HHS Public Access

Author manuscript

Am J Med Genet A. Author manuscript; available in PMC 2024 August 01.

Published in final edited form as:

Am J Med Genet A. 2023 August ; 191(8): 2156–2163. doi:10.1002/ajmg.a.63303.

TOPORS as a novel causal gene for Joubert Syndrome

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Abstract

Joubert syndrome (JBTS) is a Mendelian disorder of the primary cilium defined by the clinical triad of hypotonia, developmental delay and a distinct cerebellar malformation called the molar tooth sign. JBTS is inherited in an autosomal recessive, autosomal dominant, or X-linked recessive manner. Though over 40 genes have been identified as causal for JBTS, molecular diagnosis is not made in 30–40% of individuals who meet clinical criteria. *TOPORS* encodes topoisomerase I-binding arginine/serine rich protein, and homozygosity for a *TOPORS* missense variant (c.29C>A; p.(Pro10Gln)) was identified in individuals with the ciliopathy oral-facial-digital syndrome in 2 families of Dominican descent. Here we report an additional proband of Dominican ancestry with JBTS found by exome sequencing to be homozygous for the identical p.(Pro10Gln) *TOPORS* missense variant. Query of the Mount Sinai Bio*Me* biobank, which includes 1880 individuals of Dominican ancestry, supports a high carrier frequency of the *TOPORS* p.(Pro10Gln) variant in individuals of Dominican descent. Our data nominates *TOPORS* as a novel causal gene for JBTS

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Author Contribution Statement: Conceptualization (AS, EHZ, HH); Data Curation (AS, MM, EHZ, SC, HQ, JG, HH, EEK); Formal Analysis (AS, HH, HQ, EHZ, JG, SC); Writing-original draft (AS, EHZ, HH); Writing-reviewing and editing (AS, HH, EHZ, EEK).

Conflict of Interest:
Other authors declare no financial or competing interests related to this manuscript.

and suggests that *TOPORS* variants should be considered in the differential of ciliopathy-spectrum disease in individuals of Dominican ancestry.

Introduction:

Joubert syndrome (JBTS, OMIM #213300) is a ciliopathy syndrome characterized by the triad of hypotonia, developmental delay, and a unique cerebellar and brain stem malformation detectable on MRI called the molar tooth sign (MTS) characterized by long, thick superior cerebellar peduncles, deep interpeduncular fossa, and cerebellar hypoplasia/aplasia (Doherty, 2009; Brancati et al., 2010). Frequent comorbidities include retinopathy, nephronopthisis or cystic kidney disease, biliary dysgenesis and hepatic fibrosis, endocrinopathies, and skeletal malformations (Parisi, et al., 2009). JBTS syndrome is inherited in an autosomal recessive, autosomal dominant, and X-linked recessive manner (Parisi, 2019; Serpieri et al., 2022). Over 40 genes have been implicated in JBTS and encode structural or functional components of the primary cilium, a mechanosensory organelle that exists atop most cell types and serves as a signaling hub to coordinate organogenesis and remodeling (Vilboux et al., 2017). Importantly, molecular diagnosis is not made in 30 – 40% of individuals who meet clinical criteria for JBTS due to our incomplete understanding of the genetic landscape of this disease (Bachmann-Gagescu et al., 2015; Serpieri et al., 2022).

TOPORS encodes topoisomerase I-binding arginine/serine rich protein (Weger et al., 2003). Heterozygous *TOPORS* variants are associated with non-syndromic retinitis pigmentosa in humans (Chakarova et al., 2007; Bowne et al., 2008) while a specific homozygous *TOPORS* missense variant (c.29C>A; p.(Pro10Gln)) was identified in 3 individuals (1 fetal and 2 pediatric cases) of Dominican ancestry with the ciliopathy syndrome oral-facial-digital syndrome (OFDS), characterized by oral cavity malformations, dysmorphic facial features, and digit abnormalities (Gurrieri et al., 2007; Strong et al., 2021). OFDS and JBTS share many features; however, MTS is universal in JBTS and not OFDS, and oral cavity malformations are universal in OFDS and not JBTS.

