The correlation between multiple congenital anomalies hypotonia seizures syndrome 2 and PIGA: a case of novel PIGA germline variant and literature review

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Abstract
Background PIGA (PIG class A) gene codes for the PIG-A protein, which is a catalytic subunit of GPI-GlcNAc transferase. GPI-anchored proteins play an important role in the metabolism of mammals. Somatic variants of PIGA genes in bone marrow hematopoietic stem cells often result in paroxysmal nocturnal haemoglobinuria, and the germline PIGA variants cause multiple congenital anomalies hypotonia seizures syndrome 2 (MCAHS2) because of glycosylphosphatidylinositol metabolic abnormalities.

Methods Whole exome sequencing was performed on peripheral blood sample of the patient with MCAHS2. A novel germline PIGA variant was found, and Sanger sequencing was performed as verification for the variant. After that, we used the keywords to retrieve relevant reports and provided a literature review.

Results A novel hemizygous germline PIGA variant (NM_002641.3:c.971G>A) at exon4 was identified through whole exome sequencing. And it was a highly probable pathogenic variant. Sanger sequencing yielded consistent results. The missense variant cause change of p.(Cys324Tyr) in the transcription product according to the predicted outcomes.

Conclusion We reported a case of MCAHS2 caused by a novel PIGA variant. Following a review of the literature, we suggested that MCAHS2 should be considered as a disorder spectrum consisting of core symptoms, multi-system impairment, and premature death. The core symptoms include hypotonia, psychomotor delay, epilepsy (intractable epilepsy mostly) and early death. Core symptoms nearly happened to almost all patients. Meanwhile, MCAHS2 involves a wide range of organ and system impairments with changeable form.

Keywords PIGA gene · Multiple congenital anomalies hypotonia seizures syndrome 2 · Early Onset/Infantile Epilepsy Encephalitis · Whole exome sequencing
**Introduction**

Glycosylphosphatidylinositol (GPI) is a lipid anchor, by which at least 150 proteins are attached to the cell membrane. Those proteins are termed as GPI-anchored proteins (GPI-APs)[1, 2]. The special carboxyl terminus of GPI has a hydrophobic terminal as signal sequence[3, 4]. Precursors of GPI are synthesized through a series of enzymatic reactions in the endoplasmic reticulum. By a transamidation reaction, GPI-transamidases recognize the signal sequence of the proteins and replace it with GPI precursors[4, 5]. GPI-APs play an important role in the metabolism of mammals, involving many functional proteins such as hydrolytic enzymes, adhesion molecules, receptors, protease inhibitors and complement regulatory proteins[1, 2, 5]. These GPI-APs are particularly essential for embryogenesis, neurogenesis, fertilization and immune system[6]. At least 26 genes have been found tightly related to GPI biosynthesis, the processes of GPI binding to proteins and remodeling of side branches[1]. These genes were named PIG (Phosphatidyl Inositol Glycan), and at least three genes including class A, C and H (usually called PIGA, PIGC and PIGH) are involved in the first step of GPI biosynthesis. The PIGA (PIG class A) gene codes for the PIG-A protein, which is a catalytic subunit of GPI-GlcNAc transferase located at Xp22.2 [1].

The pathway of GPI synthesis could be divided into several subdivisions: (1) early GPI anchor synthesis, (2) late GPI anchor synthesis, (3) GPI transamidase, and (4) remodeling of fatty acids of the GPI anchor after attachment to proteins. There is also evidences proved that the interruption of GPI synthesis at a late stage may result in hyperphosphatasia[7]. Somatic variants of PIGA genes in bone marrow hematopoietic stem cells often result in paroxysmal nocturnal hemoglobinuria. The complement decay-accelerating factor (also known as CD55) and CD59 glycoprotein on the cell membrane tends to decrease due to the absence of GPI anchors[5, 8, 9]. Many studies have explored the effects of these missense mutations on the structure and function of related proteins by employing relevant computational tools[10].

