Phenotypic manifestations of C5orf42 pathogenic variants

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ABSTRACT

Joubert syndrome is a genetic disease with an autosomal-recessive or X-linked inheritance pattern caused by pathogenic variants in at least 35 genes, all encoding structures of the primary cilium involved in signaling pathways that coordinate the normal development of the kidneys, retina, brain, liver and skeletal system. The symptoms can consist in cystic renal disease and renal dystrophy, oculomotor apraxia, global developmental delay, hypotonia evolving into ataxia, skeletal and endocrine abnormalities, abnormal breathing patterns and congenital hepatic fibrosis, with the neurologic and ophthalmologic manifestations appearing early in life. The diagnostic hallmark is "the molar tooth sign", a malformation of the brainstem and cerebellum easily identifiable using brain imaging techniques. As Joubert syndrome is a highly heterogeneous disease with a large type of phenotypes, it is oftentimes underdiagnosed. This article presents a case of Joubert syndrome type 17, a rare syndrome with many features overlapping orofaciodigital syndrome type VI. Adding to the rarity of this disease, our patient is homozygous and heterozygous for two pathogenic variants in C5orf42, with one located upstream of the other, thus manifesting the phenotype of a compound heterozygous in this gene. We believe that the presentation of this rare syndrome is useful to the pediatric practice by facilitating a fast diagnosis of these patients, which helps provide genetic counselling to the patients and their families.

Keywords: Joubert syndrome type 17, C5orf42, compound heterozygous

INTRODUCTION

Cilia are microtubule-based organelles found in nearly all cell types in mammals [1] as well as in many other species, from nematodes to protozoa [2]. In the human body, these organelles are almost ubiquitous and can serve both a motile role, being involved in cellular motility and the movement of fluids, as well as a sensory role, being able to sense cues of the environment [3].

The motile cilia have also been called secondary cilia and have a motile role [4]. They can be found in the epithelial cells lining the respiratory tract, where they constantly move the mucus in an upwards manner, in the fallopian tubes, where they are involved in moving the ovule towards the uterus [4], as well as in the ependymal cells lining the cerebral ventricles, where they not only contribute to the movement of the cerebrospinal fluid, but also form a protective barrier and contribute to the neural stem cell niche [5,6].

The non-motile cilia are also called primary cilia and are found in almost all cells of the human body where they have a sensory role, detecting changes of the environment; thus, part of these types of cilia can act as mechanoreceptors, while others can be involved in the detection of chemical changes, variations of light, temperature or gravity [4,7].

The dysfunction of motile and/or non-motile cilia has been named using the umbrella term "ciliopathies" [2]. Ciliopathies represent a very heterogeneous group of genetic diseases with a large clinical variability, affecting multiple body systems [2,8]. Typically, the main organs involved are the brain (cerebral abnormalities), the eye (retinal degeneration) and the kidneys (renal disease), but these diseases can affect many other body systems such as the heart (hypertrophic or dilated cardiomyopathy, valvular defects, atrial or ventricular septal defects), the lungs (atelectasis, hypoplasia of the lungs, abnormal breathing, respiratory failure, interstitial fibrosis), ears (sensorial deafness), pancreas (pancreatic fibrosis, dysgenesis or cysts), liver (fibrosis), endocrine system (diabetes, hypothyroidism, obesity, low level of HDL cholesterol, hypertriglyceridemia, growth hormone deficiency, panhypopituitarism, hypogonadism), skeletal system (polydactyly, bowing and shortening of the long bones, brachydactyly, syndactyly), urinary system (urogenital sinus, vesicovaginal fistula, horseshoe, ectopic or absent kidneys) or genital system (ambiguous genitalia, micropenis, genital hypoplasia) [9,10]. Facial dysmorphism is also very common [10]. The genetic mechanisms responsible for this very pleiotropic disease are varied: the disease mechanism can be either monogenic, oligogenic or influenced by modifier genes or retrotransposon insertion [9,11]. As of 2021, more than 180 genes have been identified to be responsible of monogenic transmission [9]. The various genetic mechanisms involved account for a very extensive phenotypic variability [12]. Examples of ciliopathies are: the PKD complex (Autosomal Dominant Polycystic Kidney Disease – ADPKD, caused by mutations in PKD1 and PKD2 genes and Autosomal Recessive Polycystic Kidney Disease – ARPKD, caused by mutations in the PKHD1 gene; both these diseases are characterized by the presence of fluid-filled cysts in the kidneys which continue to grow throughout a patient's life), the NPH-MKS complex (Nephronophthisis – Meckel-Gruber Syndrome complex, comprised of isolated nephronophthisis – a disease characterized by tubulointerstitial fibrosis, tubular dilatation and cyst formation, as well as tubular atrophy, which is caused by more than 26 different genes, Senior-Loken syndrome – presenting with nephronophthisis and retinal degeneration, caused by mutations in more than 10 genes, Joubert syndrome, with a phenotype which can consist of specific facial dysmorphism, intellectual disability, polydactyly, hypotonia, a distinctive cerebral abnormality called the molar tooth sign, as well as breathing abnormalities, caused by mutations in at least 35 genes, and Meckel-Gruber syndrome, a lethal autosomal recessive genetic disease expressing with occipital encephalocele, bilateral dysplastic cystic kidneys and postaxial polydactyly, and caused mutations present in 6 loci on different chromosomes), or the BBS-complex comprised of various types of Bardet-Biedl syndrome (a genetic disease which can present with obesity, intellectual disability, retinitis pigmentosa, abnormal renal development, hypogonadism and polydactyly caused by mutations in more than 22 genes) [12–21].

