ABSTRACT

Joubert syndrome type 33 (JBTS33) is an extremely rare autosomal recessive disorder and characterized by developmental delay, severe renal disease, hypotonia/ataxia accompanied by cerebellar vermian hypoplasia/aplasia, optic nerve atrophy, renal atrophy, and an abnormally large interpeduncular fossa, giving the appearance of a molar tooth on transaxial slices (molar tooth sign). Here, we present genetically diagnosed a consanguineous family with JBTS33 and further expand the phenotypic literature on the disease. A novel biallelic homozygous nonsense mutation in *PIBF1*, *ENST00000326291.11:c.1231C>T (p.Arg411Ter)* identified by whole-exome sequencing and consanguinity was determined by linkage analysis. This study confirms *PIBF1* as a disease causing gene for JBTS33, and our findings expand the molecular and clinical spectrum of the syndrome. Identifying genetic fundamentals and new phenotypic expansions of the *PIBF1* gene is valuable, and more studies are essential to shed light on the phenotypic variation.

Keywords: Joubert Syndrome, PIBF1, Developmental Delay, Renal Cystic Dysplasia, Retinal, Molar tooth sign, WES.

What is already known about this topic?

Joubert syndrome type 33, is an extremely rare autosomal recessive disorder presenting with hypotonia/ataxia, apraxia, retinal, or renal abnormalities, and intellectual disability, neuroradiologically characterized by cerebellar vermian hypoplasia/aplasia, thickened and reoriented superior cerebellar peduncles, and an abnormally large interpeduncular fossa, giving the appearance of a molar tooth on transaxial slices (molar tooth sign). Disease causing gene for JBTS33 is *PIBF1* (Progesterone Immunomodulatory Binding Factor 1), however these gene-related main neurogenetic or phenotypic characteristics are not clarified yet.

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1. INTRODUCTION

Joubert Syndrome (JS, OMIM: P213300, with an incidence of 1/80.000 - 1/100.000) falls under the broad spectrum of heterogeneous ciliopathies which are characterized by central nervous system anomalies, developmental abnormalities, respiratory problems, retinal dystrophy, hypotonia, facial dysmorphism, and rarely cystic kidney disease (1). The classic form of JS characterized by three primary findings including hypotonia, developmental delays, and distinct cerebellar and brainstem malformations discernible on brain imaging, the "molar tooth" sign, however only several genes have been associated with different subtypes of the disease. In addition to these traditional manifestations, some patients with JS have disclosed abnormalities closely associated with ciliary distortion, such as familial juvenile nephronophthisis, retinal dystrophy and polydactyly (2,3). To date, almost 40 genes associated with JS have been identified. Joubert syndrome is commonly associated with pathogenic variants in genes such as CEP290, TMEM216, TMEM67, AHI1, and CC2D2A, with CEP290 pathogenic variants being the most prevalent, accounting for approximately 15-30% of cases, followed by the other genes, which are estimated to be responsible for 5-10% of each cases. Mutation of PIBF1 is a new pathogenic variant associated with Joubert syndrome type 33 (JBTS33) (4). *PIBF1*, Progesteron-Induced Blocking Factor 1, is produced during pregnancy in response to progesterone (5) located in chromosome 13q21-q22, includes 18 exons (6) and the gene encodes an approximately 757-amino acid alpha-helical protein (7). PIBF1 is the main component of the centrosome and is vital for accumulating centriolar satellites, in the event forming the primary cilia and diminishing of PIBF1 results in mitotic hold, misaligned chromosomes, and spindle pole fragmentation (1). In a study with mIMCD3 cells, exogenous expression of the human *PIBF1* gene following siRNA degradation rescues ciliogenesis (8).

This may play sensory or signalling roles in the ciliary vesicle or in the developing neocortex, and over-expression of specific ciliary proteins, or receptors, in cortical neurons causes cilia elongation, procilium at the earliest stages of ciliogenesis both in the developing brain and in other tissues (1,8).

This recent siRNA reversed genetic genome-screening study determined recessive variants with *PIBF1* in children (1). The exact cellular and molecular mechanisms that lead to the intricate JS phenotype in individuals with *PIBF1* pathogenic variants have not yet been clarified. After the first identification of *PIBF1* pathogenic variants related to JBTS33 in 2015 (8), less than 10 patients have been described in the literature (8-10). These published studies identified hypotonia and developmental delay at 9 patients, ataxia at 6 patients, cystic kidney

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disease and acute renal failure only at 1 patient, seizure at 1 patient, some ocular problems, and radiological features such as molar tooth sign, perisylvian polymicrogyria, cerebellar vermis hypoplasia were reported (11-15). Here, we present three siblings of JBTS33 with pathogenic variants of *PIBF1*, highlighting the associated genotype-phenotype link of *PIBF1* and JS with such as cystic renal disease renal failure and early onsent seizure (Table 1).

