Expanding the genetic landscape of oral-facial-digital syndrome with two novel genes

Alanna Strong1,2 | Laurie Simone3 | Anthony Krentz4 | Courtney Vaccaro2 | Deborah Watson2 | Hayley Ron1 | Jennifer M. Kalish1,5 | Helio F. Pedro3 | Elaine H. Zackai1,5 | Hakon Hakonarson1,2,5,6

1Division of Human Genetics, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania
2The Center for Applied Genomics, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania
3Center for Genetic and Genomic Medicine, Hackensack University Medical Center, Hackensack, New Jersey
4PreventionGenetics, Marshfield, Wisconsin
5Department of Pediatrics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania
6Division of Pulmonary Medicine, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania

Correspondence
Hakon Hakonarson, Director, Center for Applied Genomics, Investigator, The Joseph Stokes, Jr. Research Institute, Children’s Hospital of Philadelphia Endowed Chair in Genomic Research, Professor of Pediatrics, Perelman School of Medicine at the University of Pennsylvania, 3615 Civic Center Boulevard, Philadelphia, PA 19104, USA.
Email: hakonarson@email.chop.edu

Funding information
Medical Genetics Research Training Grant, Grant/Award Number: 5T32GM008638-22; Institutional Development Fund

Abstract
Oral-facial-digital syndromes (OFDS) are a heterogeneous and rare group of Mendelian disorders characterized by developmental abnormalities of the oral cavity, face, and digits caused by dysfunction of the primary cilium, a mechanosensory organelle that exists atop most cell types that facilitates organ patterning and growth. OFDS is inherited both in an X-linked dominant, X-linked recessive, and autosomal recessive manner. Importantly, though many of the causal genes for OFDS have been identified, up to 40% of OFD syndromes are of unknown genetic basis. Here we describe three children with classical presentations of OFDS including lingual hamartomas, polydactyly, and characteristic facial features found by exome sequencing to harbor variants in causal genes not previously associated with OFDS. We describe a female with hypothalamic hamartoma, urogenital sinus, polysyndactyly, and multiple lingual hamartomas consistent with OFDVI with biallelic pathogenic variants in CEP164, a gene associated with ciliopathy-spectrum disease, but never before with OFDS. We additionally describe two unrelated probands with postaxial polydactyly, multiple lingual hamartomas, and dysmorphic features both found to harbor an identical TOPORS missense variant, c.29 C>A; (p.Pro10Gln). Heterozygous TOPORS pathogenic gene variants are associated with autosomal dominant retinitis pigmentosa, but never before with syndromic ciliopathy. Of note, both probands are of Dominican ancestry, suggesting a possible founder allele.

Keywords
CEP164, ciliopathy, oral-facial-digital syndrome, TOPORS

1 INTRODUCTION

Ciliopathy syndromes are rare Mendelian disorders caused by dysfunction of the primary cilium, a mechanosensory organelle that exists atop most cell types that facilitates proper organ patterning and growth (Berbari et al., 2009; Fry et al., 2014). Oral-facial-digital syndromes (OFDS) represent a heterogeneous group of ciliopathies, characterized by the core features of oral cavity malformations, such as tongue hamartomas, cleft palate, lobulated tongue, and hyperplastic frenula, craniofacial dysmorphisms such as down-slanted palpebral...
fissures, hypertelorism, and broad and flat nasal bridge, and digit malformations such as brachydactyly, polydactyly, and syndactyly (Guirri et al., 2007). Affected individuals can also have mild to severe intellectual impairment, structural brain differences, hypothalamic hamartoma, facial milia, coloboma, retinopathy, missing, extra or defective teeth, clinodactyly, structural heart differences, and polycystic kidneys (Bruel et al., 2017; Franco & Thauvin-Robinet, 2016).

Importantly, features of OFD can be seen in other ciliopathy syndromes, most commonly Joubert syndrome (JS), which is defined by the clinical triad of hypotonia, developmental delays, and a pathognomonic cerebellar and brain stem malformation referred to as a “molar tooth sign” (MTS) (Parisi & Glass, 2003).