Technology for genome engineering were also used for studies involved with PIGA[11]. Of concern, the germline PIGA variants result in multiple congenital anomalies hypotonia seizures syndrome 2 (MCAHS2,MIM#300,868)[12].

MCAHS2 could also be classified as Early Onset/Infantile Epilepsy Encephalitis (EOEE/EIEE) clinically according to clinical manifestations[13, 14]. Development Epilepsy Encephalopathy (DEE) and EOEE/EIEE represent similar concepts, features of both terms are refractory epileptic seizures, developmental delay or regression associated with ongoing epileptic activity. And these features tend to occur in the first trimester of infancy and are most common in the neonatal period in the patient’s life[14–17].

Ferro-Cerebro-Cutaneous syndrome resulting from PIGA germline variants has also been reported, which is characterized by neurodegenerative encephalopathy, cutaneous manifestations and systemic iron overload[18]. EOEE can be divided into five main syndromes: Ohtahara syndrome, West syndrome, Lennox-Gastaut syndrome, Dravet syndrome, and Landau-Kleffner syndrome[15]. So far, most EOEE caused by PIGA germline variants can’t be classified as a specific syndrome. It is not difficult to understand that illumination for the relationship between genotypes and phenotypes will provide great help for the early identification of epilepsy encephalopathy. A case of MCAHS2 caused by PIGA gene variant was found by whole exome sequencing (WES), and followed by Sanger sequencing as verification. We described this patient’s phenotype as much as possible and reviewed previous literature with details of the phenotype, hoping to offer help to elucidate the relationship between genotype and phenotype.

**Method**

**Patients and DNA extraction**

DNA extraction from peripheral blood samples were performed by the Tianjin Children’s Hospital (Tianjin, China) in June 2020. DNA was extracted from the proband and submitted for exome sequencing using a Blood Genomic DNA Mini kit (cat. no. CW0541; CoWin Biosciences) according to the manufacturer’s protocol. The ratio of the absorbance at 260 and 280 nm (A260/280 ratio) were evaluated with a NanoDrop® 2000 spectrophotometer (Thermo Fisher Scientific, Inc.). We obtained a total of 100 µl DNA solution (≥ 10 ng/µl), which was stored at -20°C. The study had obtained written informed consent from parents and was approved by the ethics committee of Tianjin Children’s Hospital (Tianjin, China).  

**WES and bioinformatics analysis**

WES for proband was performed by BGI Group. Paired-end sequencing was performed with reading lengths of 150 bp and >95% of the target regions, including all coding regions and exon-intron boundarie with an average coverage depth of 100-fold. Burrows-Wheeler Aligner software was used to align the sequencing data with the human reference genome hg19. The insertion, deletion and single nucleotide polymorphism sites were analyzed by Genome Analysis Toolkit software. Variant annotations were made using the ANNOVAR tool (V20180118; https://www.ncbi.nlm.nih.gov/projects/annovar/download.shtml), dbSNP (https://database.1000genomes.org/).
ncbi.nlm.nih.gov/snp/?term=) and OMIM (https://omim.org/) data-bases. The effect of the variants on the structure and function of the proteins was predicted using Polymorphism Phenotyping v2 software (http://genetics.bwh.harvard.edu/pph2/index.shtml) and Sorts Intolerant From Tolerant software (V1.1; http://sift.jcvi.org/). In addition, Human Splicing Finder (V3.1; http://www.unmd.be/HSF/) was used to predict the splice sites in the gene.