Here we report a proband of Dominican ancestry with a clinical diagnosis of JBTS found by clinical trio exome sequencing to be homozygous for the previously identified *TOPORS* p.(Pro10Gln) missense variant. Query of the Bio*Me* biobank at the Mount Sinai Health System in New York City, which includes 1880 individuals of Dominican descent, supports a high frequency of the p.(Pro10Gln) allele in individuals of Dominican ancestry. We nominate *TOPORS* as a novel causal gene for JBTS and highlight the importance of targeted carrier screening for the p.(Pro10Gln) *TOPORS* variant in individuals of Dominican descent.

Methods:

Editorial Policies and Ethical Considerations:

Probands and their families all agreed to participate in this study and signed appropriate consent forms. This study was approved by the Children's Hospital of Philadelphia (CHOP) Institutional Review Board (Protocol #16–013278). Permission for clinical photographs was provided separately.

Clinical Methods:

Patient was evaluated by Clinical Genetics at CHOP. The Comprehensive Brain Malformation Gene Panel was performed at GeneDx (https://www.genedx.com/tests/detail/comprehensive-brain-malformations-panel-770). Chromosomal microarray and trio exome sequencing were performed in the Division of Genomic Diagnostics at CHOP (clinical CLIA-certified laboratory) on genomic DNA extracted from peripheral blood. For trio exome sequencing, targeted regions were captured with the Agilent SureSelect XT Clinical Research Exome V2 kit and sequenced on the Illumina NovaSeq 6000 platform with 100 base pair paired-end reads. Sequencing data was processed using an in-house bioinformatics pipeline that analyzed coding exons and splice sites. According to best practices for variant calling in clinical sequencing, the next generation sequencing data was visually reviewed for all reported variants in the Integrative Genomics Reviewer and passed all quality control metrics (Kobolt, 2020).

Calculating the TOPORS Allele Frequency:

Allele frequency of the c.29C>A; p.(Pro10Gln) *TOPORS* variant was calculated using data from the Bio*Me* Biobank (Belbin et al., 2021), a biorepository linked to electronic health records within the Mount Sinai healthcare system in New York City. Since its establishment in 2007, over 70,000 patients of diverse ancestries have been non-selectively enrolled. All participants were asked multiple choice questions at enrollment regarding their heritage and country-of-birth of self, parents, and grandparents (IRB Protocol 07–0529). Genetically inferred ancestry information was used to designate sub-population groups as described in detail in Belbin *et al* (Belbin et al., 2021). One genetically inferred sub-group (N=2023) had a high confidence of predicting (Positive predictive value>0.9) participants who were born, or who had parents or grandparents born, in the Dominican Republic and was designated the Dominican sub-group. Exome data is available for over 30,000 participants through a collaboration with Regeneron (Abul-Husn et al., 2021). Exome data for a sub-set of the Dominican participants (N=1880) were available and were queried for the frequency of the c.29C>A; p.(Pro10Gln) *TOPORS* variant.

Runs of Homozygosity (ROH) and Haplotype Analysis:

The ROH analysis was performed using genotyping data generated on the Illumina GSA-24v3 single-nucleotide polymorphism (SNP) array platform. Data analysis was done using the PLINK software (Purcell et al., 2007; Chang et al., 2015). Regions of homozygosity were first identified in the index patient and were subsequently aligned to regions of homozygosity identified in the previously published proband (Strong et al., 2021). Principal component analysis (PCA) was performed with these cases and 3226 controls randomly selected from the biorepository at the Center for Applied Genomics (CAG) also genotyped on the GSA-24v3 array (Supplemental Figure 1). Based on the top 20 principal components, we identified 34 PCA neighbors. From these individuals, haplotype blocks including 5 or more SNPs were examined and compared between the cases to determine shared haplotypes across different ancestries.