Identified for the first time in 1969 by doctor Marie Joubert and her colleagues [22,23], this autosomal recessive or X-linked disease can be caused by pathogenic variants in at least 35 genes, all encoding structures of the primary cilium involved in signaling pathways that coordinate the normal development of the kidneys, retina, brain, liver and skeletal system [23]. The resulting symptoms can consist in cystic renal disease and renal dystrophy, oculomotor apraxia, global developmental delay, hypotonia evolving into ataxia, skeletal and endocrine abnormalities, abnormal breathing patterns and congenital hepatic fibrosis, with the neurologic and ophthalmologic manifestations appearing early in life [23,24]. The diagnostic hallmark is "the molar tooth sign", a malformation of the brainstem and cerebellum characterized by deepened interpeduncular fossa, cerebellar vermis hypoplasia as well as thickened and elongated cerebellar peduncles, which is easily identifiable using brain imaging techniques [24,25]. The prevalence is approximatively one in 80.000 to one in 100.000 live births [26–28]. Because Joubert syndrome is a heterogeneous disorder with very variable features among patients [29], oftentimes it passes undiagnosed [30]. Certain genetic variants causing this syndrome are more prevalent in ethnic groups such as the Ashkenazi Jewish, Hutterite or French-Canadian populations [30]. Based on the affected organs and systems, Joubert syndrome can be divided into six phenotypes, namely Joubert syndrome with ocular defects (also called "pure Joubert syndrome"), Joubert syndrome with renal defects, Joubert syndrome with oculorenal defects, Joubert syndrome with orofaciodigital defects and Joubert syndrome with hepatic defects.

Case report

Presenting concerns and medical history

A 13-month-old patient was admitted in September of 2019 in Dr. Nicolae Robanescu National Clinical Center for Children's Neurorehabilitation for specialized treatment. The child was the first and only child of a non-consanguineous marriage. Family history was not relevant; the mother had a miscarriage at the gestational age of 10 weeks. The patient's fetal ultrasound revealed ventriculomegaly of the lateral ventricles, which was remitted before the end of the pregnancy. Delivery was made using

cesarean section due to fetal macrosomia (weight at birth was 4000 g). The patient's length at birth was 50 cm (50th percentile), head circumference 36 cm (50th percentile) and thoracic circumference 32 cm (50th percentile). He was diagnosed with respiratory distress syndrome and received respiratory support (continuous positive airway pressure therapy for 48 hours, followed by nasal cannula for 6 days).

The patient was born with hypotonia, hexadactyly at both hands, bifid right hallux with complete syndactyly, a bifid distal phalanx at the left hallux, a high, arched palate and mild retrognathia.

Ever since birth, he presented abnormal breathing patterns. At the age of 2-3 weeks, he had a single paroxysmal attack (perioral cyanosis), but the subsequent EEG was normal. Brain MRI performed at the age of 5 months revealed a cerebellar malformation with small cerebellar hemispheres, absent cerebellar vermis, and a retro cerebellar arachnoid space of 53/76/40 mm. Based on the finding, on the MRI, of the molar tooth sign, as well as on the patient's clinical phenotype, the suspicion of Joubert syndrome was raised.