Since its initial description in 1941 (Mohr, 1941), multiple OFDS types have been described with significant phenotypic overlap but with distinct patterns of organ system involvement and malformations (Bruel et al., 2017; Franco & Thauvin-Robinet, 2016). OFD is inherited in an X-linked dominant, X-linked recessive, and autosomal recessive manner. Causal genes have not been identified for approximately 40% of OFD types (Bruel et al., 2017; Franco & Thauvin-Robinet, 2016).

Here we describe three probands with a clinical diagnosis of OFDS VI. One patient was compound heterozygous for nonsense and frameshift CEP164 variants, and two unrelated probands of Dominican Republic descent were homozgyous for a c.29 C>A, p.(Pro10Gln) missense variant in TOPORS. We propose that CEP164 and TOPORS are novel causal genes for OFD VI, and suggest that the c.29 C>A allele is may represent a founder allele in the Dominican Republic population.

2 | METHODS

2.1 | Editorial policies and ethical considerations

All individuals and families agreed to participate in this study and signed appropriate consent forms. Permission for clinical photographs was given separately. This study was approved by the institutional IRB (Protocol # 16–013278).

2.2 | Genetic testing methodology

Chromosomal microarray for Patient 1 was performed at SUNY Upstate Medical University. Trio exome sequencing was performed on DNA from Patient 1 at GeneDx. Prenatal chromosomal microarray and Joubert Syndrome panel testing was performed on Patient 2 at GeneDx. Trio exome sequencing was performed for Patient 2 at PreventionGenetics. Prenatal chromosomal microarray and trio exome sequencing was performed for Patient 3 at GeneDx and for his affected sibling at Integrated Genetics. Patient 3 was recruited into the Center for Applied Genomics (CAG) at CHOP for exome reanalysis. Patients 2 and 3 were matched through GeneMatcher (Sobreira et al., 2015).

2.3 | Exome sequencing reanalysis

Under an Institutional Review Board approved protocol (Protocol # 16–013278), informed consent was obtained from the family of Patient 3 for enrollment in CAG. Variant annotation, filtration and prioritization was performed with GDCross, a variant annotation and prioritization platform developed within CAG. Variants with ≥5x coverage were initially filtered at 0.5% gnomAD MAF and annotated with a combination of multiple tools and databases, including Variant Effect Predictor, HGMD, ClinVar, dbsNP, OMIM, HPO, PolyPhen-2 and SIFT, and a custom-built splice-site annotator. The list of patient variants is filtered against the pedigree and HPO terms describing the patient’s phenotype, and GDCross assigns each variant a priority score of likelihood as the causal variant for the patient's disease. Variants are ranked using a weighted combination of multiple factors, including (a) overlap with HPO terms, (b) patient and family genotypes, (c) predicted functional impact, (d) inheritance modeling, and (e) presence in mutation databases such as HGMD and ClinVar.

3 | RESULTS

3.1 | Patient cohort

3.1.1 | Patient 1

Patient 1 was the product of a naturally-conceived pregnancy to a then 40-year-old GSP3-4 mother. Family history was notable for a 12-year-old brother with Trisomy 21 (conceived at 27 years of age) and a 14-year-old sister with suspected autoimmune aplastic anemia status post bone marrow transplant. There were no medications or exposures during the pregnancy. Noninvasive prenatal testing was done due to maternal age and was normal. Twenty-week ultrasound was also normal. An additional ultrasound was done at 29 weeks due to loss of the cervical mucus plug, and was notable for multiple congenital anomalies, prompting fetal MRI and echocardiogram, which were notable for polydactyly, brain cyst, absent vagina, and hydromecephalus. Patient was ultimately born via caesarian section at 33 weeks gestational age due to fetal tachycardia and worsening hydromecephalus. Birth weight was 3.115 kg (>97%; 50% for 36 weeks gestational age) and birth length was 43 cm (25–50%). She was admitted to the NICU for management. She was noted to have hypotonia, four-extremity polydactyly, syndactyly of multiple digits, multiple tongue hamartomas, and urogenital sinus. A suprapubic catheter was placed with improvement in her hydromecephalus and hydrometrocolpos. Echocardiogram and ophthalmology examination were normal. Head ultrasound showed a prominent temporal horn of the left lateral ventricle. Brain MRI showed hypothalamic hamartoma and asymmetric ventriculomegaly.

