Received: 27 January 2024 | Revised: 26 April 2024 | Accepted: 30 April 2024

DOI: 10.1002/mgg3.2452

Molecular Genetics & Genomic Medicine Open Access WILEY

CLINICAL REPORT

A case of inherited glycosylphosphatidylinositol deficiency caused by *PGAP3* variant with uniparental isodisomy on chromosome 17

Takeo Mukai[1] | **Shota Kato**[1] | **Hiroyuki Tanaka**[1] | **Yukiko Kuroda**[1] | **Hiroki Kitaoka**[1] | **Atsushi Ito**[1] | **Yoshihiko Shitara**[1] | **Kohei Kashima**[1] | **Hirokazu Takami**[2] | **Naoto Takahashi**[1] | **Motohiro Kato**[1]

[1]Department of Pediatrics, The University of Tokyo Hospital, Tokyo, Japan

[2]Department of Neurosurgery, The University of Tokyo Hospital, Tokyo, Japan

Correspondence
Takeo Mukai, Department of Pediatrics, The University of Tokyo Hospital, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 Japan.
Email: takeo-m@ims.u-tokyo.ac.jp

Funding information
Japan Society for the Promotion of Science, Grant/Award Number: 23K17188; Japan Agency for Medical Research and Development, Grant/Award Number: JP23ek0109549

Abstract

Background: Inherited glycosylphosphatidylinositol (GPI) deficiency is an autosomal recessive disease and a set of syndromes caused by different genes involved in the biosynthesis of phosphatidylinositol characterized by severe cognitive disability, elevated serum alkaline phosphatase (ALP) levels, and distinct facial features. This report presents a patient with inherited GPI deficiency caused by a homozygous frameshift variant of *PGAP3* due to uniparental isodisomy (UPiD) on chromosome 17.

Method: Clinical characteristics of the patient were collected. Microarray analysis followed by adaptive sampling sequencing targeting chromosome 17 was used for the identification of variants. Sanger sequencing was used to confirm the variant in the target region.

Results: The patient was born at 38 weeks of gestation with a birthweight of 3893 g. He had a distinctive facial appearance with hypertelorism, wide nasal bridge, and cleft soft palate. Postnatal head magnetic resonance imaging revealed a Blake's pouch cyst. The serum ALP level was 940 IU/L at birth and increased to 1781 IU/L at 28 days of age. Microarray analysis revealed region of homozygosity in nearly the entire region of chromosome 17, leading to the diagnosis of UPiD. Adaptive sampling sequencing targeting chromosome 17 confirmed the homozygous variant NM_033419:c.778dupG (p.Val260Glyfs*14) in the *PGAP3* gene, resulting in a diagnosis of inherited GPI deficiency.

Conclusion: This is the first report of inherited GPI deficiency caused by UPiD. Inherited GPI deficiency must be considered in patients with unexplained hyperphosphatasemia.

KEYWORDS

chromosome 17, HPMRS, inherited glycosylphosphatidylinositol deficiency, *PGAP3*, uniparental isodisomy

1 | INTRODUCTION

Glycosylphosphatidylinositol (GPI) is a phosphoglycerate that plays a central role in the multistep synthesis of GPI-anchored proteins (GPI-AP) and enzymes encoded by various genes. As GPI-AP are involved in several processes within the human body, genetic defects that lead to failure of its production result in a wide range of pathologies known as inherited GPI deficiency (Balobaid et al., 2018; Paprocka et al., 2021). Several subdivisions of the pathways affected by genetic variants on GPI-AP and GPI-linked substrates have been studied, including early GPI anchor synthesis, late GPI anchor synthesis, GPI transamidase, and fatty acid remodeling of GPI anchors after binding to proteins (Knaus et al., 2018). Inherited GPI deficiency is a set of syndromes caused by different genes involved in the biosynthesis of phosphatidylinositol. Variants of the six GPI-AP genes, including the *PIGV*, *PIGO*, *PGAP2*, *PGAP3*, *PIGW*, and *PIGY* genes, result in a phenotypic series of inherited GPI deficiencies (Knaus et al., 2018). Inherited GPI deficiency caused by a variant in *PGAP3* is often associated with a family history of such variants (Abdel-Hamid et al., 2018; Balobaid et al., 2018; Howard et al., 2014). This report presents a patient with inherited GPI deficiency caused by homozygous frameshift variants in *PGAP3* caused by uniparental isodisomy (UPiD) on chromosome 17.