Further evaluation diagnosed the presence of global developmental delay, palpebral ptosis in the left eye, oculomotor apraxia, laryngeal stridor and sleep apnea syndrome. He had been presenting with an abnormal breathing pattern since birth.

Prior to our consult the patient had been tested for copy number variations using microarray, with normal result.

Clinical findings

At the moment of our consult his weight and length were in the 25-50 percentile (10 kg and 75 cm, respectively), and head circumference was on the 50th percentile (46,5 cm). He had hypotonia (could not hold neck, could not sit without support), dysmorphic craniofacial features consisting in a narrow bitemporal diameter, an elongated face, bilateral epicanthus, palpebral ptosis of the left eye, nystagmus, a triangular mouth, mild retrognathia, low-set ears and a high-arched palate, as well as skeletal abnormalities such as mild pectus excavatum, hexadactyly in both hands with syndactyly of the 3rd and 4th fingers of the right hand, bifid right hallux and bifid distal phalanx of the left hallux. Patient presented important cognitive disability, absent speech and episodes of self-aggression (was biting himself during the consult, had bite marks on his hands), motor delay and abnormal breathing patterns (important hyperventilation/tachypnea). He could not follow objects with his eyes and had a permanent tendency to keep his mouth open.

Diagnostic focus and assessment

Based on the clinical and imagistic findings, diagnostic testing (NGS panel) for Joubert syndrome was recommended. Hence, 2 ml of peripheral blood on EDTA were sent to Blueprint Genetics lab (Espoo, Finland), where next generation sequencing of 36 genes involved in Joubert syndrome or genetic syndromes with a similar phenotype was performed. Coding exons, as well as exon-intron boundaries (+/- 20 base pairs) and selected non-coding, deep intronic variants were analyzed.

Sequence analysis identified a homozygous nonsense variant, namely c.7817T>A, p.(Leu2606*) in the CPLANE1 (C5orf42) gene, as well as a heterozygous frameshift variant, namely c.1819del, p.(Tyr607Thrfs*6) within the same gene.

Therapeutic focus and assessment

Currently no specific etiological treatment for Joubert syndrome exists; because of this, the treatment of the patient was based on general principles regarding the symptoms of the disease.

FIGURE 1. A and B: Following brain MRI, the molar tooth sign was detected in this patient (marked by red arrows). The molar tooth sign is characterized by cerebellar vermis hypoplasia/aplasia, deepened interpeduncular fossa and elongated superior cerebellar peduncles. **C:** Lateral view of patient's brain.

A

B

FIGURE 2 A. Overall appearance of patient at 13 months. Patient had hypotonia, could not hold his neck or sit without support. He was also displaying self-aggressive behavior (biting his hands). The red arrow shows a self-inflicted bite mark. **B.** Patient had craniofacial dysmorphism which was typical of Joubert syndrome, namely a prominent forehead, narrow bitemporal diameter, palpebral ptosis, bilateral epicanthus, triangular mouth, low-set, slightly cupped ears, high, arched eyebrows, broad nasal bridge and a high-arched palate.

A

B

C

FIGURE 3. A. Patient had hexadactyly at both hands and syndactyly of the 3rd and 4th fingers of the right hand. **B.** Bifid right hallux. **C.** Bifid distal phalanx of the left hallux

Initially a clinical and functional evaluation was performed, and the treatment recommendations were made progressively, according to the evolving clinical status of the patient.

The purpose of the treatment was the recovery of the motor deficit, the improvement of the muscle tone, and the prevention of the painful joint contractures. The complete multidisciplinary team, composed of doctors (pediatrician, medical recovery, pediatric neurologist, medical geneticist, pediatric orthopedist), nurses, kinesiotherapists, psycholo-

gists and ergotherapists established the required standards of care for the current case.

The physiotherapy and kinesiotherapy, as well as the advanced rehabilitation facilities, are necessary to maximize the rehabilitation of the motor functionality and to prevent the contractures. The occupational therapy was focused on the sensory, adaptive, motor and cognitive integration using adapted playing. The respiratory kinesiotherapy allows the maintenance/improvement of the permeability of the airways and helps prevent the respiratory inter-

current infections. The nutritional strategies were adjusted to the disability of the patient using a diet which was adapted to the clinical and developmental status of the patient as follows: the child was carefully positioned while being fed to prevent aspiration and reflux, the foods which were hard to swallow were avoided, the thin liquids, which could have been aspired, were avoided, the meals were fragmented into small snacks and small portions, the growth curves and body mass index were periodically monitored.