Results:

Case Presentation:

Pregnancy was conceived naturally to a 40-year-old G5P0→1 mother. Pregnancy was complicated by prenatal ultrasound findings of cerebellar vermis hypoplasia/aplasia, atypical cerebellar peduncles, abnormal upper extremity posturing, bilateral lower extremity post-axial polydactyly, unilateral upper extremity polydactyly, hypoplastic ears and mild nasal bone hypoplasia. Patient was born at 37 + 6 weeks gestational age via vaginal delivery. Birth weight was 3.385 kg (63%), length was 48 cm (20%), and head circumference was 36 cm (97%). Physical examination showed macrocephaly, large fontanel, hypertelorism, down-slanting palpebral fissures, overfolded superior ear helices, long philtrum, high palate, bilateral ulnar deviation, bilateral lower extremity and right upper extremity postaxial polydactyly, and axial hypotonia (Figure 1A-D). Brain ultrasound showed severe hypoplasia/aplasia of the cerebellar vermis, and MRI showed a hypoplastic cerebellar vermis with an asymmetric and small left cerebellar peduncle, consistent with “decaying MTS” (Figure 1E). EEG showed diffuse cerebral dysfunction. Endocrinology, Nephrology, Ophthalmology, Cardiology, Gastroenterology and Genetics were consulted for concern for JBTS. There was no evidence of pituitary, cardiac, liver or kidney dysfunction. Initial Ophthalmology examination showed birth-associated retinal hemorrhages; repeat examination at 5 weeks of life showed esotropia and bilateral ptosis with possible congenital fibrosis of the extra-ocular muscles. Patient is currently 8 months of age. Clinical course has evolved to include central and obstructive apnea complicated by bradycardia and desaturations with tracheostomy dependence, G-tube dependence, and global developmental delay with intermittent fixing and following and smiling.

Initial genetic testing included a chromosomal microarray and the Comprehensive Brain Malformation Panel (GeneDx, https://www.genedx.com/tests/detail/comprehensive-brain-malformations-panel-770). Microarray was notable for 2 regions of homozygosity (5.81 Mb on chromosome 1 (Chr1:171947018–177755827) and 46.92 Mb on chromosome 9, (Chr9:32427874–79348193)). Parents are both of Dominican Republic ancestry, but there is no known consanguinity. Clinical trio exome sequencing (Children’s Hospital of Philadelphia) showed homozygosity for biallelic variants of uncertain significance in *TOPORS* (c.29C>A; p.(Pro10Gln)), present in heterozygosity in the mother and father (Supplemental Figure 2).

Of note, there is a history of 3 prior first-trimester losses and one intrauterine fetal demise at 35 weeks gestation after a pregnancy complicated by macrocrania, holoprosencephaly, fixed upper extremities, and polydactyly (Supplemental Figure 3). Microarray showed a 16 Mb region of homozygosity on chromosome 9. No DNA was available for *TOPORS* testing.

Variant Allele Frequency and Shared Haplotype Structure:

All individuals identified to date with *TOPORS*-related ciliopathy spectrum disease harbor the p.(Pro10Gln) missense variant and are of Dominican ancestry. To determine if this variant is exclusive to individuals of Dominican ancestry, we queried the gnomAD database (version 3.1) and identified 9 allele carriers (9/152,230; allele frequency 0.00005912). Four

of these individuals are of African/African American descent (4/41468; allele frequency 0.00009646) and 5 are of Latino/Admixed American ancestry (5/15292; allele frequency 0.00032697).

The gnomAD database does not specifically separate individuals of Dominican ancestry. To more precisely determine the p.(Pro10Gln) allele frequency in individuals of Dominican descent, we queried the Mount Sinai Bio*Me* Biobank, which includes 1880 participants of Dominican ancestry. We identified 20 heterozygous allele carriers and no homozygous individuals, corresponding to a heterozygous allele frequency of 0.005.

