sequencing Project (see URLs) and its ongoing studies which produced and provided exome variant calls for comparison: the Lung GO Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the Heart GO Sequencing Project (HL-103010).

References

1. Gurrieri F, Franco B, Toriello H, Neri G. Oral-facial-digital syndromes: review and diagnostic guidelines. *Am J Med Genet A*. 2007 Dec 15;143A(24):3314–23.
2. Toriello HV, Franco B, Bruel A-L, Thauvin-Robinet C. Oral-Facial-Digital Syndrome Type I. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, Bird TD, Fong CT, Mefford HC, Smith RJH, Stephens K, editors. *GeneReviews*(®) [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2016 Aug 23]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK1188/>

3. Al-Gazali LI, Sztriha L, Punnose J, Shather W, Nork M. Absent pituitary gland and hypoplasia of the cerebellar vermis associated with partial ophthalmoplegia and postaxial polydactyly: a variant of orofaciogigital syndrome VI or a new syndrome? *J Med Genet.* 1999 Feb;36(2):161–6.
4. Chung WY, Chung LP. A case of oral-facial-digital syndrome with overlapping manifestations of type V and type VI: a possible new OFD syndrome. *Pediatr Radiol.* 1999 Apr;29(4):268–71.
5. Erickson RP, Bodensteiner JB. Oro-facial-digital syndrome IX with severe microcephaly: a new variant in a genetically isolated population. *Am J Med Genet A.* 2007 Dec 15;143A(24):3309–13.
6. Thauvin-Robinet C, Lee JS, Lopez E, Herranz-Pérez V, Shida T, Franco B, Jago L, Ye F, Pasquier L, Loget P, Gigot N, Aral B, Lopes CA, St-Onge J, Bruel AL, Thevenon J, González-Granero S, Alby C, Munnich A, Vekemans M, Huet F, Fry AM, Saunier S, Rivière JB, Attié-Bitach T, Garcia-Verdugo JM, Faivre L, Mégarbané A, Nachury MV. The oral-facial-digital syndrome gene C2CD3 encodes a positive regulator of centriole elongation. *Nat Genet.* 2014 Aug;46(8):905–11.
7. Ferrante MI, Giorgio G, Feather SA, Bulfone A, Wright V, Ghiani M, Selicorni A, Gammaro L, Scolari F, Woolf AS, Sylvie O, Bernard L, Malcolm S, Winter R, Ballabio A, Franco B. Identification of the gene for oral-facial-digital type I syndrome. *Am J Hum Genet.* 2001 Mar;68(3):569–76.
8. Goetz SC, Anderson KV. The Primary Cilium: A Signaling Center During Vertebrate Development. *Nat Rev Genet.* 2010 May;11(5):331–44.
9. Kobayashi T, Dynlacht BD. Regulating the transition from centriole to basal body. *J Cell Biol.* 2011 May 2;193(3):435–44.
10. Reiter JF, Blacque OE, Leroux MR. The base of the cilium: roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. *EMBO Rep.* 2012 Jul;13(7):608–18.
11. Szymanska K, Johnson CA. The transition zone: an essential functional compartment of cilia. *Cilia.* 2012;1(1):10.
12. Taschner M, Bhogaraju S, Lorentzen E. Architecture and function of IFT complex proteins in ciliogenesis. *Differentiation.* 2012 Feb;83(2):S12-22.
13. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *N Engl J Med.* 2011 Apr 21;364(16):1533–43.
14. Adly N, Alhashem A, Ammari A, Alkuraya FS. Ciliary genes TBC1D32/C6orf170 and SCLT1 are mutated in patients with OFD type IX. *Hum Mutat.* 2014 Jan;35(1):36–40.
15. Edvardson S, Shaag A, Zenvirt S, Erlich Y, Hannon GJ, Shanske AL, Gomori JM, Ekstein J, Elpeleg O. Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *Am J Hum Genet.* 2010 Jan;86(1):93–7.
16. Giorgio G, Alfieri M, Prattichizzo C, Zullo A, Cairo S, Franco B. Functional characterization of the OFD1 protein reveals a nuclear localization and physical interaction with subunits of a chromatin remodeling complex. *Mol Biol Cell.* 2007 Nov;18(11):4397–404.
17. Monroe GR, Kappen IF, Stokman MF, Terhal PA, van den Boogaard M-JH, Savelberg SM, van der Veken LT, van Es RJ, Lens SM, Hengeveld RC, Creton MA, Janssen NG, Mink van der Molen AB, Ebbeling MB, Giles RH, Knoers NV, van Haften G. Compound heterozygous NEK1 variants in

two siblings with oral-facial-digital syndrome type II (Mohr syndrome). *Eur J Hum Genet.* 2016 Aug 17;