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1. METHODS AND PATIENTS

1.1.Patients

Case 1, Index Patient:

A 5-year-old girl (current age 35, exitus) with developmental delay and hypotonia was admitted to our Pediatric Neurology Clinic. She was the first child of consanguineous (4th degree) Turkish parents delivered by vaginal spontaneously birth (Figure 1). A history of convulsion with an onset of 20 days was noted. In electroencephalography (EEG), the spread of slow waves to adjacent hemisphere regions is observed in both temporal posterior regions. The patient did respond to antiepileptic treatment with phenobarbital. On follow-up, the patient was diagnosed with global developmental delay/intellectual disability with delayed-holding head at 2.5 years of age, speaking achieved with only monosyllables but had no other verbal communication skills until 3 years, walking only with assistance at 3.5 years, and had urine and stool incontinence. The Denver Developmental Scale revealed moderate developmental delay and motor dysfunction (207–2000).

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On physical examination performed at 5 years old, height percentile was >97p (130 cm), weight percentile was >97p (46.5 kg), head circumference was 25–50 percentile (50 cm). Examination showed that strabismus was present in both eyes, esotropia in the right eye, the patient had an overweight appearance, presented with hypotonia, and dysmorphic features. The other examination findings were unremarkable. On follow-up, renal failure had developed and the patient was started on dialysis treatment due to chronic renal failure. However, she died at the age of 35, due to renal uremic syndrome related sudden cardiac death. Laboratory profile showed renal failure related anemia (haemoglobin 9.5 g/Dl), increased serum creatinine (7,1 mg/Dl) and secondary hyperparathyroidism (serum calcium 9.5 mg/dL, serum phosphorus 6.5mg/dL, serum PTH 2757pg/mL). Ultrasonographic (USG) examination revealed normal gallbladder, liver, bladder and ureter. The spleen was ˜10 cm with a homogeneous echo. Renal parenchymal USG echoes are diffusely increased (grade III Chronic Renal Failure) with the sizes of 57*24 mm (right), 73*21 (left), Cortical cysts of 12 mm and 7 mm in size were

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observed in the lower part of the left kidney and cortical cysts sized 6 mm in the lower part of the right kidney and 10 mm in the middle part were also determined. Other USG findings were normal.

Magnetic resonance imaging (MRI) revealed that the superior cerebellar peduncle, which formed the classical molar appearance, showed a thick and horizontal course with the widening of interpedicular distance (Figure 2). The cerebellar vermis was hypoplastic with the existence of a cerebellar vermian cleft and the appearance of a kissing cerebellum. Optic nerve atrophy identified during ocular assessment. Corpus callosum segments were assessed and the overall corpus callosum length was measured using normal findings. Computerized tomography (CT) of the abdomen showed bilateral kidney atrophy, hyperdense gallbladder stones, and splenomegaly.

Case 2:

The second member of the family was also admitted to our clinic at the age of 4 (current age 33) with developmental delay, ataxic gait and hypotonia. She was born on the term with a normal birth story. She could hold her head steadily after 6 months old, could not swallow solid foods until she was 5 years old. She raised her head at 2 years, unassisted sat at 3 years, crawled at 3–5 years and stood by itself at the end of 5 years old. There was an abnormal gait with truncal ataxia. She had developmental delay with severe motor retardation at the Denver Developmental Scale (206–2000). She also had urine and stool incontinence. The girl had a height of 10–25 percentiles, the weight of 25–50 percentiles, head circumference of 10 percentiles. Physical examination demonstrated similar findings to the indexed case as follows; moderate developmental delay with hypotonia, normal eyes, contractions, stereotypical hand movements, ataxic gait, and dysmorphic features. The biochemical profile also demonstrated kidney disease-related anemia (haemoglobin 9.7 g/dL), renal dysfunction (elevated kreatinin and uric acid), and secondary hyperparathyroidism (serum calcium 10.97 mg/dL, serum phosphorus 3.69, serum PTH 765 mL). Ultrasonic examination indicated that liver dimensions were larger than normal (165 mm) and the echo of the parenchyma increased. The portal system, choledoc, intrahepatic ducts, the pancreas were normal. Spleen dimensions are larger than normal (147*62 mm). The left kidney was 95*42 mm in size, and the right kidney was 82*38 mm with ultrasound increased echogenicity (grade I). The second sibling was also in pediatric nephrology follow-up with the index case and was undergoing dialysis for chronic kidney disease.