Follow-up and monitoring

The evolution of the patient was stationary, and the family has been constantly applying the standards of care, so that an optimum environment was created in order to increase the quality of life of the patient.

DISCUSSION

The CPLANE1 gene (Ciliogenesis and planar polarity effector 1, or chromosome 5 open reading frame 42, C5orf42) is located in the 5p13.2 locus [31]. The function of the protein encoded by this gene hasn't been established yet [32]. It is presumed that CPLANE1 has a putative coiled-coil motif and may be a transmembrane protein [33,34]. Defects in this gene are mainly responsible for Joubert syndrome 17 as well as orofaciodigital syndrome VI [35,36].

Joubert syndrome type 17 (JBTS 17) has an autosomal recessive inheritance pattern, has been identified mainly in individuals of French-Canadian origin and is clinically characterized by the presence of the molar tooth sign, cerebellar ataxia, mental disability, motor delay, hypotonia, oculomotor apraxia, and, in some cases, renal disease and retinal dystrophy [37,38]. According to Srour et. al [39], who described 10 patients from 7 French Canadian families with CPLANE1 pathogenic variants, cognitive impairment was present in every individual of the group (albeit variable, ranging from borderline intelligence to mild intellectual disability) and global developmental delay with onset of walking ranging between 2 years and a half and 8 years of age. Episodes of hyperventilation and oculomotor apraxia were present in most of the individuals, and two of them had limb abnormalities, namely syndactyly of the third and fourth finger at one hand in one patient, and pre- and postaxial polydactyly in another. Kidney or retinal involvement were not reported in any of these patients [39].

In a study made in 2015 by Bachmann-Gagescu et al. [40] with the purpose of developing genotype-phenotype correlations in a large cohort of 428 patients with Joubert syndrome from 363 families, after capturing using molecular inversion probes

(MIPs) all exons in the genes associated with Joubert syndrome which were known at that time as well as genes associated with the allelic disorder Meckel syndrome (in total, 27 genes), amplifying them using PCR and then sequencing them, the highest number of patients [31] had homozygous or compound heterozygous mutations in the C5orf42 (CPLANE1) gene, the majority being truncating mutations (nonsense, frameshift and canonical splice-site mutations). After correlating the non-CNS features of the patients with their genotypic findings, strong genotype-phenotype correlations were observed for four genes, namely: pathogenic variants in CEP290 were strongly associated with cystic renal disease and retinal dystrophy, mutations in TMEM 67 with liver fibrosis, variants in OFD1 with encephalocele and pathogenic mutations in C5orf42 (CPLANE1), with polydactyly. In a much smaller proportion, a positive correlation between variants in C5orf42 and encephalocele was also established [40].

Caused by mutations in the CPLANE1 gene, just like Joubert syndrome type 17, orofaciodigital syndrome VI (OFD VI) has many similarities to the above-mentioned syndrome, both presenting the molar tooth sign, dysmorphic features such as bitemporal narrowing, frontal bossing, long face, epicanthus, high palate, posteriorly situated ears, prominent nasal bridge, as well as global developmental delay, hypotonia, intellectual disability, gait disturbance, nystagmus, hand and foot polydactyly, syndactyly and/or bifid toe, but can also present other signs or symptoms such as abnormal oral frenulum morphology, bilateral cryptorchidism, conductive hearing impairment, tongue or hypothalamic hamartoma, renal agenesis and others [41–45]. Because of the high number of overlapping features between Joubert syndrome type 17 and orofaciodigital syndrome type 6, the two are, sometimes, classified using the umbrella-term Joubert syndrome with orofaciodigital defects (JS-OFD) [46].

Given the fact that the patient's clinical traits – global developmental delay, hypotonia, mental disability, nystagmus, breathing abnormalities such as hyperventilation, self-aggression, dysmorphic facial features typical to Joubert syndrome, namely elongated face, broad nasal tip, ptosis, epicanthus, posteriorly situated ears, anteverted nostrils, bitemporal narrowing, arched eyebrows, high palate, as well as limb abnormalities typical to the above-mentioned syndrome, namely hexadactyly at both hands, preaxial polydactyly at the left foot and bifid toe at the right foot - and paraclinical features, such as the molar tooth sign [44,45] corresponded to the Joubert syndrome phenotype, our patient was diagnosed with Joubert syndrome type 17.