**Variant screening and Sanger sequencing**

PCR and further Sanger sequencing were performed for confirming the candidate variants and analyze the cosegregation pattern for the both two variants respectively. For the PIGA located at X chromosome, PCR and Sanger sequencing were performed for the proband and his mother. Amplification was performed in a final volume of 50 µl, containing 25 µl 2X GC buffer I, 20 mM deoxynucleoside triphosphates mixture, 100,200 ng DNA, 0.5 µM forward and reverse primers and 2.5 U LA Taq polymerase (cat. no. RR02AG; Takara Biotechnology Co., Ltd.). The following thermocycling conditions of initial denaturation for 2 min at 94˚C, followed by 35 cycles of 94˚C for 30 s, 58˚C for 30 s and 72˚C for 40 s; and a final extension step at 72˚C for 5 min were used for the PCR. The PCR products were separated by 1.5% agarose gel electro-phoresis and the proper DNA was purified from agarose gel using a Gel Extraction kit (CoWin Biosciences) and sent to Genewiz (Beijing, China) for Sanger sequencing. Chromas software (version 1.62; Technelysium Pty Ltd.) was used to compare the sequencing data with the reference sequences (NM_002641.3) in GenBank (https://www.ncbi.nlm.nih.gov/nuccore/NC_00015319451&to=15335554&strand=true).

**Literature review**

Genetic association studies or case reports published before the end of Jun, 2021 about germline PIGA variants on PubMed, web of Science, EMBASE were acquired, using combinations of the following keywords “early onset epilepsy encephalopathy”, “development Epilepsy encephalopathy”, “early infantile epilepsy encephalopathy”, “PIGA”, “early infantile epilepsy encephalopathy”, “germline PIGA variants”, “early onset development and epilepsy encephalopathy”, “glycosylphosphatidylinositol biosynthesis defects”, “multiple cogenital anomalies-hypotonia- seizures syndrome 2”. We reviewed the retrieved literature and drew those phenotypes into the Table 1 to describe the clinical phenotype of PIGA germline variants visually. IBM SPSS 23.0 was used for statistical analysis of the data obtained from previously published articles and our clinical records. Non-normally distributed measurement data was represented by $M(P_{25},P_{75})$.

**Results**

**Clinical phenotypes**

The proband was admitted to Tianjin Children’s Hospital because of repeated nonfebrile seizures 7~8 times within 30 h. His parents came from nonconsanguineous families without family history of epilepsy. However, the birth history of the proband was not clear. The patient began to have repeated nonfebrile seizures at the age of six months. The initial clinical manifestations were lips cyanosis, rigidity of extremities and lack of response to shouts. The interphase of onset lasted from one hour to over ten hours, each episode lasted about 1 min. Symptoms were controlled effectively with phenobarbital until recurrence at the age of 9 months with the epilepsy form of focal epilepsy. Generalized tonic seizures were observed frequently. Seizures were intractable in spite of taking a variety of antiepileptic drugs including topiramate, phenobarbital and oxcarbazepine. The patient was exhausted after remission of seizures, and no language or motor disorders were observed. In this relapse, the patient presented with significant hypotonia. Thus, the boy was admitted to Tianjin children's hospital (Tianjin, China), then high fever took place frequently which was difficult to drop to normal with febrifuge, seizures occurred more than 10 times a day at the most. The physical examination revealed that the patient had general psychomotor development delay, sporadic dark brown skin rashes scattered around the right lower limb and would not fade when pressed it. No obvious facial malformation was observed. No obvious abnormalities in cardiovascular, respiratory or other systems were observed. The outcome of brain magnetic resonance imaging (MRI) showed abnormal images with small symmetrical dots of high signal shadows in the dorsal pons, and the extracephalic space was slightly wider. Video-Electroencephalogram (EEG) showed bilateral frontal pole, frontal lobe, temporal lobe and frontal midline areas release sharp wave, sharp slow wave and tip type of slow wave and awake generalized slow wave short-long-range paroxysm with slow background.