Three of the 4 identified *TOPORS* probands have a stretch of homozygosity on chromosome 9 that includes the *TOPORS* gene (Figure 2). We performed SNP-based microarray genotyping and identified extended regions of homozygosity (ROH) in the index proband (Chr9[hg19]:22920663 – 40087758, 17.2 MB) and the previously-reported proband (Chr9[hg19]:32427874 – 79348193, 46.9 MB) for an overlapping identical-by-descent (IBD) haplotype of Chr9[hg19]:32427874 – 40087758 (7.66 MB).

Further analysis using the 34 PCA neighbors identified a single 140 kB haplotype block (Chr9[hg19]:34170980 – 34310927) within the shared region of homozygosity 1.6 MB upstream of *TOPORS*, which contains homozygous variants that are shared by this proband and our previously-reported proband (rs2769710_G-rs4319185_C-rs7020934_T-rs2890545_T-rs10972043_T-rs17350674_C-rs10972048_C). This block also harbors 2 OMIM genes, *KIF24* and *UBAP1*. Query of biobank at the Center for Applied Genomics at CHOP demonstrates that this haplotype is common in individuals of European ancestry but is rare in individuals of African and Asian ancestry (Table 1).

Discussion:

We report biallelic missense *TOPORS* variants in a proband with JBTS, nominating *TOPORS* as a novel causal gene for JBTS. The c.29C>A; p.(Pro10Gln) *TOPORS* missense variant identified in this patient has been previously described in homozygosity in 3 probands from two unrelated families with the ciliopathy OFDS (Strong et al., 2021).

The current proband and the 3 previously reported individuals with biallelic *TOPORS* variants (1 fetal and 2 pediatric cases) have significant clinical overlap, including macrocephaly, hypertelorism, down-slanting palpebral fissures, ptosis, polydactyly, respiratory failure, and severe developmental delay with seemingly normal liver and kidney structure and function (Table 2). Of note, all reported *TOPORS* probands are below 3 years of age, and kidney and liver disease is often a later disease manifestation.

Patient 2 in Strong *et al.* carried a clinical diagnosis of OFDS due to oral cavity malformations, polydactyly and dysmorphisms, but also had MTS on MRI. MTS, the pathognomonic structural brain difference seen in JBTS has been seen in individuals with a clinical diagnosis OFDS in a unique OFDS subtype called OFDS VI or Varadi-Papp syndrome (Munke et al., 1990; Poretti et al., 2012; Darmency-Stamboul et al., 2013). Interestingly, congenital fibrosis of the extra-ocular muscles, as suspected in our patient, has been reported Varadi--Papp syndrome (Rajamani et al., 2017).

Genetic overlap between JBTS and OFDS is also a well-described phenomenon. Pathogenic variants in *OFD1, CPLANE1, TMEM107,* and *TMEM216* are associated both with OFDS and JBTS (Franco and Thauvijn-Robinet, 2016; Bujakowska, Liu and Pierce, 2017; Mitchison and Valente, 2017). This case adds *TOPORS* to the growing list of genes associated with both OFDS and JBTS.

The molecular basis of the specific overlap between JBTS and OFDS is unknown, though many of the causative genes implicated in both syndromes localize to the centriole/basal body and have known roles in ciliogenesis, suggesting that the JBTS/OFDS pathology may be related to basal body dysfunction (Parisi, 2019).