MRI and CT images of the second patient revealed normal corpus callosum, no cortical malformation but also findings similar to the index case such as molar tooth sign, cerebellar vermis hypoplasy, and optic nerve atrophy accompanying of CT findings with renal atrophy.

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Case 3:

The 28-month-old (current age 19) third sibling of the family, admitted to our clinic with similarly affected but presented with more severe developmental delay findings, including delayed head holding, delayed holding objects, delayed walking or sitting, and delayed recognition of parents accompanied by seizures. The Denver Developmental Scale similarly showed global developmental delay and he had urine and stool incontinence too. Upon physical examination, height percentile was 97p, weight percentile was >97p, head circumference was 50 percentile at 28 months old. Examination showed stereotypical hand movements, self-harming tendency, reduced eye contact, social disability, ataxic gait, hypotonia, and dysmorphic features including ptosis, prognathism, lower lip eversion, strabismus with esotropia in the left eye, low set and posteriorly rotated ears with thick ear lobes, telecanthus, and synophrys. Laboratory findings were normal besides mild anemia (11.5 g/dL). Ultrasonic examination of the liver, gallbladder, intrahepatic ducts, portal vein, pancreas were normal. Bilateral kidney echoes increase in line with grade I. However, cystic kidney findings had not occurred yet and there was no renal failure at the time of examination.

MRI images of the third patient showed that both brainstem and cerebellar vermis were mildly hypoplasic. Optic nerve atrophy and a deep cleft between prominent cerebellar peduncles were also noted. The bilateral middle cerebellar peduncle was thick and elongated. Corpus callosum was normal. All findings were correlated with a molar tooth malformation (Figure 2). CT examination with similar findings of bilateral kidney atrophy and gallbladder stones were noted.

Patients had unspecific symptoms of developmental abnormalities, hypotonia, ocular movement abnormalities, and renal problems leading to JS-related subtypical features. Hence, as previously described, a whole-exome sequencing (WES) study by the Yale Center for Genome Analysis (YCGA) was performed. Clinical and genetic findings in all three siblings suggested a diagnosis of *PIBF1* mutations relating to JBTS33 (Figure 1A).

1.2. Whole-Exome Sequencing

Whole exome sequencing (WES) was performed out in the index patient (NG116-2) as previously described (16). The mean 20x coverage of all targeted bases was 87%. Whole-exome sequencing analysis identified a biallelic homozygous nonsense mutation in ENST00000326291.11:c.1231C>T (p.Arg411Ter) in exon 10 of the *PIBF1* gene. This pathogenic variant introduces a premature stop codon, which terminates the amino acid sequence at arginine 411. This truncated protein is predicted to undergo nonsense-mediated

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consanguinity was determined by linkage analysis.¶
The inbreeding coefficient was calculated for the index case (NG116-2) to be 0.07 by whole genome genotyping. After homozygosity mapping and multipoint parametric linkage analysis using genotyping results of six DNA samples, we found only one homozygous genomic interval with LOD scores above 2 (Figure 1B). This region (LOD score of 2.5) was located on chromosome 4 between heterozygous SNP markers, rs776847 (70,892,919) and rs12510756 (111,132,892) (GRCH37/hg19). Therefore, focused our subsequent analyses on the identification of potential disease-causing variants within this region (35.68 cm). Homozygosity analysis showed a single region shared only by the three affected individuals located at 4q13.3-4q25 between 75,718,547 and 109,296,917 bps (29.55cM) by PLINK software (Supplementary Table 1).¶

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decay. Both parents were identified to be heterozygous for the variant by Sanger sequencing. Finally, exome CNV analysis demonstrated no big events within the coding regions of the genome.