The neurological features could be explained by the presumption that CPLANE1 gene (C5ORF42)

could play a role in neurodevelopment [47]. In fact, the CPLANE1 protein was found to be expressed in the plasmalemma and cytosol of many tissues, such as the brain (mainly in the cerebral cortex), the eye (detection of CPLANE1 mRNA expression), the endocrine tissues, the digestive tract, namely the colon, the liver, gallbladder and pancreas, the kidney and urinary bladder, the testes, the muscles, the connective tissue, the skin and the bone marrow and lymphoid tissues [48]. Although the exact pathways in which pathogenic mutations contribute to the impairment of the above-mentioned tissues haven't been elucidated yet, the variety of mRNA and protein expression of CPLANE1 explains the vast phenotypic expression, the extended variability of the features and the strong pleiotropic nature of this versatile disease.

So far, the variant CPLANE1 c.7817T>A, p.(Leu2606*) has been reported ten times in ClinVar, each time as pathogenic. The variant causes a premature translational stop signal (p.Leu2606* in the CPLANE1 gene) and is thus predicted to lead to loss of normal protein function either through protein truncation (2606 out of 3197 amino acids) or nonsense mediated mRNA decay [49–51]. As expected, the variant is absent from populational databases [50]. The variant has been reported in compound heterozygous Joubert syndrome patients [40,51] as well as in a patient with orofaciodigital syndrome type 6 [51].

The variant CPLANE1 c.1819del, p.(Tyr607Thrfs*6) has been reported 14 times in ClinVar as pathogenic and twice as likely pathogenic [52,53]. The variant deletes 1 base pair in exon 12 and generates a frameshift leading to a premature stop codon at position 6 in a new reading frame. It is predicted to cause loss of normal protein function either through protein truncation (611 out of 3197 amino acids) or nonsense-mediated mRNA decay [53]. In literature, several authors have reported this variant as pathogenic [40,54,55].

As the variant CPLANE1 c.1819del, p.(Tyr607Thrfs*6) is located upstream of the variant CPLANE1 c.7817T>A, p.(Leu2606*), it is likely that the variant CPLANE1 c.1819del, p.(Tyr607Thrfs*6) will lead to loss-of-function of one allele and the variant CPLANE1 c.7817T>A, p.(Leu2606*), of the other. This is consistent with autosomal recessive inheritance. If the patient's parents are each found to be carriers of these variants, each of the patient's siblings would have a 25% chance of being homozygous for the variant CPLANE1 c.7817T>A, p.(Leu2606*) and heterozygous for CPLANE1 c.1819del, p.(Tyr607Thrfs*6) and thus affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being an unaffected non-carrier. To establish the mode of segregation and the recurrence risk, the patient's parents were recommended genetic testing, which they refused.

In HGMD (Human Gene Mutation Database), more than 60 mutations in C5orf42 have been described as associated with Joubert syndrome type 17 or orofaciodigital syndrome type 6 [39,46,51,56–58]. C5orf42 has a propensity to truncating mutations [35]. According to Zhang et.al [35], the reason for less severe phenotypes in spite of the fact that the majority of mutations in C5orf42 are truncating could be the fact that this protein might have a less important role than the other proteins which, when defective, are responsible for the occurrence of Joubert syndrome. Another explanation these authors give could be that the function of C5orf42 could be compensated by other ciliary proteins which are involved in the same pathways during embryonic development [35].

The rarity of our case consists in the fact that our patient is both homozygous as well as heterozygous for two pathogenic variants in the same gene – CPLANE1, and, due to the fact that the variant CPLANE1 c.1819del, p.(Tyr607Thrfs*6) is located upstream of the variant CPLANE1 c.7817T>A, p.(Leu2606*), most likely the variant CPLANE1 c.1819del, p.(Tyr607Thrfs*6) leads to loss-of-function of one allele and the variant CPLANE1 c.7817T>A, p.(Leu2606*), to a loss of function of the other, and thus the patient presents the phenotype of a compound heterozygous for c.1819del, p.(Tyr607Thrfs*6)/ c.7817T>A, p.(Leu2606*) variants. According to ClinVar, 125 pathogenic mutations have been registered so far in the C5orf42 gene, among which 33 nonsense and 47 frameshift mutations [59]. The fact that so far, 1177 pathogenic mutations have been registered in this database as causing Joubert syndrome [59] showcases even more the rarity of our case.