18. Roberson EC, Dowdle WE, Ozanturk A, Garcia-Gonzalo FR, Li C, Halbritter J, Elkhartoufi N, Porath JD, Cope H, Ashley-Koch A, Gregory S, Thomas S, Sayer JA, Saunier S, Otto EA, Katsanis N, Davis EE, Attié-Bitach T, Hildebrandt F, Leroux MR, Reiter JF. TMEM231, mutated in orofacioidigital and Meckel syndromes, organizes the ciliary transition zone. *J Cell Biol.* 2015 Apr 13;209(1):129–42.
19. Romio L, Fry AM, Winyard PJD, Malcolm S, Woolf AS, Feather SA. OFD1 is a centrosomal/basal body protein expressed during mesenchymal-epithelial transition in human nephrogenesis. *J Am Soc Nephrol.* 2004 Oct;15(10):2556–68.
20. Saari J, Lovell MA, Yu H-C, Bellus GA. Compound heterozygosity for a frame shift mutation and a likely pathogenic sequence variant in the planar cell polarity-ciliogenesis gene WDPCP in a girl with polysyndactyly, coarctation of the aorta, and tongue hamartomas. *Am J Med Genet A.* 2015 Feb;167(2):421–7.
21. Shamseldin HE, Rajab A, Alhashem A, Shaheen R, Al-Shidi T, Alamro R, Al Harassi S, Alkuraya FS. Mutations in DDX59 implicate RNA helicase in the pathogenesis of orofacioidigital syndrome. *Am J Hum Genet.* 2013 Sep 5;93(3):555–60.
22. Thomas S, Legendre M, Saunier S, Bessières B, Alby C, Bonnière M, Toutain A, Loeuillet L, Szymanska K, Jossic F, Gaillard D, Yacoubi MT, Mougou-Zerelli S, David A, Barthez MA, Ville Y, Bole-Feysot C, Nitschke P, Lyonnet S, Munnich A, Johnson CA, Encha-Razavi F, Cormier-Daire V, Thauvin-Robinet C, Vekemans M, Attié-Bitach T. TCTN3 mutations cause Mohr-Majewski syndrome. *Am J Hum Genet.* 2012 Aug 10;91(2):372–8.
23. Thauvin-Robinet C, Cossée M, Cormier-Daire V, Van Maldergem L, Toutain A, Alembik Y, Bieth E, Layet V, Parent P, David A, Goldenberg A, Mortier G, Héron D, Sagot P, Bouvier AM, Huet F, Cusin V, Donzel A, Devys D, Teyssier JR, Faivre L. Clinical, molecular, and genotype-phenotype correlation studies from 25 cases of oral-facial-digital syndrome type 1: a French and Belgian collaborative study. *J Med Genet.* 2006 Jan;43(1):54–61.
24. Thauvin-Robinet C, Callier P, Franco B, Zuffardi O, Payet M, Aral B, Gigot N, Donzel A, Mosca-Boidron AL, Masurel-Paulet A, Huet F, Teyssier JR, Mugneret F, Faivre L. Search for genomic imbalances in a cohort of 20 patients with oral-facial-digital syndromes negative for mutations and large rearrangements in the OFD1 gene. *Am J Med Genet A.* 2009 Aug;149A(8):1846–9.
25. Poretti A, Brehmer U, Scheer I, Bernet V, Boltshauser E. Prenatal and neonatal MR imaging findings in oral-facial-digital syndrome type VI. *AJNR Am J Neuroradiol.* 2008 Jun;29(6):1090–1.
26. Amato R, Morleo M, Giaquinto L, di Bernardo D, Franco B. A network-based approach to dissect the cilia/centrosome complex interactome. *BMC Genomics.* 2014;15:658.
27. Ishikawa H, Thompson J, Yates JR, Marshall WF. Proteomic analysis of mammalian primary cilia. *Curr Biol.* 2012 Mar 6;22(5):414–9.
28. Ivliev AE, 't Hoen PAC, van Roon-Mom WMC, Peters DJM, Sergeeva MG. Exploring the transcriptome of ciliated cells using in silico dissection of human tissues. *PLoS ONE.* 2012;7(4):e35618.