**Genetic analysis: results of WES and Sanger sequencing**

A missense variant was revealed by the WES within PIGA gene (NM_002641.3). The hemizygous variant c.971 in PIGA gene was located in exon 4. It led to an amino acid exchange from Cystine to Tyrotine in the position of the
sive epilepsy, however, whether the variant was pathogenic was not clear[20]. Though Yang et al.[21] reported a patient without epileptic symptoms, it was possible that the patient died due to respiratory complications before the onset of seizures. There was no doubt that epilepsy was still an important feature. Cash et al.[22] reported 2 cases of PIGA-related disorders, both of which showed the symptoms of autism. Accelerated linear growth[19] and overgrowth[17] were also recorded. Various developmental abnormalities could occur, such as wide-spaced small teeth[12, 23] and cleft palate[24]. Facial malformations were common with diverse patterns, details were recorded in the annotations of Table 1. Tarailo et al. [25]reported a patient with bilateral facial asymmetry caused by inconsistent degree of bilateral brachycephaly. Congenital abnormalities could occur in multiple systems. Abnormalities of cardiovascular system included atrial septal defect[18, 19, 26], right ventricular hypertrophy and arrhythmia[25] and left ventricular dilated[26]. Various cutaneous lesions could appear, such as psoriasis-like skin lesions[17, 23]. The reduction of range of motion of extremities joints with camptodactyly and deep palmar creases has also been reported[18, 25]. Neuhof et al.[12] reported the condition of dyskinesia and Low et al.[24] reported the condition of scoliosis. Abnormalities of motor and nervous system also manifold, hypotonia is the core feature according to the Table 1, whether the axial hypotonia with or without normal peripheral tone even peripheral hypertonia or the generalized hypotonia[20, 23, 25]. Abnormal reflexes[17, 23, 26], dystonia and chorea of the distal extremities[20, 24, 27], and repetitive stereotyped behavior[27] could also occur. Neonatal hemochromatosis caused by PIGA germline variants has also been reported[28]. Cerebral visual impairment, bilateral strabismus and other ophthalmic problems could also occur[12]. Besides, abnormalities of other systems, including the urinary system, digestive system, respiratory system and auditory and visual system may also occur[18, 25, 26]. Surface expression levels of CD16, CD24, CD58 and CD59 as the representative of GP-APs were obviously lower compared with health control[29, 30]. Serological abnormalities of elevated ferritin levels were observed in addition to elevated alkaline phosphatase levels[17]. Abnormalities in brain development were also observed by brain MRI. Kim et al.[29] reported a patient with normal brain MRI image at 1 years, and MRI presented bilateral hippocampal sclerosis at 7 years. Some marks could be detected by MRI, such as white-matter immaturity[17, 18, 26], thin corpus callosum[17, 19, 25, 26], slightly enlarged ventricles and subarachnoid space[12, 17, 26], delayed myelination[19], diffuse leukoencephalopathy[25] and small cerebellum[18, 26], prominent sulci[18], all of which signified brain atrophy. Image of patchy areas of myelin damage also has been reported[24]. Some microcephalies were also observed.

**Literature review**

Dozens of PIGA gene variants have been detected by gene sequencing technologies. However, it is still a challenge to clarify the genotype-phenotype correlation of MCAHS2 due to a lack of systematic description and relatively few case reports with accurate medical history, which is not beneficial for early identification. So we filtered the reports with detailed medical history and summarized these reports to characterize the MCAHS2, hoping to offer help for early identification. In order to get concise results, we tabulated information into Table 1. According to the review results of Table 1 MCAHS2 usually occurs relatively early in the stage of infancy. The median age of epilepsy onset was 6.00 months (6.00(3.00,7.00), 95%CI:3.33~8.67). In the data obtained, all the patients were males. 83.3% of patients’ epilepsy were intractable (15/18). Delayed psychomotor development was observed in all the patients assessed, and hypotonia was present in 78.3% of the patients (18/23). Alkaline phosphatase elevations were observed in 40.0% of patients (6/15), facial malformations were observed in 70.0% of patients (14/20), and abnormal MRI images were observed in 70.0% patients(14/20). Something worth noticing was that an unaffected male was reported by Crabben et al.[26]. Meanwhile, a female patient (the proband’s maternal aunt with serial number of ZY03) with heterozygous variant (c.356G>A) shows symptoms of pharmacoresponsive epilepsy, however, whether the variant was pathogenic...
Table 1 Clinical summary of patients with PIGA