She was evaluated by Genetics at 3 months of age. Growth parameters were notable for a weight of 3.65 kg (10–25% corrected), height of
48.3 cm (<3%; 50% for newborn), and head circumference of 36.5 cm (75% corrected). Physical examination was notable for hypotonia, relative macrocephaly, down-slanting palpebral fissures, hypertelorism, broad and flat nasal bridge, upper alveolar ridge notch, multiple lingual hamartomas, accessory frenula, high palate, mild microglossia, and linear nevus simplex extending from the forehead to the upper lip (Figure 1a,b). Extremity examination was notable for a left hand with 2,3 and 4,5 syndactyly (Figure 1c), postaxial polydactyly of the right hand with 7 digits total and complete syndactyly of digits 5,6 (Figure 1d), postaxial polydactyly with complete 5,6 syndactyly of the left foot (Figure 1e), and postaxial polydactyly of the right foot (Figure 1f).

Presence of hypothalamic hamartoma, lingual hamartomas, polydactyly, and syndactyly was concerning for OFD VI (Poretti et al., 2012). Kidney ultrasound was performed, which showed moderate, symmetric hydronephrosis with no kidney cysts. An EEG was performed, which showed intermittent focal slowing in the bifrontal region and rare epileptiform discharges in the left temporal and right occipital regions. She underwent surgical removal of her lingual hamartomas and accessory frenula, and was admitted postoperatively due to concern for silent aspiration, later confirmed on video swallow study. She is currently NPO. Patient is now 6 months of age, gestationally corrected to 4 months of age. Motor milestones are delayed with minimal head control. Social skills are normal; patient fixes and follows, smiles, and is extremely interactive.

3.1.2 | Patient 2

Patient 2 was born to a 31-year-old G4P3→4 mother. Family history was noncontributory. Both parents were from the Dominican Republic. Pregnancy was complicated by gestational diabetes treated with metformin. Prenatal ultrasounds and follow up fetal MRI were notable for four extremity polydactyly, severely hypoplastic/absent cerebellar vermis, mildly enlarged posterior fossa, and micropenis. Patient was born via repeat caesarian section at 38 weeks gestational age. APGARS were 9 and 9 at 1 and 5 min of life, respectively. Birth weight was 3.73 kg (90%), birth length was 50.5 cm (75–90%), and head circumference was 37 cm (>97%).

Patient was diagnosed postnatally with hypotonia, multiple lingual hamartomas, four-extremity postaxial polydactyly, and micropenis. Echocardiogram demonstrated pulmonic valve stenosis, tricuspid valve regurgitation, and patent foramen ovale. MRI demonstrated arachnoid cyst and MTS.

Genetics evaluated the baby at 1 day of life and again at 3 months of life. At 4 months of life, weight was 8.9 kg (>97%; 50% for 9 months), length was 60.5 cm (25%), and head circumference was 44 cm (98%). Physical examination was notable for bilateral ptosis worse on the left, down-slanting palpebral fissures, broad nasal bridge, slightly low and posteriorly-rotated ears, multiple lingual hamartomas, 2/6 murmur, and hypotonia with significant head lag. Patient did not
3.2 | Genetics testing

3.2.1 | Patient 1

Patient 1 had a chromosomal microarray performed, which showed a 470 base pair duplication of unclear clinical significance at 5q35.2 (hg19: 175,668,563–176,138,247) including the NOP16, FAF2, SIMC1, CLTB, and SNCB genes. Parental testing was declined. Trio exome sequencing was performed at GeneDx, and was notable for a paternally-inherited pathogenic CEP164 frameshift variant (c.2535_2536dupGG; p. Glu846Glyfs*10) and a maternally-inherited pathogenic CEP164 nonsense variant (c.3055 C>T; p.Gln1019*).