2 | CASE PRESENTATION

A 30-year-old primigravid patient without *PGAP3* variants (Figure 1a) no history of infection or gestational diabetes mellitus had conceived naturally. At 31 weeks of gestation, Dandy–Walker malformation with hypoplasia of cerebellum was diagnosed via fetal magnetic resonance imaging (MRI). An elective cesarean section was performed at 38 weeks of gestation. The baby had a birth weight of 3893 g and Apgar scores of 8 at 1 and 5 min. He had a unique facial appearance with hypertelorism, a wide nasal bridge, and a cleft soft palate (Figure 1b,d). The baby's muscle tone was good, and an ultrasound examination revealed no cardiac or renal malformations. He showed severe respiratory distress after birth, and tracheal intubation was initially required; however, his respiratory condition improved over time, and extubation was performed on day of life 9. A postnatal head MRI performed on day of life 7 revealed a Blake's pouch cyst in which the displaced cerebellum in the upper part of the posterior fossa was not atrophied (Figure 1c). No hydrocephalus was observed. The serum ALP level at birth was 940 IU/L and increased to 1781 IU/L at 28 days of age. The ALP-2 isozyme accounted for 9.2% while ALP-3 accounted for 90.8%. Most of the ALP was derived from the bone, which is a nonspecific finding. The auditory

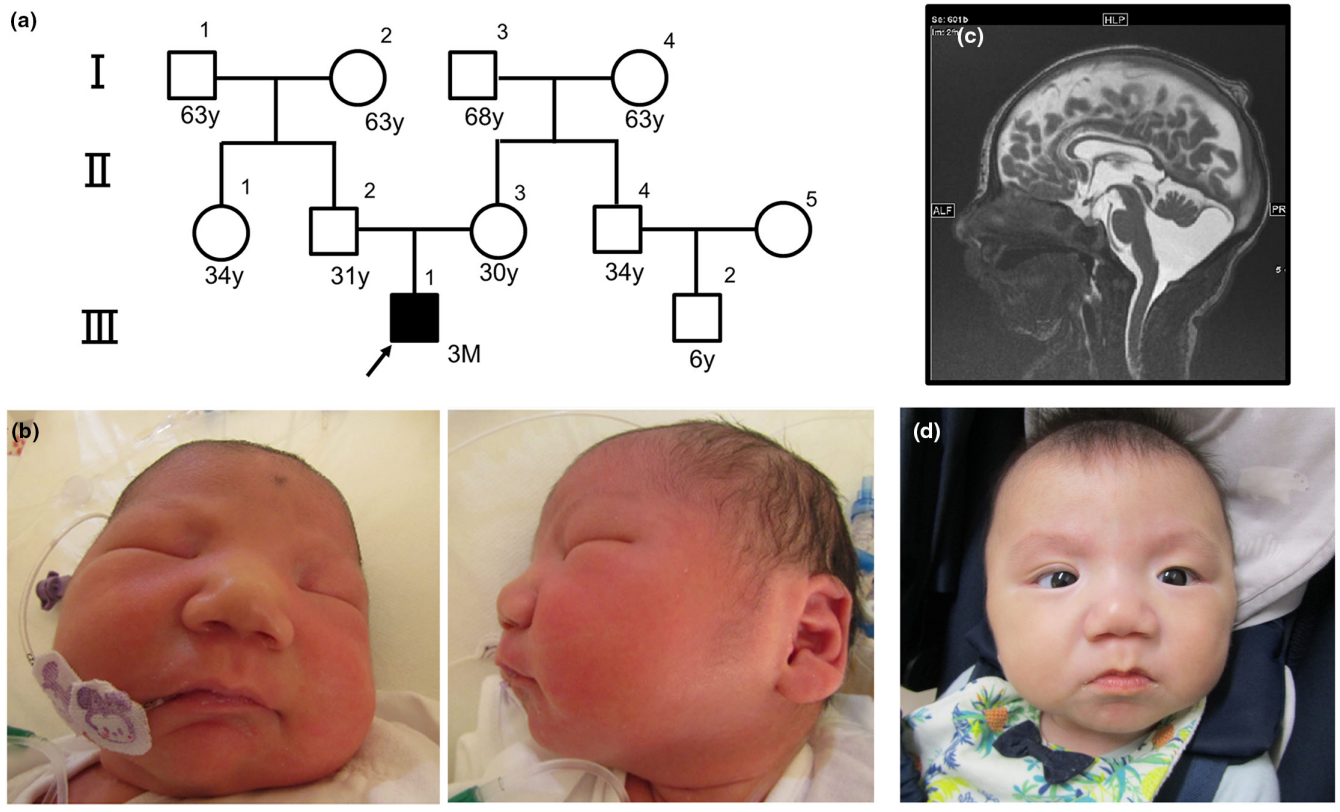

FIGURE 1 (a) Family tree of the patient. (b) Photograph of the patient at admission. (c) Postnatal head MRI showed Blake's pouch cyst. (d) Photograph of the patient at 7 months old.

brainstem response (ABR) revealed mild hearing loss. The baby was stable and discharged at the age of 58 days. Due to his multiple phenotypes, including the Blake's pouch cyst, cleft soft palate, peculiar facial appearance, and hearing loss, the parents consented to further evaluation.