The c.1231C>T variant was reported in the heterozygous state thrice with a 1.19x 10^{-5} allele frequency in GnomAD exomes and 2 times with 0.63x10^{-5} allele frequency in gnomAD genomes (17). The c.1231C>T variant has never been reported in a homozygous state in the dbSNP, NHLBI GO ESP Exome Variant Server, Exome Aggregation Consortium (ExAC), 1000 Genomes, or Greater Middle East Variome Project databases. The detected variant is rare and predicted to be pathogenic by most of the in silico prediction tools, including five function prediction methods (FATHMM (18), LRT (19), MutationTaster (20), PolyPhen2 (21) and SIFT (22), three conservation methods (GERP++(23), phastCons (24) and PhyloP (25) and five ensemble methods (CADD (26), DANN (27), Eigen (28), FATHMM-MKL (29), GenoCanyon (30) and there is no other disease causing variant detected related to the patient's phenotype (31). Furthermore, no aberrant structural variants were detected that could explain the phenotype of the patient. Applying American College of Medical Genetics and Genomics and the Association for Molecular Pathology criteria for PIBF1: c.1231C>T, variant is detected as PVS1 and PM2 leading to a likely pathogenic variant (32). These findings strongly suggest that the identified *PIBF1* variant is probably the disease-causing variant in the siblings.

2. DISCUSSION

We evaluated three members of a consanguineous family with global developmental delay, cystic kidney disease, neurological features, and molar tooth sign. Exome sequencing indicated a unique pathogenic variant in exon 10 of *PIBF1* as the likely cause of the reported condition. JBTS33, is one of the *PIBF1*/chromosome 13q21 related subtypes of JS, presenting ciliopathic features such as renal disease, retinal dystrophy, and polydactyly but also the other features of JBTS33 are the combinations of radiological features related to brain abnormalities (molar tooth sign), dysmorphisms (hypotonia, absent deep tendon reflexes, and an enlarged liver), feeding difficulties, seizures, and neurological abnormalities (developmental delay, hypotonia, ataxia) (8,33).

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Wheway et al. (2015) first reported six *PIBF1* pathogenic variant in patients (homozygous c.1910A> p.Asp637Ala) from a consanguineous Canada Hutterite family presenting with mild-to-moderate developmental delay, hypotonia, and ataxia accompanied by imaging findings ranging from the typical "molar tooth sign'' and dysplastic cerebellum to moderate vermis

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hypoplasia with thick cerebellar peduncles (34). Following case-based studies identified Indian (homozygous for c.1181_1182ins36, p.(Gln394_Leu395ins12) and German (heterozygous for c.1453C > T, p.Gln485 & c.1508A > G, p.Tyr503Cys) girls with similar neurological features including molar tooth sign, and thinning of the corpus callosum (9,35). Additionally, they defined facial dysmorphisms such as frontal prominence, retinal dystrophy, deep-set eyes, and midface hypoplasia (9). The index patient had distinct truncal ataxia and the third sibling had more severe retardation. This variable expression and phenotypic alterations in a single population are possibly related to incomplete penetrance, pleiotropy, or differential age of diagnosis.

A recent study by Yue Shen et al. (2020) involved a Chinese boy (heterozygous for c.1147delC, p.Gln383LysfsTer4 & c.1054A > G, p.Lys352Glu) and subsequently confirmed that JBTS33 was characterized by hypotonia, developmental delay, radiological features including molar tooth sign, foramen magnum cephalocele and atypical ocular movements similar to Ott et al. (10). Our current findings in patients add substantially to our understanding of the phenotypic expansion of the JBTS33 disorder with the following symptoms: hypotonia, ataxic gait, developmental delay with early-onset seizures, and severe cystic renal disease with the combinations of accompanying molar tooth sign, cerebellar vermis hypoplasy, vermian cleft, optic atrophy.

Epileptic seizures are uncommon in JS, however in recent cases, only Hebbar et al. (2018) reported a neonatal seizure (33,36). Thus, we also assumed that the neonatal or early onset seizures might be a potential phenotypic expansion of *PIBF1* pathogenic variants and seizure presentation is quite critical to further study to manage JS patients. Unlikely our patients did not have any cortical diplasia; early onset of seizures might be a result of dysplastic changes in the frontoparietotemporal and perisylvian regions of the brain (33). So one of the potent MRI techniques, DTI-tractography, may greatly help explore potential unidentifiable axonal dystrophies in future studies to further understand *PIBF1*-related potential seizures and guide appropriate treatments (37).

However, our patients have not shown findings of cortical malformation, corpus callosum abnormalities or and cerebellar dysplasia one of them had cerebellar vermis hypoplasia and the typical molar tooth indication on brain imaging was assessed in only two of our patients. Two of the patients had renal atrophy, they also revealed gallbladder stones with splenomegaly. However, the 28-month-old third case probably had not developed renal cysts yet. Since now in only one patient was reported with hepatosplenomegaly associated with ADAMTS9

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mutation, none of them was reported with gallbladder stones (38). Our patient also had optic nerve atrophy. Only a minority of patients with other JS subtypes describe optic nerve atrophy (about %22 with optic nerve atrophy), on the other hand, this is one of the first JBTS33 series with optic nerve atrophy findings (39).