3.2.2 | Patient 2

Patient 2 had a prenatal microarray and JS gene panel performed at GeneDx, which were nondiagnostic. Trio exome sequencing was performed postnatally at PreventionGenetics, and was notable for biallelic missense variants of uncertain significance in the candidate gene TOPORS (c.29 C>A; p.Pro10Gln) and a 6 Mb region of homozygosity on chromosome 9 (hg19: 32,425,909–38,423,826) including the TOPORS gene.

3.2.3 | Patient 3

Patient 3 had a prenatal microarray and trio exome sequencing performed at GeneDx. Microarray was notable for two regions of homozygosity on chromosomes 8 (hg19: 128,664,940–140,805,582) and 9 (hg19: 22,920,663–40,087,758) but no copy number variations. Exome sequencing was notable for a paternally-inherited pathogenic variant in PMM2 (c.713G>A; p.(Arg238His)), associated with the autosomal recessive congenital disorder of glycosylation PMM2-CDG. Microarray and trio exome sequencing were also performed on the previously affected fetus. Microarray was notable for two regions of homozygosity on chromosomes 7 (hg19: 44,098,864–58,019,983) and 9 (hg19: 25,000,261–40,087,758). Patient and family were enrolled in CAG for research exome reanalysis given negative clinical testing. Reanalysis revealed biallelic variants of uncertain significance in TOPORS (c.29 C>A; p.Pro10Gln) in the affected proband. These variants were clinically confirmed via gene sequencing at PreventionGenetics. Integrated Genetics subsequently confirmed that the affected fetus also harbored the TOPORS variants. The Pro10Gln variant was subsequently upgraded to likely pathogenic for both patients by PreventionGenetics given the similar clinical presentations and identical variants. Of note, TOPORS maps to the region of homozygosity shared by both children.

4 | DISCUSSION

Oral facial digital syndromes (OFDS) are a group of rare ciliopathy syndromes characterized by the core features of oral cavity
malformations, facial dysmorphism, and digit anomalies (Bruel et al., 2017; Franco & Thauvin-Robinet, 2016). OFDS subtypes are classified based on pattern of associated features such as structural brain differences, intellectual disability, skeletal differences, congenital heart disease and kidney disease, with over 18 subtypes described to date (Table 1).

Over 16 genes have now been identified as causal for OFDS (Bruel et al., 2017). These genes encode components of the primary cilium, a mechanosensory organelle that facilitates organ growth and patterning (Berbari et al., 2009; Fry et al., 2014). OFDS genes localize to virtually every ciliary subsegment, including the basal body, centriole, transition zone, and axoneme (Figure 2). The exact mechanism by which ciliary dysfunction causes the clinical features of OFDS is unknown.

Though initially described 80 years ago, up to 40% of the described OFDS subtypes do not have an associated gene and many patients with a clinical diagnosis of OFDS have negative molecular testing, highlighting our incomplete understanding of the genetic landscape of these conditions (Bruel et al., 2017; Franco & Thauvin-Robinet, 2016). Additionally, many genes associated with OFDS are also associated with other ciliopathy syndromes, most commonly JS; however, genotype–phenotype correlation remains elusive, and it is difficult to use mutation type to predict which ciliopathy phenotype a patient will ultimately have (Lambacher et al., 2016; Shaheen et al., 2013; Valente et al., 2010).