| Reported by                        | Variant types | Sex | Age | Seizure onset | Seizure types | Seizure prognosis | Psychomotor delay | Hypotonia | Alkaline phosphatase levels | Facial malformations | MRI |
|-----------------------------------|---------------|-----|-----|---------------|---------------|-------------------|-------------------|-----------|-----------------------------|---------------------|-----|
| Xie et al.[30]                    | c.110 T>C     | M   | 10d | /             | GTCS,ME,SE    | intractable       | +                 | +         | normal                       | +                   | normal |
| Perk et al.[17]                   | c.992 C>T     | M   | 6 m | /             | ES,AS,ME      | intractable       | +                 | -         | normal                       | +                   | normal |
| Van der Crabben et al.[19]       | c.278 C>T     | M   | 8.5 m | Died at 2y6m | GCS           | intractable       | +                 | +         | elevated                     | abnormal            | |
| Lin et al.[20]                    | c.356G>A      | M   | 2 m | /             | ME,ES         | intractable       | +                 | +         | normal                       | +                   | abnormal |
| Cash et al.[22]                   | c.368 C>T     | M   | 1 w | 8y            | AS,ME         | self-limited      | +                 | +         | normal                       | normal              | |
| Kim et al.[29]                    | c.427 A>G     | M   | 5 m | 11y           | FE            | intractable       | +                 | -         | normal                       | normal              | abnormal |
| Joshi et al.[27]                  | c.535 A>T     | M   | 4 m | 6y            | Partial SE    | intractable       | +                 | +         | normal                       | normal              | |
| Tarailo et al.[25]                | c.989G>A      | M   | 2.4 y| Died at 3.4 y | ME            | intractable       | +                 | +         | elevated                     | abnormal            | |
| Johnston et al.[26]               | c.1234 C>T    | M   | /   | Died at 11 w | ME            | /                 | +                 | +         | elevated                     | abnormal            | |
| Yang et al.[21]                   | c.849-5 A>G   | M   | /   | Died at 2 m  | /             | /                 | +                 | +         | normal                       | normal              | abnormal |
| Neuhofer et al.[12]               | C.154 C>T     | M   | 8 m | 9y            | FE,GTCS,ME,TS | intractable       | +                 | +         | normal                       | +                   | normal |
| Kato et al.[14]                   | c.616 A>T     | M   | 3 m | 10y           | MS,SLM        | intractable       | +                 | -         | elevated                     | normal              | abnormal |
|                                  | c.230G>T      | M   | 7 m | 8y            | TS,SGS        | pharmaco-responsive | +                 | -         | normal                       | abnormal            | abnormal |
|                                  | c.230G>T      | M   | 7 m | 18 m          | GCS,TS        | self-limited      | +                 | -         | normal                       | abnormal            | abnormal |
|                                  | c.355 C>T     | M   | 3 m | 15 m          | MS,TS         | intractable       | +                 | +         | elevated                     | abnormal            | |
| Fauth et al.[18]                  | c.1234 C>T    | M   | 5d  | Died at 15d  | TS             | intractable       | /                 | +         | elevated                     | abnormal            | |
|                                  | c.1234 C>T    | M   | /   | Died at 3 m  | ME             | intractable       | /                 | +         | elevated                     | abnormal            | |
| Low et al.[24]                    | c.293 A>C     | M   | 4 m | 17y           | FE,AS,TS      | intractable       | +                 | +         | normal                       | normal              | abormal |
Table 1 (continued)