29. Wheway G, Schmidts M, Mans DA, Szymanska K, Nguyen T-MT, Racher H, Phelps IG, Toedt G, Kennedy J, Wunderlich KA, Soroush N, Abdelhamed ZA, Natarajan S, Herridge W, van Reeuwijk J, Horn N, Boldt K, Parry DA, Letteboer SJ, Roosing S, Adams M, Bell SM, Bond J, Higgins J, Morrison EE, Tomlinson DC, Slaats GG, van Dam TJ, Huang L, Kessler K, Giessl A, Logan CV, Boyle EA, Shendure J, Anazi S, Aldahmesh M, Al Hazzaa S, Hegele RA, Ober C, Frosk P, Mhanni AA, Chodirker BN, Chudley AE, Lamont R, Bernier FP, Beaulieu CL, Gordon P, Pon RT, Donahue C, Barkovich AJ, Wolf L, Toomes C, Thiel CT, Boycott KM, McKibbin M, Inglehearn CF; UK10K Consortium.; University of Washington Center for Mendelian Genomics., Stewart F, Omran H, Huynen MA, Sergouniotis PI, Alkuraya FS, Parboosingh JS, Innes AM, Willoughby CE, Giles RH, Webster AR, Ueffing M, Blacque O, Gleeson JG, Wolfrum U, Beales PL, Gibson T, Doherty D, Mitchison HM, Roepman R, Johnson CA. An siRNA-based functional genomics screen for the identification of regulators of ciliogenesis and ciliopathy genes. *Nat Cell Biol.* 2015 Jul 13;
30. Lambacher NJ, Bruel A-L, van Dam TJP, Szymańska K, Slaats GG, Kuhns S, McManus GJ, Kennedy JE, Gaff K, Wu KM, van der Lee R, Burglen L, Doummar D, Rivière JB, Faivre L, Attié-Bitach T, Saunier S, Curd A, Peckham M, Giles RH, Johnson CA, Huynen MA, Thauvin-Robinet C, Blacque OE. TMEM107 recruits ciliopathy proteins to subdomains of the ciliary transition zone and causes Joubert syndrome. *Nat Cell Biol.* 2015 Nov 23;
31. Li C, Jensen VL, Park K, Kennedy J, Garcia-Gonzalo FR, Romani M, De Mori R, Bruel AL, Gaillard D, Doray B, Lopez E, Rivière JB, Faivre L, Thauvin-Robinet C, Reiter JF, Blacque OE, Valente EM, Leroux MR. MKS5 and CEP290 Dependent Assembly Pathway of the Ciliary Transition Zone. *PLoS Biol.* 2016 Mar;14(3):e1002416.
32. Thevenon J, Duplomb L, Phadke S, Eguether T, Saunier A, Avila M, Carmignac V, Bruel AL, St-Onge J, Duffourd Y, Pazour GJ, Franco B, Attie-Bitach T, Masurel-Paulet A, Rivière JB, Cormier-Daire V, Philippe C, Faivre L, Thauvin-Robinet C. Autosomal Recessive IFT57 hypomorphic mutation cause ciliary transport defect in unclassified oral-facial-digital syndrome with short stature and brachymesophalangia. *Clin Genet.* 2016 Apr 7;
33. Toriyama M, Lee C, Taylor SP, Duran I, Cohn DH, Bruel A-L, Tabler JM, Drew K, Kelly MR, Kim S, Park TJ, Braun DA, Pierquin G, Biver A, Wagner K, Malfroot A, Panigrahi I, Franco B, Al-Lami HA, Yeung Y, Choi YJ; University of Washington Center for Mendelian Genomics., Duffourd Y, Faivre L, Rivière JB, Chen J, Liu KJ, Marcotte EM, Hildebrandt F, Thauvin-Robinet C, Krakow D, Jackson PK, Wallingford JB. The ciliopathy-associated CPLANE proteins direct basal body recruitment of intraflagellar transport machinery. *Nat Genet.* 2016 May 9;
34. Chevrier V, Bruel A-L, Dam TJP van, Franco B, Scalzo ML, Lembo F, Audebert S, Baudalet E, Isnardon D, Bole A, Borg JP, Kuentz P, Thevenon J, Burglen L, Faivre L, Rivière JB, Huynen MA, Birnbaum D, Rosnet O, Thauvin-Robinet C. OFIP/KIAA0753 forms a complex with OFD1 and FOR20 at pericentriolar satellites and centrosomes and is mutated in one individual with Oral-Facial-Digital Syndrome. *Hum Mol Genet.* 2015 Dec 7;ddv488.
35. Lopez E, Thauvin-Robinet C, Reversade B, Khartoufi NE, Devisme L, Holder M, Ansart-Franquet H, Avila M, Lacombe D, Kleinfinger P, Kaori I, Takanashi J, Le Merrer M, Martinovic J, Noël C, Shboul M, Ho L, Güven Y, Razavi F, Burglen L, Gigot N, Darmency-Stamboul V, Thevenon J, Aral B, Kayserili H, Huet F, Lyonnet S, Le Caignec C, Franco B, Rivière JB, Faivre L, Attié-Bitach T. C5orf42 is the major gene responsible for OFD syndrome type VI. *Hum Genet.* 2014 Mar;133(3):367–77.