| Gene          | OFD1 | NERI | TMEM231 | TCTN3 | DDX59 | C9ORF72 | OFD1 | TMEM107 | TMEM216 | CPLANE1 | CEP164 | TOPORS |
|---------------|------|------|---------|-------|-------|---------|-------|---------|---------|---------|--------|--------|
| Inheritance   | XLD  | AR   | AR      | AR    | AR    | AR      | AR    | AR      | AR      | AR      | AR     | AR     |
| Craniofacial features | | | | | | | | | | | | |
| Hypertelorism | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Telecanthus   | ++   | ++   | ++      | ++    | ++    | ++      | ++    | ++      | ++      | ++      | ++     | ++     |
| Cell lip      | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Cell polity   | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Accessory tine | + | + | | + | + | + | + | + | + | + | + |
| Lobulated tongue | + | | + | + | + | + | + | + | + | + | + |
| Cleft tongue  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Lingual hamartoma | + | + | + | + | + | + | + | + | + | + | + |
| Tooth defects | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Bilateral clefts | + | + | + | + | + | + | + | + | + | + | + |
| Micrognathia  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Polydactyly   | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Clinodactyly  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Brachydactyly | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Syndactyly    | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Oligodactyly  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Fimbrial aplasia | + | + | + | + | + | + | + | + | + | + | + |
| Short stature  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Corpus callosum agenesis | + | + | + | + | + | + | + | + | + | + | + |
| Ventriculomegaly | + | + | + | + | + | + | + | + | + | + | + |
| Molar tooth sign | + | + | + | + | + | + | + | + | + | + | + |
| Cardiac ventricle hypoplasia | + | + | + | + | + | + | + | + | + | + | + |
| Hypohydramnios | + | + | + | + | + | + | + | + | + | + | + |
| Hamartoma      | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Renal hypoplasia | + | + | + | + | + | + | + | + | + | + | + |
| Neural tube defect | + | + | + | + | + | + | + | + | + | + | + |
| Intellectual disability | + | + | + | + | + | + | + | + | + | + | + |
| Congenital heart disease | + | + | + | + | + | + | + | + | + | + | + |
| Renal disease  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Liver disease  | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Hearing loss   | +    | +    | +       | +     | +     | +       | +     | +       | +       | +       | +      | +      |
| Structural eye differences | + | + | + | + | + | + | + | + | + | + | + |

Notes: Overlapping features are highlighted in red. Given the hypertelorism, lingual hamartomas, tongue lobulations, polysyndactyly, hypothalamic hamartoma, renal tube defects, molar tooth sign and intellectual disability seen in our patients, we propose that our patients’ phenotypes are most consistent with OFD VI.

We also describe two novel genes identified in patients with clinical features consistent with OFD VI: CEP164, previously identified as causal for the ciliopathies nephronophthisis, JS and Bardet Biedl syndrome, and TOPORS, a gene associated with the autosomal dominant ciliopathy RP, but never before with syndromic ciliopathy. CEP164 maps to 11q23.3 and encodes centrosomal protein of 164 kDa, a component of the centriolar distal appendage. CEP164 is required for vesicular docking at the centriole, thereby enabling ciliogenesis (Graser et al., 2007). CEP164 has also been associated with coordination of the DNA damage response (Pan & Lee, 2009; Sivasubramaniam et al., 2008); however, this has recently been challenged (Daly et al., 2016). Morpholino-mediated cep164 knockdown in zebrafish disrupts the DNA damage pathway and causes nephronophthisis-spectrum disease and hydrocephalus (Chaki et al., 2012). Total body cep164 deficiency is embryonic lethal in mice, associated with holoprosencephaly, abnormal heart development, and gross patterning defects, and conditional cep164 deficiency in multiciliated cells causes hydrocephalus, lung disease, and infertility, supporting a role for CEP164 in ciliary function (Siller et al., 2017). Biallelic CEP164 variants have been identified in multiple individuals affected by ciliopathy-spectrum disease, including Leber congenital amaurosis, Bardet-Biedl syndrome, and JS with associated features including obesity, bronchiectasis, liver fibrosis, and intellectual disability (Chaki et al., 2012; Shamseldin et al., 2020). Pathogenic CEP164