TOPORS maps to 9p21.1 and encodes topoisomerase I-binding arginine/serine rich protein, a protein that localizes to the nucleus and basal body (Weger, Hammer and Engstler, 2003; Chakarova et al., 2011). *TOPORS* plays a critical role in protein ubiquitination and sumoylation and genome stability (Pungaliya et al., 2007; Marshall et al., 2010). Though heterozygous *TOPORS* variants are associated with non-syndromic retinitis pigmentosa in humans (Chakarova et al., 2007; Bowne et al., 2008), studies in animal models and the addition of these clinical cases suggest a role for *TOPORS* beyond the retina. Specifically, morpholino-mediated *topors* knockdown in zebrafish causes microphthalmia, kinked tail and body edema, and *topors* deficient mice have poor growth and decreased viability of unclear etiology (Marshall et al., 2010; Chakarova et al., 2011). The mechanism by which pathogenic *TOPORS* variants cause ciliopathy-spectrum disease is unknown. The role of genome stability and DNA damage in the pathogenesis of ciliopathy-spectrum disease is becoming increasingly appreciated (Chaki et al., 2012; Choi et al., 2013; Airik et al., 2014; Grampa et al., 2016; Johnson et al., 2016; Macia et al., 2017). Further studies are required to determine the effect of pathogenic *TOPORS* variants on ciliogenesis, ciliary signaling and the DNA damage response. It is also interesting that although ciliopathy-spectrum disease is associated with DNA damage and cilia are intricately linked to the cell cycle, ciliopathy syndromes are not classically associated with malignancy, suggesting a distinct cell cycle regulation and DNA damage response coordinated by the cilium.

The etiology for the disparate phenotypes in individuals with heterozygous versus homozygous *TOPORS* variants (isolated retinal disease versus syndromic ciliopathy) is unknown. The heterozygous variants associated with retinitis pigmentosa could act via a dominant-negative mechanism or could be true loss-of-function alleles to which the retina is particularly sensitive. Conversely, the p.(ProGln10) *TOPORS* variant associated with autosomal recessive disease may represent a loss-of-function, hypomorphic allele, with sufficient residual activity in heterozygotes to be clinically silent. Of note, none of the parents of the published probands with *TOPORS* variants (confirmed carriers) have retinal disease. Heterozygous versus homozygous/compound heterozygous variants in ciliopathy genes causing isolated versus multiorgan disease has been described (Senum et al., 2022).

All probands identified with the missense p.(Pro10Gln) variant are of Dominican ancestry. Query of gnomAD and the Mount Sinai Bio*Me* Biobank suggests an enrichment of the p.(Pro10Gln) variant in individuals of Dominican ancestry (allele frequency of 0.00006 versus 0.005, respectively). Array-based homozygosity mapping identified an

extended IBD region in our 2 probands at Chr9[hg19]:32427874 – 40087758 that includes *TOPORS*. This region also includes *KIF24* and *DCTN3*, which encode proteins involved in cilium assembly (https://reactome.org/PathwayBrowser/#/R-HSA-5617833), but no other ciliopathy-associated genes (https://pathcards.genecards.org/card/ciliopathies). Our two probands share an identical homozygous haplotype within this block localizing 1.6 MB upstream of *TOPORS*. Interestingly, this haplotype is common in individuals of European and Latin American ancestry, but is much less common in individuals of African and East Asian ancestry. Two OMIM genes map within this haplotype: *UBAP1*, a component of the endosomal sorting machinery associated with autosomal dominant spastic paraplegia, and *KIF24*, encoding a kinesin motor protein that plays a role in ciliary disassembly associated with ciliopathy-spectrum skeletal dysplasia (Farazi Fard et al., 2019 and Reilly et al., 2022). SNP markers in this haplotype have been associated with an eQTL of *KIF24* in brain (cerebellum, frontal cortex), GI tract (sigmoid colon, esophagus, gastroesophageal junction), skeletal muscle, and testes (https://www.gtexportal.org/home/gene/KIF24). It is possible that genetic polymorphisms within this common European haplotype ameliorate the ciliopathy phenotype seen with biallelic *TOPORS* variants, explaining the seeming absence of *TOPORS*-related ciliopathy in individuals of European ancestry. It is also possible that *TOPORS*-related disease has not been reported in individuals of European ancestry due to the lower variant allele frequency and the only recent discovery of *TOPORS* as a ciliopathy-associated gene. Further work is required to determine the significance of this upstream haplotype to *TOPORS*-related disease.