Although currently the treatment of Joubert syndrome is symptomatic and an etiologic treatment doesn't exist yet [26], the evaluation and multidisciplinary therapies are essential to these patients, and they include the participation of a multidisciplinary team (pediatric neurology, pediatrics, medical recovery, ophthalmology, medical genetics, kinesiotherapists, psychologists, nurses) [26,60,61]. The patient was investigated in this regard in our hospital as well as in other clinical facilities, such as the "Prof. Dr. Alexandru Obregia" Psychiatry hospital from Bucharest, having received symptomatic treatment consisting of kinesiotherapy, occupational therapy, respiratory kinesiotherapy and nutritional support, under which he presented a stationary evolution.

CONCLUSION

Because of the numerous genes involved and multitude of affected tissues, Joubert syndrome is a

highly heterogeneous disease with pleiotropic effects and hence, oftentimes it passes undiagnosed. Furthermore, in many cases the overlap with other similar syndromes – in our case, orofaciodigital syndrome type 6, makes a correct diagnosis even harder to accomplish. Further studies on the genotype-phenotype correlations as well as the genetic causes – including the discovery of new genes – are needed. This way, many yet undiagnosed cases would be solved and light would be shed on the crucial impor-

tance of these small, yet essential organelles: the cilia. By presenting one of the rare cases of Joubert type 17, our article has aimed to shed light on this pleiotropic disease. Furthermore, a very interesting aspect of this case is the fact that our patient is homozygous and heterozygous for two pathogenic variants in the same gene, with one located upstream of the other, thus manifesting the phenotype of a compound heterozygous in the C5orf42 gene.

