Renpenning Syndrome in a Turkish Patient: de novo Variant c.607C>T in *PACS1* and Hypogammaglobulinemia Phenotype

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Established Facts

- *PQBP1* is directly associated with Renpenning syndrome.
- *PACS1* is associated with Schuurs-Hoeijmakers syndrome.

Novel Insights

- Our patient showed changes in both *PACS1* and *PQBP1* genes. We found the novel variant c.640C>T in the *PQBP1* gene.
- The patient also has hypogammaglobulinemia which did not respond to treatment.

Keywords

Novel variant · *PACS1* · *PQBP1* · Renpenning syndrome · c.640C>T

Abstract

Renpenning syndrome is an X-linked intellectual disability syndrome caused by mutations in the human polyglutamine binding protein 1 (*PQBP1*) gene characterized by intellectual disability (ID), microcephaly, and dysmorphic facial features. We report a Turkish child with a novel pathogenic variant in *PQBP1* and a likely pathogenic variant in the *PACS1* gene presenting with growth restriction, microcephaly, ID, micropenis, bilateral iris coloboma, and hypogammaglobulinemia. Cytogenetic investigations, including a high-resolu-

tion-banded karyotype, were normal. Clinical exome sequencing was performed. We found the novel *PQBP1* variant, c.640C>T; p.(Arg214Trp), and the known *PACS1* variant, c.607C>T; p.(Arg203Trp), in the proband. The patient's hypogammaglobulinemia did not respond to treatment. This condition was detected for the first time in a patient with Renpenning syndrome.

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Renpenning syndrome is an X-linked intellectual disability syndrome caused by mutations in the human polyglutamine binding protein 1 (*PQBP1*) gene in Xq11.2 characterized by intellectual disability (ID), microcephaly, short stature, microorchidia, and dysmorphic facial

Fig. 1. **A** Facial features of the patient with the *PACSI* variant. **B** Third generation pedigree of a family with novel *PQBP1* variant c.640C>T; p.(Arg214Trp) and *PACSI* c.607C>T; p.(Arg203Trp). The proband is indicated by an arrow. **C** NGS results of the novel missense mutation and comparison of other family members. The altered base is highlighted in red (in the proband and mother). **D** Protein structure of the *PQBP1* gene.

features (prevalence: <1/1,000,000 Worldwide). *PQBP1* is thought to interact with RNA polymerase, polyQ-containing transcription factors, and spliceosome proteins. It contains 6 exons and encodes 38 kDa nuclear proteins predominantly expressed in the central nervous system. *PQBP1* is the first molecule that is directly associated with neurodegenerative diseases accompanying dementia, brain atrophy, developmental symptoms such as ID, and microcephaly [Okazawa, 2018]. In this case, in addition to changes in the *PQBP1* gene, a pathogenic variant was found in the *PACSI* gene. This variant in *PACSI* has been

previously reported in patients with Schuurs-Hoeijmakers syndrome by exome sequencing. Schuurs-Hoeijmakers syndrome (OMIM 615009) is an autosomal dominant disorder characterized by ID, distinct craniofacial features, and variable additional congenital anomalies [Schuurs-Hoeijmakers et al., 2016].

Here, in addition to the novel variant in the *PQBP1* gene, we will determine the effect of changes in the *PACSI* gene on the phenotype with the contribution of a likely pathogenic variant.

Case Presentation

A 6-year-old male patient presented with ID and dysmorphic features. There was no exposure to the toxic effects during pregnancy (especially alcohol), infectious disease during pregnancy, or perinatal anoxia. The parents are not related; the mother was 31 years old (G2P2Y2). The boy's birth weight was 3,000 g. During prenatal follow-up, at 26 weeks, his developmental growth retardation was noticed, and a brain cyst was diagnosed. He had jaundice and hypoglycemia in the postnatal period and received phototherapy for jaundice. Following, he was hospitalized for pulmonary infection at 3 and 6 months of age. Later, he had intermittent bronchitis. Aortic coarctation was suspected immediately after birth, followed by patent foramen ovale. He was able to hold his head when he was 6 months old and was able to walk without support at 18 months. When the patient was 61 months old, according to the Ankara Development Screening Inventory (AGTE), gross motor skills were at 20 months, fine motor skills were at 3.5 years, language development 18–19 months, and his general development was at 16–17 months. It was reported as mild cognitive retardation, markedly in speech and social skills. The patient has a nutritional problem. The hearing test was normal. The patient's general neurological condition was good.