Here we describe two novel genes identified in patients with clinical features consistent with OFD VI: CEP164, previously identified as causal for the ciliopathies nephronophthisis, JS and Bardet Biedl syndrome, and TOPORS, a gene associated with the autosomal dominant ciliopathy RP, but never before with syndromic ciliopathy. CEP164 maps to 11q23.3 and encodes centrosomal protein of 164 kDa, a component of the centriolar distal appendage. CEP164 is required for vesicular docking at the centriole, thereby enabling ciliogenesis (Graser et al., 2007). CEP164 has also been associated with coordination of the DNA damage response (Pan & Lee, 2009; Sivasubramaniam et al., 2008); however, this has recently been challenged (Daly et al., 2016). Morpholino-mediated cep164 knockdown in zebrafish disrupts the DNA damage pathway and causes nephronophthisis-spectrum disease and hydrocephalus (Chaki et al., 2012). Total body cep164 deficiency is embryonic lethal in mice, associated with holoprosencephaly, abnormal heart development, and gross patterning defects, and conditional cep164 deficiency in multiciliated cells causes hydrocephalus, lung disease, and infertility, supporting a role for CEP164 in ciliary function (Siller et al., 2017). Biallelic CEP164 variants have been identified in multiple individuals affected by ciliopathy-spectrum disease, including Leber congenital amaurosis, Bardet-Biedl syndrome, and JS with associated features including obesity, bronchiectasis, liver fibrosis, and intellectual disability (Chaki et al., 2012; Shamseldin et al., 2020). Pathogenic CEP164
variants are believed to cause ciliopathy-spectrum disease by disrupting ciliogenesis and possibly also through modulation of the DNA damage response and cell cycle control (Chaki et al., 2012; Graser et al., 2007; Pan & Lee, 2009; Sivasubramaniam et al., 2008). We hypothesize that Patient 1 presented with severe ciliopathy-spectrum disease because her nonsense and frameshift CEPI64 variants more significantly impair CEPI64 function. Indeed, patients with biallelic nonsense CEPI64 variants presented with Bardet-Biedl and JS phenotypes, as opposed to patients harboring missense variants, who presented with nephronophthisis and retinal disease (Chaki et al., 2012; Shamseldin et al., 2020).

**TOPORS** maps to 9p21.1 and encodes topoisomerase I-binding arginine/serine rich protein, a ubiquitously-expressed protein that localizes to the nucleus and basal body (Chakarova et al., 2011). TOPORS was originally identified in a screen for topoisomerase interacting proteins, and was later found to also interact with p53 (Haluska Jr et al., 1999; Zhou et al., 1999). TOPORS functions as an E3 ubiquitin ligase, and plays a role in protein ubiquitination and sumoylation (Pungaliya et al., 2007; Rajendra et al., 2004). Morpholin-mediated topors knockdown in zebrafish causes microphthalmia, kinked tail and body edema, all established ciliopathy phenotypes in fish (Chakarova et al., 2011). Heterozygous TOPORS variants are associated with the retinal ciliopathy retinitis pigmentosa (RP) (Bowne et al., 2008; Chakarova et al., 2007).

The mechanism by which heterozygous TOPORS variants cause disease is unknown. Biallelic TOPORS variants have not been previously reported. Given the localization of TOPORS at the ciliary base and in the nucleus, loss of TOPORS function could cause ciliopathy-spectrum disease by disrupting ciliary homeostasis directly or through dysregulated gene expression. We hypothesize that loss of TOPORS function disrupts ciliogenesis and ciliary function by interfering with the proper ubiquitination and turnover of ciliary proteins and morphogens (Figure 3). Disruption of the ciliary ubiquitin-proteasome system disrupts ciliogenesis, and biallelic variants in genes involved in regulation of the ubiquitin-proteasome system have been identified in ciliopathy patients (Gerhardt et al., 2015; Izawa et al., 2015; Kasahara et al., 2014).