REFERENCES

1. Sengupta P. Cilia and sensory signaling: The journey from "animalcules" to human disease. *PLoS Biol.* 2017;15(4):e2002240.
2. Mitchison HM, Valente EM. Motile and non-motile cilia in human pathology: from function to phenotypes. *J Pathol.* 2017;241(2):294–309.
3. Dishinger JF, Kee HL, Verhey KJ. Chapter Five - Analysis of Ciliary Import. In: Marshall WFBT-M in E, editor. Cilia, Part A [Internet]. Academic Press; 2013:p. 75–89. Available from: https://www.sciencedirect.com/science/article/pii/B9780123979452000056
4. Adams M. The primary cilium: An orphan organelle finds a home. *Nat Educ.* 2010;3(9):54.
5. Mahuzier A, Shihavuddin A, Fournier C et al. Ependymal cilia beating induces an actin network to protect centrioles against shear stress. *Nat Commun* [Internet]. 2018;9(1):2279.
6. Tramontin AD, García-Verdugo JM, Lim DA, Alvarez-Buylla A. Postnatal development of radial glia and the ventricular zone (VZ): a continuum of the neural stem cell compartment. *Cereb cortex.* 2003;13(6):580–7.
7. Satir P, Pedersen LB, Christensen ST. The primary cilium at a glance. *J Cell Sci.* 2010;123(4):499–503.
8. Reiter JF, Leroux MR. Genes and molecular pathways underpinning ciliopathies. *Nat Rev Mol cell Biol.* 2017;18(9):533–47.
9. Focşa IO, Budişteanu M, Bălgrădean M. Clinical and genetic heterogeneity of primary ciliopathies. *Int J Mol Med.* 2021;48(3):1–15.
10. Grochowsky A, Gunay-Aygun M. Clinical characteristics of individual organ system disease in non-motile ciliopathies. *Transl Sci Rare Dis.* 2019;4(1–2):1–23.
11. Tavares E, Tang CY, Vig A et al. Retrotransposon insertion as a novel mutational event in Bardet-Biedl syndrome. *Mol Genet genomic Med.* 2019;7(2):e00521.
12. Habbig S, Liebau MC. Ciliopathies-from rare inherited cystic kidney diseases to basic cellular function. *Mol Cell Pediatr.* 2015;2(1):1–6.
13. Gupta S, Ozimek-Kulik JE, Phillips JK. Nephronophthisis-Pathobiology and Molecular Pathogenesis of a Rare Kidney Genetic Disease. *Genes* (Basel). 2021;12(11):1762.
14. Meng X, Long Y, Ren J et al. Ocular Characteristics of Patients With Bardet–Biedl Syndrome Caused by Pathogenic BBS Gene Variation in a Chinese Cohort. *Front cell Dev Biol.* 2021;9:378.
15. McConnachie DJ, Stow JL, Mallett AJ. Ciliopathies and the kidney: a review. *Am J Kidney Dis.* 2021;77(3):410–9.
16. Simms RJ, Eley L, Sayer JA. Nephronophthisis. *Eur J Hum Genet.* 2009;17(4):406–16.
17. Senior-Loken syndrome [Internet]. Orphanet. 2022 [cited 2022 Feb 28]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=en&Expert=3156
18. Zhongling K, Guoming L, Yanhui C, Xiaoru C. Case Report: Second Report of Joubert Syndrome Caused by Biallelic Variants in IFT74. *Front Genet.* 2021;12.
19. Parisi MA. The molecular genetics of Joubert syndrome and related ciliopathies: The challenges of genetic and phenotypic heterogeneity. *Transl Sci rare Dis.* 2019;4(1–2):25–49.
20. Kheir AEM, Imam A, Omer IM et al. Meckel-Gruber syndrome: A rare and lethal anomaly. *Sudan J Paediatr.* 2012;12(1):93.
21. Hartill V, Szymanska K, Sharif SM et al. Meckel–Gruber syndrome: An update on diagnosis, clinical management, and research advances. *Front Pediatr.* 2017;5:244.
22. Joubert M, Eisenring JJ, Robb JP, Andermann F. Familial agenesis of the cerebellar vermis. *Neurology.* 1969;19(9):813–25.
23. Shaik L, Ravalani A, Nelekar S et al. Joubert Syndrome: A Molar Tooth Sign in Disguise. *Cureus.* 2020;12(8).
24. Romani M, Micalizzi A, Valente EM. Joubert syndrome: congenital cerebellar ataxia with the molar tooth. *Lancet Neurol.* 2013;12(9):894–905.
25. Wang SF, Kowal TJ, Ning Kb et al. Review of ocular manifestations of Joubert syndrome. *Genes* (Basel). 2018;9(12):605.
26. Joubert Syndrome [Internet]. National Organization for Rare Disorders (NORD). 2021. Available from: https://rarediseases.org/rare-diseases/joubert-syndrome/
27. Parisi M, Syndrome GIJ. Jul 9 [updated 2017 Jun 29]. GeneReviews®[Internet] Seattle Univ Washington, Seattle. 1993;2020.
28. Brancati F, Dallapiccola B, Valente EM. Joubert Syndrome and related disorders. *Orphanet J Rare Dis* [Internet]. 2010;5(1):20.
29. Zhang X, Shen Y, Li P et al. Clinical heterogeneity and intrafamilial variability of Joubert syndrome in two siblings with CPLANE1 variants. *Mol Genet Genomic Med.* 2021;9(6):e1682.
30. Joubert syndrome [Internet]. HealthJade. 2019. Available from: https://healthjade.net/joubert-syndrome/
31. Ciliogenesis and planar polarity effector 1; CPLANE1 [Internet]. Online Mendelian Inheritance in Man. 2019. Available from: https://www.omim.org/entry/614571
32. Ciliogenesis and planar polarity effector 1 [Internet]. InterPro. Available from: https://www.ebi.ac.uk/interpro/entry/InterPro/IPR028236/
33. CPLANE1 ciliogenesis and planar polarity effector complex subunit 1 [Internet]. GTR: Genetic Testing Registry. 2022. Available from: https://www.ncbi.nlm.nih.gov/gtr/genes/65250/
34. CPLANE1 [Internet]. Antibodypedia. Available from: https://antibodypedia.com/gene/51274
35. Zhang C, Sun Z, Xu L, Che F, Liu S. Novel compound heterozygous CPLANE1 variants identified in a Chinese family with Joubert syndrome. *Int J Dev Neurosci.* 2021;81(6):529–38.
36. Gene: CPLANE1 (ciliogenesis and planar polarity effector complex subunit 1) Homo sapiens [Internet]. Rat Genome Database (RGD). Available from: https://rgd.mcw.edu/rgdweb/report/gene/main.html?id=1606513
37. Joubert Syndrome 17 (JBTS17) [Internet]. MalaCards Human Disease Database. 2021. Available from: https://www.malacards.org/card/joubert_syndrome_17
38. Disease - Joubert syndrome 17 [Internet]. Universal Protein Resource (UniProt). Available from: https://www.uniprot.org/diseases/DI-03439
39. Srour M, Schwartzentruber J, Hamdan FF, Ospina LH, Patry L, Labuda D, et al. Mutations in C5ORF42 cause Joubert syndrome in the French Canadian population. *Am J Hum Genet.* 2012;90(4):693–700.