Methods and Results

On physical examination, his height was 98 cm, weight was 20 kg, and his head circumference was 50 cm. The boy's face was flat, eyebrows were sparse, and hypertelorism, wide palpebral fissure, lower eyelid eversion, epicanthus, downturned corners of the mouth, and bilateral inferior iris coloboma were observed (Fig. 1A). The patient had microtia, trichomegaly, a micropenis, and both testes were found in the proximal part of the inguinal canal. In the ultrasound, the right testis dimension was 15 × 10 × 7 mm, and the volume was 0.5 cm³. The dimension of the left testis was 14 × 10 × 8 mm, and the volume was 0.6 cm³. The contours were smooth, the parenchyma echoes were normal. Both epididymides showed normal echo structure. No hydrocele was detected.

The patient's tonus was normal; there was no lateralizing motor deficiency. Mild cerebral atrophy and secondary enlargement of the third lateral ventricles, hypoplasia of the inferior vermis, and delayed myelination areas in the posterior periventricular white matter were detected. TSH level was high (TSH: >8, St4: 1.25). The right lobe dimension of the thyroid gland was 15 × 8 × 10 mm and the left lobe was 13 × 8 × 7 mm. Isthmus AP diameter was 2 mm. Both lobe parenchyma were homogenous and the echo intensity was normal. There were no space-occupying cystic or solid nodules detected in the lobes. Recurrent urinary tract infections and bronchitis were reported. Despite treatment, Ig levels were low, causing suspicion of hypogammaglobulinemia (IgG: 705 mg/dL [776–1195], IgA: 31 mg/dL [54–129], IgM: 29 mg/dL [65–146], IgE: <18). It was observed that the values were still low after 6 months of follow-up (IgG: 717 mg/dL [776–1195], IgA: 34 mg/dL [54–129], IgM: 29 mg/dL [65–146], IgE: <18) (Table 1).

Molecular Genetic Studies

The pedigree analysis shows that he has a healthy brother; a cousin is noted to have unspecified epilepsy (Fig. 1B, III3).

Table 1. Phenotypic features of the present case with overlapping syndromes

Features	Phenotypic features of the present case
<i>Renpenning syndrome, PQBP1</i>	
Microcephaly	
Short stature	+
Small testes	+
Anal anomalies	
Intellectual disability	Mild
Speech	+
Language problems	+
Cardiac malformations	PFO
Dysmorphic facial features	
Long narrow face	
Upslanting palpebral fissures	+
Abnormal nasal configuration	
Cupped ears	
Short philtrum	
Long or bulbous nose	+
<i>Schuurs-Hoeijmakers syndrome, PACS1</i>	
Cryptorchidism	+
Prefer soft foods	
Autism spectrum disorder	
Impaired communication and social interaction	+
Seizures	
Hypotonia	
Delayed in walking	+
Dysmorphic facial features	
Arched eyebrows	+
Long eyelashes	+
Hypertelorism	+
Downslanting palpebral fissures	
Ptosis	+
Rounded nasal tip	+
Wide mouth, downturned corners	+
Thin upper lip	+
Philtrum	
	Recurrent urinary tract infections and bronchitis
	Hypogammaglobulinemia
	High TSH level
PFO, patent foramen ovale; TSH, thyroid-stimulating hormone.	

Cytogenetic investigations, including a high-resolution-banded karyotype, were also normal in the patient. Clinical exome sequencing was performed in the proband. Peripheral blood samples were collected from the proband and his parents. Genomic DNA of the patient was examined with Clinical exome solution (Sophia Genetics, 4493 genes) associated with human disease by NGS-

based sequencing technology. The generated library was sequenced on the Illumina NextSeq 500 device (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The obtained variants were evaluated considering the >Q30 reading quality and a >50 confidence score. The software (Sophia DDM 5.2.1) was used for the analysis of the data. Various filtering options were used for the identification of the phenotypes of the variants that were performed by annotation procedure. The defined variants were labeled in accordance with the recommendation standards of the American College of Medical Genetics, and Genomics (ACMG) [Richards et al., 2008]. A novel hemizygous missense variant in the *PQBP1* gene, NM_001032381: c.640C>T; p.(Arg214Trp), and a heterozygous variant in the *PAC1* gene, NM_018026: c.607C>T; p.(Arg203Trp) was detected in the patient. This new variant was confirmed by using NGS. The de novo variant, also found in the *PAC1* gene, was not seen in the patient's parents. It was predicted to be "probably damaging" by PolyPhen-2, and SIFT, and "disease causing" by MutationTaster. Cosegregation analysis was performed in the individuals who were likely to be affected in the family. The c.640C>T variant in *PQBP1* was found in heterozygote state in the asymptomatic mother (Fig. 1C); X-inactivation study did not follow.