36. Iglesias A, Anyane-Yeboa K, Wynn J, Wilson A, Truitt Cho M, Guzman E, Sisson R, Egan C, Chung WK. The usefulness of whole-exome sequencing in routine clinical practice. *Genet Med*. 2014 Dec;16(12):922–31.
37. Shylo NA, Christopher KJ, Iglesias A, Daluiski A, Weatherbee SD. TMEM107 is a Critical Regulator of Ciliary Protein Composition and is Mutated in Orofaciodigital Syndrome. *Human Mutation*. 2015 Oct 1;n/a-n/a.
38. Novarino G, Akizu N, Gleeson JG. Modeling Human Disease in Humans: the Ciliopathies. *Cell*. 2011 Sep 30;147(1):70–9.
39. Coene KLM, Roepman R, Doherty D, Afroze B, Kroes HY, Letteboer SJF, Ngu LH, Budny B, van Wijk E, Gordien NT, Azhimi M, Thauvin-Robinet C, Veltman JA, Boink M, Kleefstra T, Cremers FP, van Bokhoven H, de Brouwer AP. OFD1 is mutated in X-linked Joubert syndrome and interacts with LCA5-encoded lebercilin. *Am J Hum Genet*. 2009 Oct;85(4):465–81.
40. Field M, Scheffer IE, Gill D, Wilson M, Christie L, Shaw M, Gardner A, Glubb G, Hobson L, Corbett M, Friend K, Willis-Owen S, Gecz J. Expanding the molecular basis and phenotypic spectrum of X-linked Joubert syndrome associated with OFD1 mutations. *Eur J Hum Genet*. 2012 Jul;20(7):806–9.
41. Kroes HY, Monroe GR, van der Zwaag B, Duran KJ, de Kovel CG, van Roosmalen MJ, Harakalova M, Nijman IJ, Kloosterman WP, Giles RH, Knoers NV, van Haften G. Joubert syndrome: genotyping a Northern European patient cohort. *Eur J Hum Genet*. 2015 Apr 29;
42. Webb TR, Parfitt DA, Gardner JC, Martinez A, Bevilacqua D, Davidson AE, Zito I, Thiselton DL, Ressa JH, Aperi M, Schwarz N, Kanuga N, Michaelides M, Cheetham ME, Gorin MB, Hardcastle AJ. Deep intronic mutation in OFD1, identified by targeted genomic next-generation sequencing, causes a severe form of X-linked retinitis pigmentosa (RP23). *Hum Mol Genet*. 2012 Aug 15;21(16):3647–54.
43. Valente EM, Logan CV, Mougou-Zerelli S, Lee JH, Silhavy JL, Brancati F, Iannicelli M, Travaglini L, Romani S, Illi B, Adams M, Szymanska K, Mazzotta A, Lee JE, Tolentino JC, Swistun D, Salpietro CD, Fede C, Gabriel S, Russ C, Cibulskis K, Sougnez C, Hildebrandt F, Otto EA, Held S, Diplas BH, Davis EE, Mikula M, Strom CM, Ben-Zeev B, Lev D, Sagie TL, Michelson M, Yaron Y, Krause A, Boltshauser E, Elkhartoufi N, Roume J, Shalev S, Munnich A, Saunier S, Inglehearn C, Saad A, Alkindy A, Thomas S, Vekemans M, Dallapiccola B, Katsanis N, Johnson CA, Attié-Bitach T, Gleeson JG. Mutations in TMEM216 perturb ciliogenesis and cause Joubert, Meckel and related syndromes. *Nat Genet*. 2010 Jul;42(7):619–25.
44. Bisschoff IJ, Zeschnigk C, Horn D, Wellek B, Rieß A, Wessels M, Willems P, Jensen P, Busche A, Bekkebraten J, Chopra M, Hove HD, Evers C, Heimdal K, Kaiser AS, Kunstmann E, Robinson KL, Linné M, Martin P, McGrath J, Pradel W, Prescott KE, Roesler B, Rudolf G, Siebers-Renelt U, Tyshchenko N, Wiczorek D, Wolff G, Dobyns WB, Morris-Rosendahl DJ. Novel mutations including deletions of the entire OFD1 gene in 30 families with type 1 orofacioidigital syndrome: a study of the extensive clinical variability. *Hum Mutat*. 2013 Jan;34(1):237–47.
45. Shaheen R, Faqeih E, Alshammari MJ, Swaid A, Al-Gazali L, Mardawi E, Ansari S, Sogaty S, Seidahmed MZ, AlMotairi MI, Farra C, Kurdi W, Al-Rasheed S, Alkuraya FS. Genomic analysis of Meckel-Gruber syndrome in Arabs reveals marked genetic heterogeneity and novel candidate genes. *Eur J Hum Genet*. 2013 Jul;21(7):762–8.

46. Shaheen R, Ansari S, Mardawi EA, Alshammari MJ, Alkuraya FS. Mutations in TMEM231 cause Meckel-Gruber syndrome. *J Med Genet*. 2013 Mar;50(3):160–2.
47. Kim SK, Shindo A, Park TJ, Oh EC, Ghosh S, Gray RS, Lewis RA, Johnson CA, Attie-Bittach T, Katsanis N, Wallingford JB. Planar cell polarity acts through septins to control collective cell movement and ciliogenesis. *Science*. 2010 Sep 10;329(5997):1337–40.
48. Cortés CR, McInerney-Leo AM, Vogel I, Rondón Galeano MC, Leo PJ, Harris JE, Anderson LK, Keith PA, Brown MA, Ramsing M, Duncan EL, Zankl A, Wicking C. Mutations in human C2CD3 cause skeletal dysplasia and provide new insights into phenotypic and cellular consequences of altered C2CD3 function. *Sci Rep*. 2016;6:24083.
49. Williams CL, Li C, Kida K, Inglis PN, Mohan S, Semene L, Bialas NJ, Stupay RM, Chen N, Blacque OE, Yoder BK, Leroux MR. MKS and NPHP modules cooperate to establish basal body/transition zone membrane associations and ciliary gate function during ciliogenesis. *J Cell Biol*. 2011 Mar 21;192(6):1023–41.
50. Perrault I, Halbritter J, Porath JD, Gérard X, Braun DA, Gee HY, Fathy HM, Saunier S, Cormier-Daire V, Thomas S, Attié-Bitach T, Boddaert N, Taschner M, Schueler M, Lorentzen E, Lifton RP, Lawson JA, Garfa-Traore M, Otto EA, Bastin P, Caillaud C, Kaplan J, Rozet JM, Hildebrandt F. IFT81, encoding an IFT-B core protein, as a very rare cause of a ciliopathy phenotype. *J Med Genet*. 2015 Aug 14;jmedgenet-2014-102838.