40. Bachmann-Gagescu R, Dempsey JC, Phelps IG et al. Joubert syndrome: a model for untangling recessive disorders with extreme genetic heterogeneity. *J Med Genet.* 2015;52(8):514–22.

41. Orofaciodigital syndrome type 6 [Internet]. Orphanet. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp. php?lng=en&Expert=2754

42. Orofaciodigital syndrome 6 [Internet]. Genetic and Rare Diseases Information Center (GARD). Available from: https://rarediseases.info. nih.gov/diseases/4412/orofaciodigital-syndrome-6

43. Orofaciodigital syndrome VI; OFD6 [Internet]. Online Mendelian Inheritance in Man. Available from: https://www.omim.org/ entry/277170

44. Bonnard C, Shboul M, Tonekaboni SH, Ng AYJ, Tohari S, Ghosh K, et al. Novel mutations in the ciliopathy-associated gene CPLANE1 (C5orf42) cause OFD syndrome type VI rather than Joubert syndrome. *Eur J Med Genet.* 2018;61(10):585–95.

45. Joubert syndrome [Internet]. Genetic and Rare Disease Information Center (GARD). Available from: https://rarediseases.info.nih.gov/ diseases/6802/joubert-syndrome

46. Wentzensen IM, Johnston JJ, Keppler-Noreuil K, Acrich K, David K, Johnson KD, et al. Exome sequencing identifies novel mutations in C5orf42 in patients with Joubert syndrome with oral–facial–digital anomalies. *Hum Genome Var.* 2015;2(1):1–3.

47. Ben-Salem S, Al-Shamsi AM, Gleeson JG, Ali BR, Al-Gazali L. Mutation spectrum of Joubert syndrome and related disorders among Arabs. *Hum genome Var.* 2014;1(1):1–10.

48. CPLANE1 [Internet]. The Human Protein Atlas. Available from: https:// www.proteinatlas.org/ENSG00000197603-CPLANE1/tissue

49. ClinVar. c.7817T>A [Internet]. National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/ clinvar/?term=c.7817T%3EA

50. ClinVar; [VCV000217575.18] [Internet]. National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih. gov/clinvar/variation/VCV000217575.18

51. Romani M, Mancini F, Micalizzi A et al. Oral-facial-digital syndrome type VI: is C5orf42 really the major gene? *Hum Genet.* 2015;134(1):123–6.

52. ClinVar. c1819del. [Internet]. National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/ clinvar/?term=c.1819del

53. ClinVar; [VCV000217590.28] [Internet]. National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih. gov/clinvar/variation/VCV000217590.28

54. Marinakis NM, Svingou M, Veltra D et al. Phenotype-driven variant filtration strategy in exome sequencing toward a high diagnostic yield and identification of 85 novel variants in 400 patients with rare Mendelian disorders. *Am J Med Genet Part A.* 2021;185(8):2561–71.

55. Vilboux T, Doherty DA, Glass IA, Parisi MA, Phelps IG, Cullinane AR, et al. Molecular genetic findings and clinical correlations in 100 patients with Joubert syndrome and related disorders prospectively evaluated at a single center. *Genet Med.* 2017;19(8):875–82.

56. Bayram Y, Aydin H, Gambin T et al. Exome sequencing identifies a homozygous C5orf42 variant in a Turkish kindred with oral-facial-digital syndrome type VI. *Am J Med Genet Part A.* 2015;167(9):2132–7.

57. Kroes HY, van de Putte DEF, Ravesloot CJ, Lindhout D. The birth prevalence of Joubert syndrome: a population based study in the Netherlands. *Eur J Hum Genet.* 2007;15(S1):68.

58. Lopez E, Thauvin-Robinet C, Reversade B et al. C5orf42 is the major gene responsible for OFD syndrome type VI. *Hum Genet.* 2014;133(3):367–77.

59. ClinVar [Internet]. National Center for Biotechnology Information. Available from: https://www.ncbi.nlm.nih.gov/clinvar/

60. Ahmetgjekaj I, Rahman M, Hyseni F et al. A case report of Joubert syndrome with renal involvement and seizures in a neonate. *Radiol Case Reports.* 2021;16(5):1075–9.

61. Bachmann-Gagescu R, Dempsey JC, Bulgheroni S et al. Healthcare recommendations for Joubert syndrome. *Am J Med Genet Part A.* 2020;182(1):229–49.