| Reported by | Variant types | Sex | Seizure onset | Age | Seizure types | Seizure prognosis | Psychomotor delay | Hypotonia | Alkaline phosphatase levels | Facial malformations | MRI |
|-------------|---------------|-----|---------------|-----|--------------|-------------------|------------------|-----------|----------------------------|----------------------|-----|
| Swoboda et al.[23] | c.1030_1032delCTT | M | Died at 7y | FE | / | + | + | / | / | / | / |
| c.1030_1032delCTT | M | Died at 16y | FE,UN | / | / | / | / | / | / | / |
| c.1030_1032delCTT | M | 9m | / | / | / | / | / | / | / | / |
| Present case | M | 6m | 9m | FE,GCS | / | / | / | / | / | / |

Xie et al. reported a pair of monozygotic twins with allied clinical manifestation. Age: age at last recorded or age of death; GTCS: generalized tonic-clonic seizures, GCS: generalized clonic seizure, MS: myoclonic epilepsy, ES: epileptic spasms, SL:spasm-like movement, AS: atonic seizures, TS: tonic seizures, SGS: secondarily generalized seizures; MRI: abnormal imaging finded by cranial, FE: focal epilepsy; UN: unclassified seizures. MR1; “+” indicates that the content is not recorded or evaluated. “-” is just the opposite. The “y”, “m”, “d” on behalf of the “Years”, “months”, “days” respectively. *Wide-set eyes, depressed nasal bridge, and short anteverted nose; *Long coarse face, prominent chin, macrostomia, macroglossia, fleshy lips, gingival hypertrophy; *High anterior hairline, mildly upslanted palpebral fissures, a thin vermilion, a long philtrum, alveolar ridge overgrowth, absence of teeth, deepened palmar creases; *Depressed nasal bridge, large mouth, high-arched palate, and micrognathia; *Flat nasal bridge and deep-set eyes; *Pierre Robin sequence, prominent occiput, enlarged fontanelle, depressed nasal bridge, short and anteverted nose, malar flattening, up slanted palpebral fissures, overfolded helix, gingival overgrowth, small mouth with downturned corners, triangular shape, short neck, globulous chest, small nails; *Concave nasal bridge, low-set ears; *Upslanting eyes, a long philtrum and a thin upper lip; *High anterior hairline, upslanted palpebral fissures, a depressed nasal bridge, short nose with anteverted nares, malar flattening, a long philtrum, a thin vermilion of the lips, down-turned corners of the mouth, micrognathia, a cleft soft palate, large and uplifted earlobes, an overfolded helix of the left ear, and a short neck; *High anterior hairline, bitemporal narrowing, a prominent metopic ridge with a midline naevoid simplex, widely spaced eyes, a depressed nasal bridge, a short nose with anteverted nares, a prominent, fleshy philtrum, a wide mouth with thick vermilion of the lip.

easily[12, 25]. It was not strange that intellectual disability (ID/DD) appeared[17, 22, 24]. Children who survive to older ages have speech problems[23, 24]. High degree of dysrhythmia and burst-suppression pattern could be observed in EEG[18, 23, 26].

About the treatment, Joshi et al.[27] reported that with therapeutic levels of multiple antiepileptics, ketogenic diet decreased the frequency of episodes of status epilepticus significantly, and the situation of development improved appropriately. However, no significant improvement was observed on the similar therapy reported by Kim et al.[29] and Xie et al.[30]. Though the reason of deaths were still unclear, deaths due to respiratory abnormalities were more common according to those cases that have been reported[19, 21, 26]. Respiratory complications are common causes of death[18, 19, 21]. Both of the patients reported by Yang et al.[21] and Crabben et al.[19] died because of respiratory complications at an early stage, especially the latter one illuminated that progressive hypotonia may be the root reason. The patients with normal muscle strength have a relatively long survival time[17]. Although individual cases could not be regarded as conclusive evidence, relief of hypotonia may delay disease progression according to simply inferring only, LEvodopa (1 mg/kg/day) alleviated dyskinesia reported by Lin et al.[20] to some extent. By resecting the mild dysplastic cortical at 2 years old, the seizures improved 50%-90%. Though, unfortunately, the patient was still near non-ambulatory with decreased muscular tone and strength at the age of 13[22].