Legends

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-shaped 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 S1: Clinical data of all OFD cases with exome analysis (patients 1-24) and only OFD patients from the replication cohort (patients 25-29) with causal mutations. NA: Not Available, AO: oculomotor apraxia, AVSD: atrio-ventricular septal defects, B: brachydactyly, C: clinodactyly, CCA: Corpus callosum agenesis, DWM: Dandy-Walker malformation, F: female, FB: frontal bossing, PF: upslanting palpebral fissures, HH: Hypothalamic hamartoma, HM: hypermetropia, HN: hypoplasia of the alae of nose, ID: Intellectual disability, IVC: Intra-ventricular communication, LSE: low-set ears, M: male, MP: mesoaxial polydactyly, MR: micro/retrognathia, MTS: Molar Tooth Sign, NL: The Netherlands, P: polydactyly, PMD: psychomotor delay, PoP: Postaxial polydactyly, PrP: Pre-axial polydactyly, PSD: primary septal defect, S: syndactyly, ToF: teratology of Fallot, Y: Y-shaped metacarpal.

Table 1: OFD genes identified by whole-exome sequencing or targeted gene sequencing

Table 2: Summary of OFD phenotypes as well as localization and function of OFD proteins

Table 3: Novel classification of OFDs based on the association between clinical and molecular features