Based on the now 4 published patients in the literature, we nominate *TOPORS* as an important contributor to the genetic landscape of ciliopathy-spectrum disease, identify a novel genetic cause of JBTS syndrome, and recommend that *TOPORS* be included in pre-conception carrier screening for individuals of Dominican descent and be on the differential for individuals of Dominican ancestry presenting with ciliopathy-spectrum disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements:

The authors wish to acknowledge the participating families for their time and contribution to this study.

Funding Sources:

The authors also wish to acknowledge their funding sources: NIH - K08DK128606 (AS), U01HG009080 (EEK and SC).

Ethics Declaration:

The Institutional Review Boards of the Children's Hospital of Philadelphia (Protocol number 16–013278) and Mount Sinai (IRB Protocol 07–0529) approved this study. All studies were performed in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants and/or their legal guardians. Permission for use of clinical photographs was given separately. All clinical data was deidentified as part of this study.

Eimear Kenny has received personal fees from Regeneron Pharmaceuticals, 23&Me, Allelica, and Illumina; has received research funding from Allelica; and serves on the advisory boards for Encompass Biosciences, Foresite Labs, and Galateo Bio.

Data Availability Statement:

Microarray genotyping data may be provided upon request where individual consent permits.

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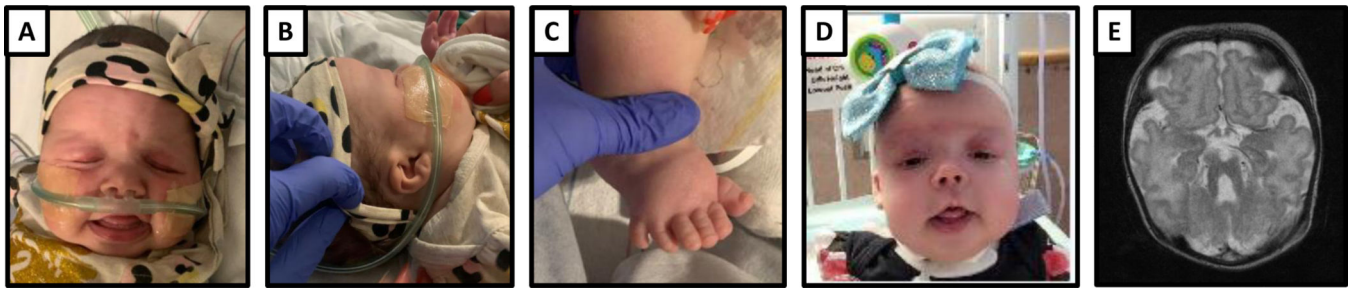

Figure 1:

Patient Photos A) Proband at 3-months of life with macrocephaly and downslanting palpebral fissures B) Proband right ear at 3-months of life with overfolded helix C) Right lower extremity at 3-months of life demonstrating post-axial polydactyly D) Proband at 7-months of life with macrocephaly, hypertelorism and down-slanting palpebral fissures E) T2-weighted MRI images on day of life 4 demonstrating decaying molar tooth sign