Discussion

The patient with a novel pathogenic variant in *PQBP1* and likely pathogenic variant in the *PAC1* gene presented with growth restriction after birth, dysmorphic facial features, ID, micropenis, and bilateral iris coloboma. *PQBP1* has been suggested to be one of the major contributors to coronary heart disease [Talukdar et al., 2016]. The patient was followed for patent foramen ovale (Table 1).

PQBP1 is generally considered as one of the major causative genes for ID. The clinical diagnoses of these patients include Renpenning syndrome, Golabi-Ito-Hall syndrome, and Sutherland-Haan syndrome. All these syndromes share ID, microcephaly, short stature, lean body, and hypogonadism [Stevenson et al., 2005]. Exome analysis revealed the novel pathogenic *PQBP1* variant, c.640C>T; p.(Arg214Trp), which was not previously reported in Renpenning syndrome. The mother is clinically asymptomatic.

In the Renpenning syndrome spectrum, pathogenic mutations are found in the WW domain, polar amino acid rich region, and C-terminal domain (CTD) [Germanaud et al., 2011]. Mutations in the CTD cause the amino acid reading frame to shift. The CTD of *PQBP1* binds to U5–15 kD, a component of the U5 small ribonucleoprotein particle, which is one of the components of the spliceosome [Waragai et al., 2000]. Once *PQBP1* captures U5–15 kD using the CTD, the spliceosome may be located in close proximity to Pol II because *PQBP1* binds to Pol II via its

WWD. When *PQBP1* connects the spliceosome and Pol II, *PQBP1* may act as a spacer molecule between these large multiprotein complexes [Takahashi et al., 2009]. Previous mutations in the *PQBP1* gene were mostly found in the middle of exon 4, but the c.640C>T; p.(Arg214Trp) missense variant was found in the CTD region. This novel missense mutation does not cause the change of a YPSPGAVL motif in the CTD region of *PQBP1*. This motif is essential for the U5–15 kD binding. The variant can destroy a canonical splice donor site and is predicted to cause abnormal gene splicing, leading to either an abnormal message that is subject to nonsense-mediated mRNA decay or result in a disrupted protein product.

We also found a c.607C>T; p.(Arg203Trp) variant in *PAC1* in the exome analysis of the proband. *PAC1* (OMIM 607492) encodes the *PAC1* protein, which is a trans-Golgi membrane traffic regulator. *PAC1* is a 962 aa protein, consisting of 4 domains: the atrophin-1-related region, the furin-(cargo-) binding region (FBR), a middle region, and the C-terminal region. The FBR is responsible for the binding of cargo proteins, playing a major role in the trans-Golgi network pathway. The autoregulatory domain is phosphorylated by casein kinase 2 (CK2); only if it is phosphorylated, cargo binding is possible. The de novo variant c.607C>T is located in close proximity to the CK2-binding motif (RRKRY200) and therefore possibly influences the binding properties of *PAC1* [Schuurs-Hoeijmakers et al., 2012; Gadzicki et al., 2015]. The *PAC1* mutation is known to cause Schuurs-Hoeijmakers syndrome characterized by ID, characteristic facial features (arched eyebrows, widely spaced eyes, a wide mouth with downturned corners, and thin upper lips with a cupid's bow shape), oral aversion, seizures, hypertelorism, and brain structural abnormalities [Gadzicki et al., 2015]. Unlike other patients, our patient had frequent recurrent urinary tract infections and bronchitis. The mentioned hypogammaglobulinemia did not respond to treatment. A 3-year-old male patient with Schuurs-Hoeijmakers syndrome reported by Gadzicki et al. [2015] was found to have low immunoglobulin levels.

In summary, it is difficult to diagnose intellectual disorders and other neurodevelopmental disorders in terms of genetic heterogeneity and clinical variability. The use of technologies such as microarray analysis and exome sequencing increased the diagnostic efficiency. In our case, the novel variant c.640C>T in the *PQBP1* gene gave evidence of Renpenning syndrome, and it was also clear that the c.607C>T variant in *PAC1* was the cause of the low level of immunoglobulin in a patient who had previously shown a Schuurs-Hoeijmakers syndrome.

Statement of Ethics

All procedures, including the informed consent process, were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration.

Disclosure Statement

The authors have no conflicts of interest to declare.

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Author Contributions

F.C.K., N.E. designed and coordinated the study. C.A., A.E.S. assessed the clinical and radiological findings of the case. F.C.K. and N.E. performed molecular genetic studies and analyzed the data. F.C.K. and N.E. wrote the draft of the manuscript. All authors read and approved the final manuscript.