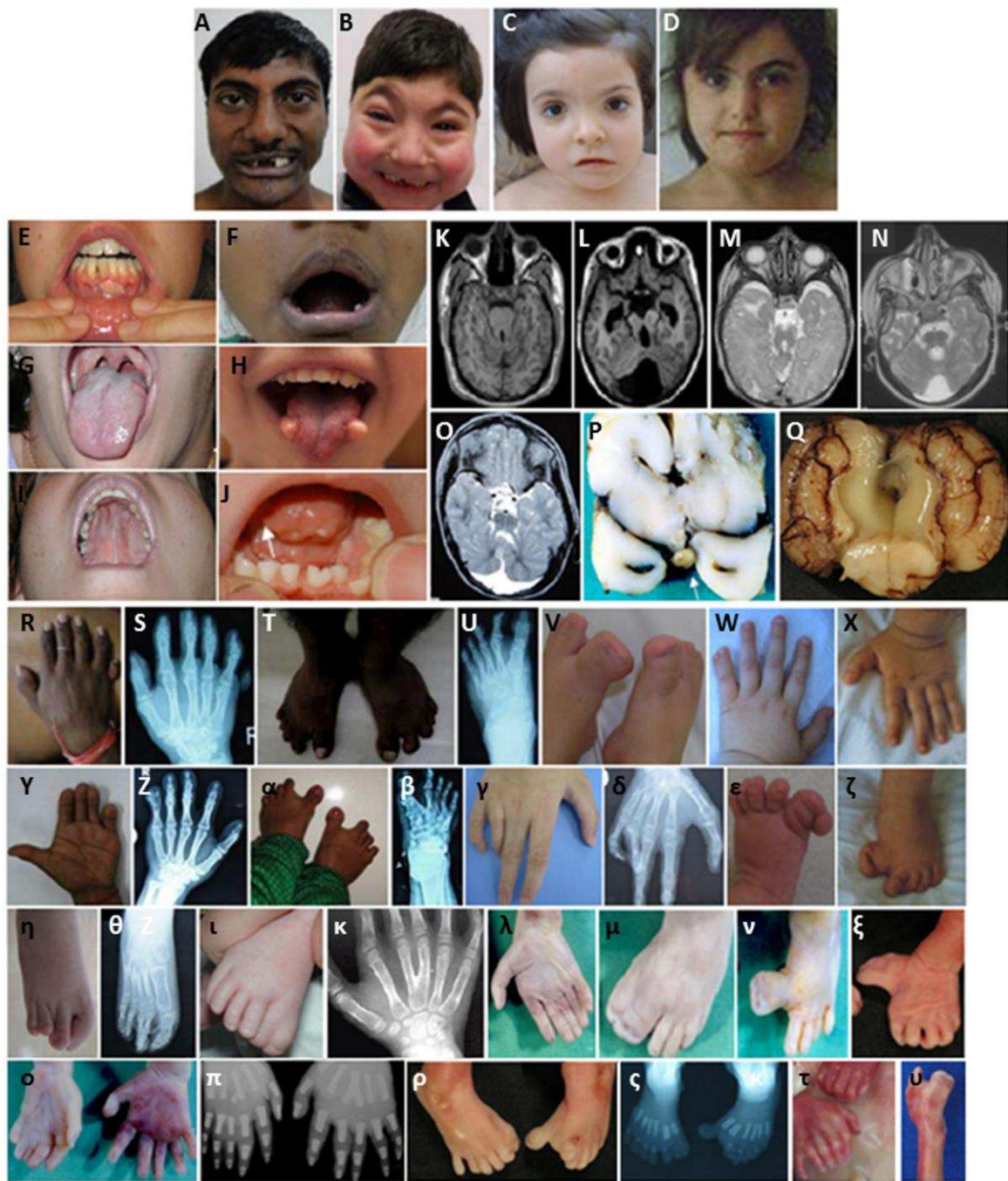


Figure1

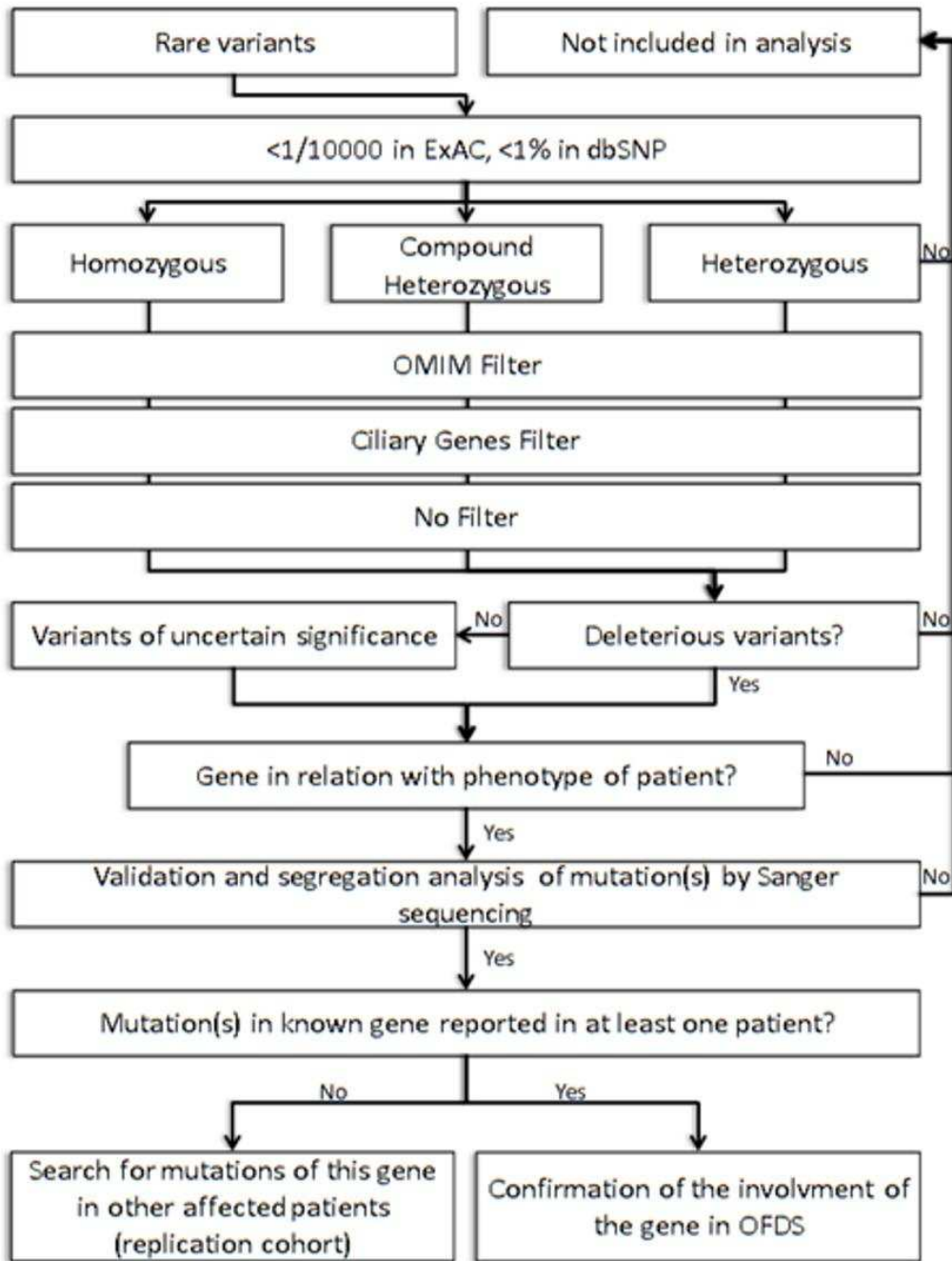


Figure 2

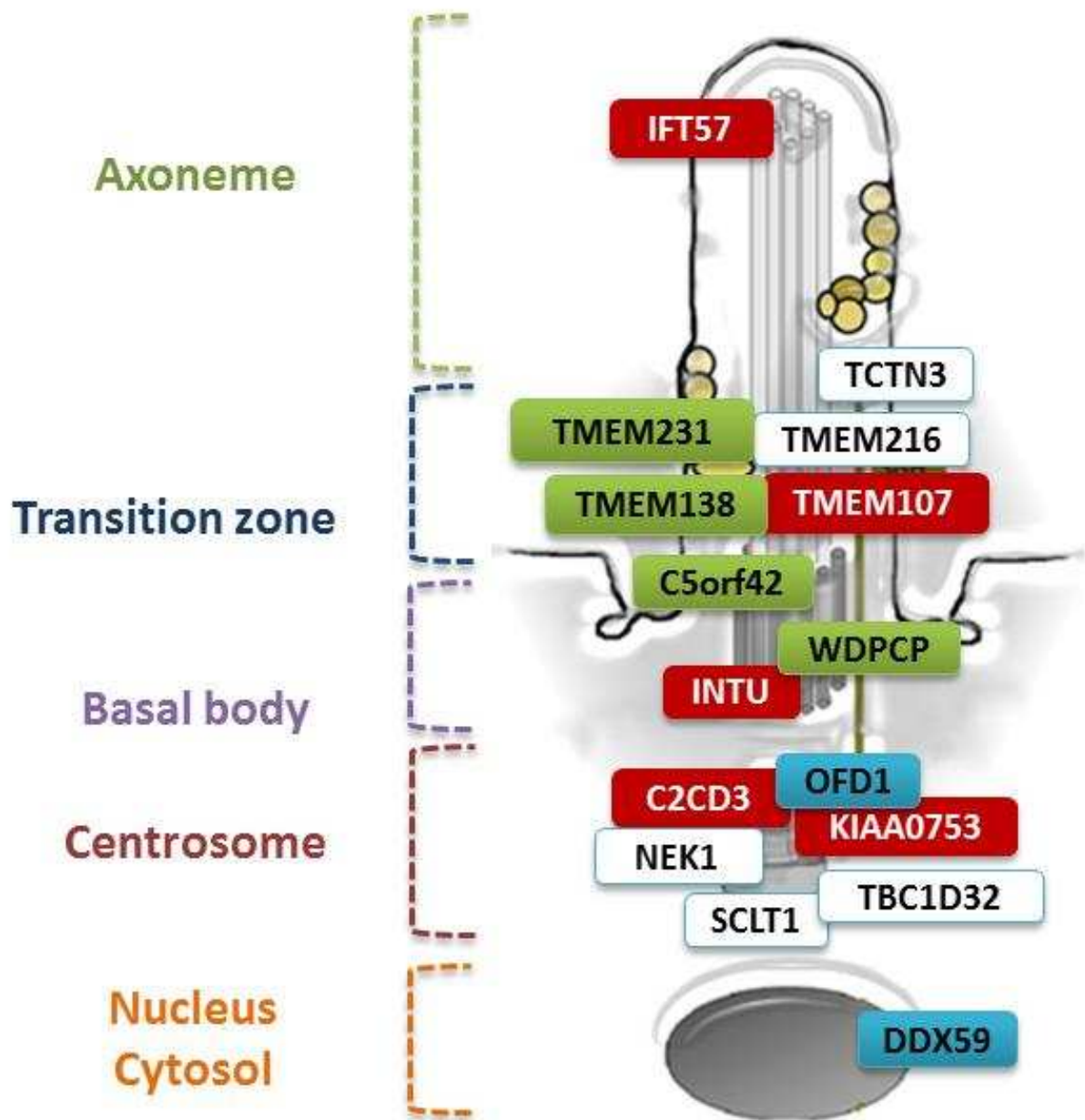


Figure 3

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 c.754G>A	p.Gly252Arg p.Gly252Arg	Maternal Paternal	-	-	-
2	TMEM138	+	Joubert syndrome 16 [614465]	c.352A>T c.352A>T	p.Met118Leu p.Met118Leu	NA NA	-	-	-
3a/b	TMEM107	+	-	c.134A>G c.134A>G	p.Glu45Gly p.Glu45Gly	Maternal Paternal	-	-	-
4	C2CD3	+	Oral-facial-digital syndrome XIV [615948]	c.184C>T c.184C>T	p.Arg62* p.Arg62*	Maternal Paternal	-	-	-
5	INTU	+	-	c.396delT c.396delT	p.Asn132Lysfs*11 p.Asn132Lysfs*11	NA NA	-	-	-
6a	IFT57	+	-	c.777G>A c.777G>A	p.Lys259Lys p.Lys259Lys	NA NA	-	-	Splice defect
7	C5orf42	+	Joubert syndrome 17 [614615]	3557delA c.3577C>T	Lys1186Argfs*22 p.Arg1193Cys	NA NA	-	-	-
8	C5orf42	+	Joubert syndrome 17 [614615]	c.3290-2A>G c.493delA	- p.Ile165Tyrfs*17	Maternal Paternal	- 1/6155	-	-
9	TMEM231	+	Joubert syndrome 20 [614970] Meckel syndrome 11 [615397]	c.656C>T c.532C>G	p.Pro219Leu p.Pro178Ala	Maternal Paternal	- -	-	-
10	WDPCP	+	Bardet-Biedl syndrome 15 [209900]	c.160G>A c.526_527delTT	p.Asp54Asn Leu176Ilefs*21	Paternal Maternal	1/11827 -	7/119586 -	-
11	KIAA0753	+	-	c.1546-3C>A c.1891A>T	- p.Lys631*	de novo Maternal	- -	-	Splice defect -
12	OFD1	+	Oral-facial-digital syndrome I [3111200]	c.260A>G	p.Tyr87Cys	de novo	-	-	-
13	OFD1	+	Joubert syndrome 10 [300804] Simpson-Golabi-Behmel syndrome 2 [300209]	c.1840delG	p.Ala614Hisfs*15	de novo	-	-	-