Renal cystic diseases and optic atrophy and early onset seizures are rare in Jouberts, but were common at PIBF1-related mutation in this series. Perhaps JBTS33 patients will need to be followed more closely in this respect. Because of the insufficient cases to ascertain the correlation between seizures and PIBF1 pathogenic variant, long-term follow-up and large series are required to establish potential direct relations.

In conclusion, this study confirms *PIBF1* as an initiative genes for JBTS33, and our findings expand the molecular and clinical spectrum of the disease. The definition of other variants in *PIBF1* and advanced confirmation is important for the clarification of the JS subtypes' pathogenic mechanism. A systematic study would identify how JBT33 interacts with pathogenic variants of *PIBF1* that are believed to be linked to phenotypical expansion and clinical features. Next-generation sequencing will possibly provide a complete picture of genetic variants of rare inherited diseases such as JBTS33. We highlight the associated phenotypes linked to *PIBF1* pathogenic variant leading to clinical features of JBTS33.

3. CONCLUSIONS

These cases expanded the spectrum of PIBF1 associated JBTS33. Identifying genetic fundamentals and new phenotypic expansions of the PIBF1 gene is valuable, and more studies are essential to shed light on the phenotypic variation. Our study may help establish appropriate genetic counseling and diagnosis for undiagnosed patients with mild to severe developmental delay potentially accompanied by renal disease, epilepsy and ataxia. To summarize, JBTS33 should be suspected in abnormal development in children with progressive renal disease and/or ataxia, abnormal eye movements, and distinctive radiological findings such as polymicrogyria, hypoplasia of the vermis cerebelli, molar tooth sign with optic nerve atrophy, renal atrophy, cerebellar vermis hypoplasia. Our biallelic homozygous nonsense mutation in ENST00000326291.11:c.1231C>T (p.Arg411Ter) in exon 10 of *PIBF1* has never been reported in homozygous state in the publicly available databases such as dbSNP, 1000 Genomes or GnomAD which is adding to the association of a *PIBF1* pathogenic variant to the clinical features of JBTS33. This paper describes the clinical and genetic characterization of 3 novel patients with inherited PIBF1 defect

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causing psychomotor retardation, dysmorphic features, hypotonia/ataxia, kidney failure, and potentially seizures. This paper has expanded the spectrum of the *PIBF1* gene mutations with the newly identified c.1231C>T (p.Arg411Ter) variant, and proposes further diagnostic awareness that will aid clinicians to establish a faster and more accurate diagnosis so that early interventions and diagnosis are considered. Further studies are required to understand the fundamental mechanisms and clinical correlations between JS and JBTS33.

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Disclosure

No financial disclosure exists for this study or any author.

Ethics and permissions

According to the Erciyes University Medical Faculty Clinical Research Committee model, the patient signed the Free and Informed Consent Form. The study was approved by Erciyes University 2019. The permission and consent forms were obtained from both parents to use the patient's images and information.

FIGURE LEGENDS

Figure 1 **A.** Pedigree of the patients and *PIBF1* Gene & Exons (The novel variants of this study in exon10) marked in red

B. Genotyping results of six DNA samples

Figure 2 Radiological characteristics of patients with distinctive brain malformations including molar tooth sign, cerebellar vermis hypoplasia, optic nerve atrophy. *Axial T1W image shows the ''molar tooth'' appearance of the secondary to the large superior cerebellar peduncles (red arrows, A) and deepened interpeduncular fossa (white arrow, A); bilateral optic nerves (red arrows, B) appear thinner than the superior ophthalmic veins (black arrows, B), related to optic nerve atrophy; Coronal T1W image shows the vermian cleft (white arrow, C); Axial T1W image at the level of the pons shows the ''bat-wing'' appearance of the fourth ventricule (red arrow, D) and cerebellar hemispheres are in opposition in the midline (white arrow, D); Sagittal T1W MR image shows hypoplastic cerebellar vermis (white arrows, E) Note the abnormal folial pattern. The isthmus is abnormally narrow (black arrow, E).*

Table 1 *PIBF1* related to JBTS33 variants and the review of the literature.

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