All three TOPORS patients harbor identical biallelic missense variants (c.29 C>A; p.Pro10Gln) in TOPORS. TOPORS is intolerant to loss-of-function variants, with a pLI of 1. The Pro10Gln variant has been reported in gnomAD and ExAc at low allele frequency, and there are no homozygotes. In silico prediction tools such as polyphen and SIFT support pathogenicity. Given the overlapping patient phenotypes, the Pro10Gln variant was reclassified by PreventionGenetics as likely pathogenic. Interestingly, neither parent nor any relatives report vision difficulties or RP, suggesting that this variant is a hypomorph, insufficient to cause retinal disease in the heterozygous state, but severe enough to cause syndromic ciliopathy in the homozygous state.

Combined autosomal dominant and autosomal recessive inheritance has been reported for ciliopathies. Heterozygous PKD1 variants are the most common cause of adult-onset autosomal dominant polycystic kidney disease, while biallelic PKD1 variants are associated with
early and potentially neonatal-onset, severe polycystic kidney disease (Al-Hamed et al., 2019; Audrézet et al., 2016; Durkie et al., 2021). Heterozygous DNAJB11 variants are associated with autosomal dominant polycystic kidney disease, while biallelic variants cause Uvemark II syndrome or renal-hepatic-pancreatic dysplasia syndrome (Jordan et al., 2021). We propose that TOPORS has similar properties, with heterozygous variants causing the milder, organ-specific ciliopathy retinitis pigmentosa, and biallelic variants causing OFDS-spectrum syndromic ciliopathy.

Our identified TOPORS patients both presented with the clinical stigmata of OFDS, including oral cavity malformations, dysmorphic features, and polydactyly; however, Patient 2 also presented with MTS, which is pathognomonic for JS. The overlap between OFDS and JS is well established, with several reports of individuals harboring features of both syndromes (Aljeaid et al., 2019; Bhardwaj et al., 2018; Johnston et al., 2017; Wentzensen et al., 2015). Several genes are associated with both conditions, including C5ORF42, OFD1, TCTN3, TMEM107, TMEM216, and TMEM231, and JS and OFDS can be allelic to each other (Bruel et al., 2017; Coene et al., 2009; Franco & Thauvin-Robinet, 2016). We propose that TOPORS also bridges OFDS and JS. It is unknown at this time whether this TOPORS phenotype is specific to the Pro10Gln variant identified in our probands, or whether it can be generalized to other gene variants. It is also unclear why Patient 3 and his affected sibling presented with neural tube defects while Patient 2 presented with MTS. Of note, both families trace their ancestry to the Dominican Republic, suggesting a possible founder allele.

In conclusion, we report on two novel genes associated with OFD VI, TOPORS and CEP164, further expanding the genetic differential of OFDS. Our cases highlight the phenotypic and genetic diversity of this family of diseases, and again implicate the ubiquitin-proteasome system in ciliary disease.

ACKNOWLEDGMENT
Medical Genetics Research Training Grant 5T32GM008638-22 (Alanna Strong) and Institutional Development Fund (Hakon Hakonarson).

CONFLICT OF INTEREST
None.

AUTHOR CONTRIBUTIONS
Alanna Strong conceptualized and designed the study, evaluated Patients 1 and 3 clinically, helped in variant interpretation and data analysis, and drafted the manuscript. Laurie Simone and Hello Fernando Pedro helped evaluate Patient 2 and helped analyze phenotypic and genetic data from all patients. Hayley Ron and Jennifer Kalish helped evaluate Patient 3 and guided the phenotypic workup. Elaine Zackai helped evaluate Patient 3, guide the phenotypic workup and interpret the genetic variants identified. Anthony Krentz helped analyze the exome for Patient 2. Deborah Watson and Courtney Vaccaro facilitated genetic data collection and analysis for Patient 3. Hakon Hakonarson helped conceptualize and design the study, contributed to data analysis, critically reviewed and edited the manuscript, and provided funding for the work. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

DATA AVAILABILITY STATEMENT
Data sharing is not applicable to this article as no new data were created or analyzed in this study.