20	OFD1	+	Retinitis pigmentosa 23 [300424]	c.655-8A>G	-	de novo	-	-	-
Replication cohort									
25	C5orf42	+	Joubert syndrome 17 [614615]	c.3550C>T c.9121C>T	p.Arg1184Cys p.Gln3041*	Paternal Maternal	- -	-	-
26a/b	C5orf42	+	Joubert syndrome 17 [614615]	c.3150-1G>T c.3150-1G>T	- -	Maternal Paternal	- -	-	Splice defect Splice defect
27	C5orf42	+	Joubert syndrome 17 [614615]	c.2377C>T c.8509G>T	p.Gln793* p.Val2837Leu	Paternal Maternal	- -	2/ 22038 -	-
28b	C5orf42	+	Joubert syndrome 17 [614615]	c.493delA c.3380C>T	p.Ile165Tyrfs*17 p.Ser1127Leu	Paternal Maternal	- -	-	-

29	C2CD3	+	-	c.3085T>C c.3911-2A>T	p.Cys1029Gly -	NA	- 6/ 12978	- 31/ 120818	- Splice defect
----	-------	---	---	--------------------------	-------------------	----	---------------	-----------------	--------------------

NA: Not Available

Table 1: OFD genes identified by whole-exome sequencing or single gene sequencing

Gene	Protein localization	Functional protein complex	Protein function	OFD subtype	Pre-axial polydactyly	Post-axial polydactyly	Retinopathy	Renal anomaly	Cerebral malformation	MTS	Tibial dysplasia	Reference
OFD1	Centrosome/BB	OFD1-KIAA0753-FOPNL	Negative regulator of centriole elongation	OFDI	x			x	x			Ferrante et al., 2001
C2CD3	Centrosome/TF	-	Positive regulator of centriole elongation	OFDXIV		x	x		x			Thauvin-Robinet et al., 2014
KIAA0753/OFIP	Centrosome	OFD1-KIAA0753-FOPNL	Recruitment of OFD1 at centriole	OFD VI		x			x	x		Chevrier et al., 2015
SCTL1	Centrosome/TF	-	Unknown, ciliogenesis	OFDIX					x			Adly et al., 2013