Discussion

We reported a case of MCAHS2 due to novel PIGA variant (NM_002641.3:c.971G>A), which expanded variant spectrums of PIGA and provided help for genetic counseling. Then we fully demonstrated how we discovered the variant using sequencing technologies and identified it as a pathogenic variant. It would be of great help in finding likely pathogenic variants for clinicians. Many genes have been proven to cause EOEE, including SCN1A, SCN2A, SCN8A, SCNQ2, KCNT1, KCNA2, STXBP1, CDKL5, PIGA, SPTAN1, GNAO1, PNPO, ARX, SIK1, SLC25A22 and CHD22[13–15, 31, 32]. About 80% of Dravet syndrome is caused by SCN1A gene variants. Ohara syndrome is related to ARX and STXBO1 gene variants and West syndrome is related to ARX, CDKL5, SPTAN1 gene variants mostly[7, 14, 15, 31]. However, the correlation between genotypes and phenotypes remains elusive. Elucidation of the correlation between phenotypes and genotypes of diseases is undoubtedly significant. In fact, MCAHS2 should be treated as a disease spectrum[19, 22].

An exhaustive literature review was conducted to describe the spectrum of MCAHS2 as clearly as possible. The spectrum can be summarize into core symptoms and
scoliosis and long bones with reduced mineral impairment (8/73)
elevated level of alkaline phosphatase (11/73)
basicall normal, polyhydramnios (10/49)
Pernatal history
Ophthalmological features
basically normal, polyhydramnios (10/49)
cortical visual impairment (15/39), refractive error (8/39), retinal coloboma or retinal dystrophy (not quantified)
Otological features
hearing impairment (8/73)
Cardiovascular features
atrial septal defect (7/76), cardiomyopathy (7/76), persistent foramen ovale (1/76)
Genitourinary features
Hydronephrosis (5/76), vesicoureteral reflux (4/76), and dysplastic kidneys (1/76), nephrolithiasis (2/76), one of the two spontaneously resolved, bilateral inguinal hernia (2/76), small penis (3/76), bilateral cryptorchidism (2/76)
Gastrointestinal features
feeding difficulties (17/73), Gastroesophageal reflux (10/73)
Endocrine features
elevated level of alkaline phosphatase (11/73)
Dermatological anomalies
dermatitis (12/73), excess of skin (10/73), ichthyosis (8/73), deep palmar creases (2/73), psoriasis (4/73)
Skeletal anomalies
scoliosis and long bones with reduced mineralization (8/73)
ID/DD
mild (4/39), moderate (4/39), severe (13/39), profound (18/39)
Movement disorders
dyskinesia (8/73), dystonia (7/73), choreo-athetosis (7/73), tremor (2/73), ataxia (1/73), hypertrekplexia (1/73), spasticity (3/73), overactive or overresponsive reflexes (16/73)
Behavioral and psychiatric features
autistic features (8/73), stereotypies (4/73), autistic disturbances anger tantrums (1/73), obsessive-compulsive disorder (1/73)

Table 2 multiple systems impairments obtained by Bayat et al.[33]