ORCID
Alanna Strong https://orcid.org/0000-0001-9261-244X
Jennifer M. Kalish https://orcid.org/0000-0003-1500-9713
Hakon Hakonarson https://orcid.org/0000-0003-2814-7461

REFERENCES
Al-Hamed, M. H., Alsaahan, N., Rice, S. J., Edwards, N., Nooreddeen, E., Alotaibi, M., Kurdi, W., Alnemer, M., Alileb, N., Ali, W., Al-Numair, N., Almejaish, N., Sayer, J. A., & Imtiaz, F. (2019). Biallelic PKD1 mutations underlie early-onset autosomal dominant polycystic kidney disease in Saudi Arabian families. Pediatric Nephrology, 34(9), 1615–1623.
Aljeaid, D., Lombardo, R. C., Witte, D. P., & Hopkin, R. J. (2019). A novel pathogenic variant in OFD1 results in X-linked Joubert syndrome with orofaciodigital features and pitting aplasia. American Journal of Medical Genetics. Part A, 179(6), 1010–1014.
Audrézet, M. P., Corbiere, C., Lebhab, S., Moriníere, V., Broux, F., Louillet, F., Fischbach, M., Zaloszyc, A., Cloarec, S., Merieau, E., Baudouin, V., Deschénes, G., Roussy, G., Maestri, S., Visconti, C., Boyer, O., Abel, C., Lahoche, A., Randrianiaivo, H., … Heidet, L. (2016). Comprehensive PKD1 and PKD2 mutation analysis in prenatal autosomal dominant polycystic kidney disease. Journal of the American Society of Nephrology, 27(3), 722–729.
Berbari, N. F., O’Connor, A. K., Haycraft, C. J., & Yoder, B. K. (2009). The primary cilium as a complex signaling center. Current Biology, 19(13), R526–R535.
Bhardwaj, P., Sharma, M., & Ahiuwalla, K. (2018). Joubert syndrome with Orofacial digital features. Journal of Neurosciences in Rural Practice, 9(1), 152–154.
Bowne, S. J., Sullivan, L. S., Gire, A. I., Birch, D. G., Hughbanks-Wheaton, D., Heckenlively, J. R., & Daiger, S. P. (2008). Mutations in the TOPORS gene cause 1% of autosomal dominant retinitis pigmentosa. Molecular Vision, 14, 922–927.
Bruel, A. L., Franco, B., Duffourd, Y., Thevenon, J., Jego, L., Lopez, E., Deleuze, J. F., Doummar, D., Giles, R. H., Johnson, C. A., Huynen, M. A., Chevrier, V., Burglen, L., Morleo, M., Desguerre, I., Pierquin, G., Doray, B., Gilbert-Dussardier, B., Reversade, B., … Thauvin-Robinet, C. (2017). Fifteen years of research on oral-facial-digital syndromes: From 1 to 16 causal genes. Journal of Medical Genetics, 54(6), 371–380.
Chakarova, C. F., Khanna, H., Shah, A. Z., Patil, S. B., Sedmak, T., Murga-Zamalloa, C. A., Papaioannou, M. G., Nagel-Wolfrum, K., Lopez, I., Munro, P., Cheetham, M., Koenekoop, R. K., Rios, R. M., Matter, K., Wolfrum, U., Swaroop, A., & Bhattacharya, S. S. (2011). TOPORS, implicated in retinal degeneration, is a cilia-centrosomal protein. Human Molecular Genetics, 20(5), 975–987.
Chakarova, C. F., Papaioannou, M. G., Khanna, H., Lopez, I., Waseem, N., Shah, A., Theis, T., Friedman, J., Maubaret, C., Bujakowska, K., Veralitch, B., el-Aziz, M. M. A., Prescott, D. Q., Parapuram, S. K., Bickmore, W. A., Munro, P. M. G., Gal, A., Hamel, C. P., Marigo, V., … Bhattacharya, S. S. (2007). Mutations in TOPORS cause autosomal dominant retinitis pigmentosa with perivascular retinal pigment epithelium atrophy. American Journal of Human Genetics, 81(5), 1098–1103.