TBC1D32	Centrosome	-	Unknown	OFDIX		x			x			Adly et al., 2013
DDX59	Cytosol/?	-	Regulation of ciliary signalling	OFDV		x						Present study
INTU	BB	CPLANE	IFT-A pre-assembly	OFDII?		x		x				Toriyama et al., 2016
WDPCP	BB	CPLANE	IFT-A pre-assembly	-		x						Toriyama et al., 2016
C5orf42	BB/TZ	CPLANE	IFT-A pre-assembly	OFDVI	x	x			x	x		Lopes et al., 2014
TCTN3	TZ	-	Regulation of ciliary signalling	OFDIV	x	x		x	x		x	Thomas et al., 2012
TMEM216	TZ	MKS	Ciliary gate formation	OFDVI	x	x			x			Valente et al., 2012
TMEM231	TZ	MKS	Ciliary gate formation	OFDVI?		x			x			Li et al., 2016
TMEM107	TZ	MKS	Ciliary gate formation	OFDVI		x	x		x	x		Lambacher et al., 2015
TMEM138	TZ	-	Vesicular transport	OFDVI					x	x		Li et al., 2016
IFT57	BB/Axoneme	IFT-B	Intraflagellar transport	-		x						Thevenon et al., 2016

Table 2: Summary of OFD phenotype as well as localization and function of OFD proteins

BB: Basal Body, OFD: Oral-Facial-Digital, TF: Transition fibers

OFD subtype	Clinical data	Genes
OFDI	Polycystic kidney disease, Corpus callosum agenesis	<i>OFD1</i>
OFDIV	Tibial dysplasia	<i>TCTN3</i>
OFDVI	Molar tooth sign	<i>TMEM216, TMEM231, TMEM138, C5orf42, TMEM107, KIAA0753</i>
Classification based on the genotype for other patients	Median cleft of the upper lip	<i>DDX59, NEK1</i>
	Cardiac defects	<i>INTU, WDPCP</i>
	Retinopathy	<i>SCLT1, TBC1D32/C7orf170</i>
	Severe microcephaly	<i>C2CD3</i>
	Chondrodysplasia	<i>IFT57</i>

Table 3: Novel classification of OFDS based on associated clinical feature and molecular basis