| Organs or systems and abnormalities | Clinical manifestation with ratio |
|------------------------------------|----------------------------------|
| Pernatal history                   | basically normal, bolyhydramnios (10/49) |
| Ophthalmological features          | cortical visual impairment (15/39), refractive error (8/39), retinal coloboma or retinal dystrophy (not quantified) |
| Otological features                | hearing impairment (8/73) |
| Cardiovascular features            | atrial septal defect (7/76), cardiomyopathy (7/76), persistent foramen ovale (1/76), atrial septal aneurysm (1/76), bicupid aorta valve (1/76), mildly dilated ascending aorta (1/76), first-degree atrioventricular block (1/76) |
| Genitourinary features             | Hydronephrosis (5/76), vesicoureteral reflux (4/76), and dysplastic kidneys (1/76), nephrolithiasis (2/76), one of the two spontaneously resolved, bilateral inguinal hernia (2/76), small penis (3/76), bilateral cryptorchidism (2/76) |
| Gastrointestinal features          | feeding difficulties (17/73), Gastroesophageal reflux (10/73) |
| Endocrine features                 | elevated level of alkaline phosphatase (11/73) |
| Dermatological anomalies           | dermatitis (12/73), excess of skin (10/73), ichthyosis (8/73), deep palmar creases (2/73), psoriasis (4/73) |
| Skeletal anomalies                 | scoliosis and long bones with reduced mineralization (8/73) |
| ID/DD                              | mild (4/39), moderate (4/39), severe (13/39), profound (18/39) |
| Movement disorders                 | dyskinesia (8/73), dystonia (7/73), choreo-athetosis (7/73), tremor (2/73), ataxia (1/73), hypertrekplexia (1/73), spasticity (3/73), overactive or overresponsive reflexes (16/73) |
| Behavioral and psychiatric features| autistic features (8/73), stereotypies (4/73), autistic disturbances anger tantrums (1/73), obsessive-compulsive disorder (1/73) |

symptoms of multisystem impairments. The core symptoms include hypotonia, psychomotor delay, epilepsy (intractable epilepsy mostly) and early death, which nearly happen to almost all patients. MCAHS2 involves a wide range of organ and system impairments with changeable form. Bayat et al.[33] conducted a cohort study, in which abnormalities of multiple organs and system were evaluated. We summarize the results into Table 2 for quick browsing. If you want to understand the frequency and forms of multisystem impairment, the Bayat et al.’s result can be referred. Then, facial deformities are also common signs.

We hope to provide a deeper understanding of PIGA-related encephalopathy for clinical workers, particularly for the early identification of MCAHS2. A key component is that amelioration of the epileptiform activity may have the potential to improve the developmental consequences of the disorder[32]. But the problem is that most PIGA-related EOEE are refractory according to the literature review. Finally, too many terms are confusing to some extent, which may increase the difficulty for deep understanding. “PIGA encephalopathy” and MCHAS2 are two more concise and precise terms. A more complete, more concise classification system is needed to facilitate clinical workers to understand the relevant content of EOEE easier. It is obvious that there are many challenges for EOEE exploration, which needs researchers’ joint efforts. The novel variants reported by us provided a lot of help.

In conclusion, we reported a case of MCAHS2 due to novel PIGA variant (NM_002641.3:c.971G>A), which expanded variant spectrums of PIGA and would be helpful to genetic counseling for human beings. What’s more, the outcomes of the literature review would be of great help for clarifying the correlation between genotype and phenotype and early identification for MCAHS2. In the future, more research is needed to explore effective treatment options.

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Authors’ contributions Xiangyu Liu and Jing Meng participated in the conception of the study and writing of the manuscript. Jinhui Ma made great contributions to clinical information collection of the case and provided abundant knowledge of electroencephalogram. Jianbo Shu, Xiangyu Liu, Jing Meng Chuny Gu performed the experiments and provided the planning and analysis of the application of sequencing technology. Chunquan Cai, Dong Li and Xiaofang Chen made a review of previous literature. Chunyu Gu and Jing Meng revised the article critically for important intellectual content. Chunquan Cai revised the manuscript and submitted the manuscript. All of the authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that there were not any financial or non-financial interests related to the work that could be constructed as a potential conflict of interest.

Ethics Statement The study was approved by the Ethics Committee of Tianjin Children’s Hospital (Tianjin University Children’s Hospital). Written informed consent of the patient was obtained from his parents. All study procedures adhered to the tenets of the Declaration of Helsinki.

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