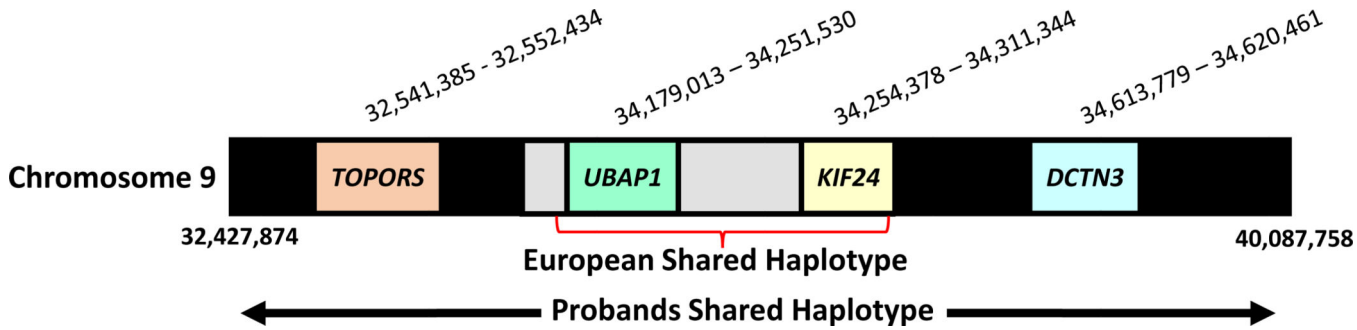

Figure 2:

Schematic of the identified haplotype blocks and select genes on chromosome 9. The shaded black area represents the common haplotype identified in the 2 probands (7.66 MB). The gray shaded area represents the common European haplotype (140 kB). Coordinates are based on the hg19 reference genome sequence.

Table 1:

Frequency of the shared haplotype in different populations

Population	Number of Individuals Homozygous for The Common Upstream Haplotype	Number of Individuals Heterozygous for The Common Upstream Haplotype	Number of Individuals With The Common Upstream Haplotype	Total Number of Individuals Queried	Allele Frequency
European	1616	3864	5480	8270	0.429
African	4	82	86	996	0.045
East Asian	2	60	62	453	0.071

Table 2:

Phenotypes of previously-reported and current *TOPORS* patients

	Current Patient	Strong *et al.* Patient 2	Strong *et al.* Patient 3
Genetic Information			
Inheritance	Autosomal Recessive	Autosomal Recessive	Autosomal Recessive
Base Pair Change	c.29 C > A	c.29 C > A	c.29 C > A
Amino Acid Change	p.(Pro10Gln)	p.(Pro10Gln)	p.(Pro10Gln)
TOPORS ROH	Yes	No	Yes
Clinical Diagnosis	Joubert Syndrome	Oral-facial-digital syndrome with Joubert features	Oral-facial-digital syndrome
Craniofacial			
Head	Macrocephaly	Macrocephaly	Macrocephaly
Hypertelorism	+	No	+
Ptosis	+	+	+
Downslanting Palpebral Fissures	+	+	+
Palate	Arched palate	Normal	Cleft palate
Tongue	Normal	Lingual hamartomas	Cleft tongue, lingual hamartomas
Ears	Overfolded superior helices	Low set, posteriorly rotated	
Ophthalmology			
Eye Malformations	Congenital fibrosis of the extra-ocular muscles	Unknown	Optic nerve colobomas, small optic nerves
Cardiac			
Congenital Heart Disease	No	Pulmonic stenosis, tricuspid regurgitation	No
Pulmonary			
Central/Obstructive Apnea	+	No	+
Tracheostomy Dependence	+	No	+
Gastroenterology			
Feeding	G-tube dependence	No	G-tube dependence
Liver	Normal	Normal	Normal
Endocrine			
Short Stature	+	No	+
Pituitary Insufficiency	No	No	+
Genitourinary			
Genitalia		Micropenis	Ambiguous genitalia
Kidney	Normal Ultrasound	Normal ultrasound	Normal Ultrasound
Musculoskeletal			
Polydactyly	+	+	+
Other	No	No	Arthrogryposis, talipes equinovarus
Neurology			
Molar tooth sign	+	+	No

	Current Patient	Strong *et al.* Patient 2	Strong *et al.* Patient 3
Cerebellar vermis hypoplasia	+	+	+
Neural tube defect	No	No	Occipital meningocele
Hypotonia	+	+	+
Developmental Delay	+	+	+