3 | MATERIALS AND METHODS

3.1 | Editorial policies and ethical considerations

The parents of the infant provided written informed consent, and the study was approved by the Institutional Review Board of the Ethics Committee of the University of Tokyo Hospital (approval ID: 2022058G).

3.2 | Microarray analysis

Microarray-based comparative genomic hybridization (array-CGH) was conducted using the blood of the infant at LSI Medience Corporation. GenetiSure Dx Postnatal Assay (Agilent Technologies, Inc.) was used for the array and the data were analyzed by CytoDx Software.

3.3 | Adaptive sampling using Nanopore sequencer

DNA was extracted from the patient's peripheral blood samples and fragmented by needle shearing, as described previously (Longreadclub. org/mountain-protocol/). After needle shearing, 2 μg of DNA was used for library preparation using a Ligation Kit (SQK-LSK-114; Oxford Nanopore Technologies, OX, UK) according to the manufacturer's instructions. The library was sequenced on R10.4.1, flow cells (FLO-MIN114; ONT), and a GridION sequencer (ONT) using the high-accuracy mode and adaptive sampling option. Adaptive sampling is a software-controlled targeted sequencing approach that uses a nanopore sequencer to obviate the need for capture or amplicon generation (Loose et al., 2016). The entire chromosome 17 region was designated as the target region (chr17:1–83,257,441 according to GRCh38).

FAST5 files were re-base called using the super-accuracy mode in Guppy (version 6.4.2), and FASTQ files were mapped to the GRCh38 human reference genome using minimap2 (version 2.24). Then, PEPPER-Margin-DeepVariant (version 0.8) was used for detecting single nucleotide variants and insertions/deletions with the "--ont_r10_q20" option, followed by annotation with ANNOVAR.

3.4 | Sanger sequencing and reverse-transcript polymerase chain reaction

Polymerase chain reaction (PCR) and Sanger sequencing were performed to validate the detected *PGAP3* variant using the following primers: 5′-ttgaggtgggagagagaggag-3′ (forward) and 5′-agcccattgaggcacac-3′ (reverse).

Total RNA was extracted from peripheral blood samples with or without incubation with puromycin to inhibit nonsense-mediated decay (NMD). Complementary DNA was synthesized using SuperScript VILO Master Mix (Invitrogen, CA, USA) and was subjected to PCR with the following primers: 5′-gagccagggagaaaggatg-3′ (forward) and 5′-agactcgctccaaggtcttc-3′ (reverse).

4 | RESULTS

G-banding revealed a normal karyotype (46, XY), and microarray analysis revealed a region of homozygosity in nearly the entire region of chromosome 17 (cytoband p13.3-p11.2; 21 Mb, and q11.1-q25.3; 55 Mb), indicating UPiD (Figure 2a).

As UPiD of chromosome 17 alone is insufficient to account for the patient's phenotype, it was hypothesized that a heterozygous variant on chromosome 17 derived from one of the parents became homozygous through UPiD, resulting in the manifestation of an autosomal recessive genetic disorder. Adaptive sampling detected six homozygous variants with low prevalence in the general population on chromosome 17. The *PGAP3* variant (NC_000017.11:g.3967317_3967318insC, NM_033419:c.778dup (p.Val260Glyfs*14)) was concordant with the patient's phenotype (Figure 2b) and classified as pathogenic based on the American College of Medical Genetics guidelines (PVS1, PM2, PM3, and PP3) (Richards et al., 2015). This *PGAP3* frameshift variant was validated using Sanger sequencing (Figure 2c). In the reverse-transcript PCR assay, the patient's sample without puromycin incubation exhibited decreased expression compared with that of a healthy control sample. This reduction was mitigated by puromycin treatment, indicating the occurrence of NMD (Figure 2d). Therefore, the patient was diagnosed with inherited GPI deficiency caused by a homozygous frameshift variant in *PGAP3* due to UPiD of chromosome 17.

5 | DISCUSSION

Inherited GPI deficiency is an autosomal recessive disease and some of patients present phenotypic features such as hyperphosphatasia, neurodevelopmental disorders,

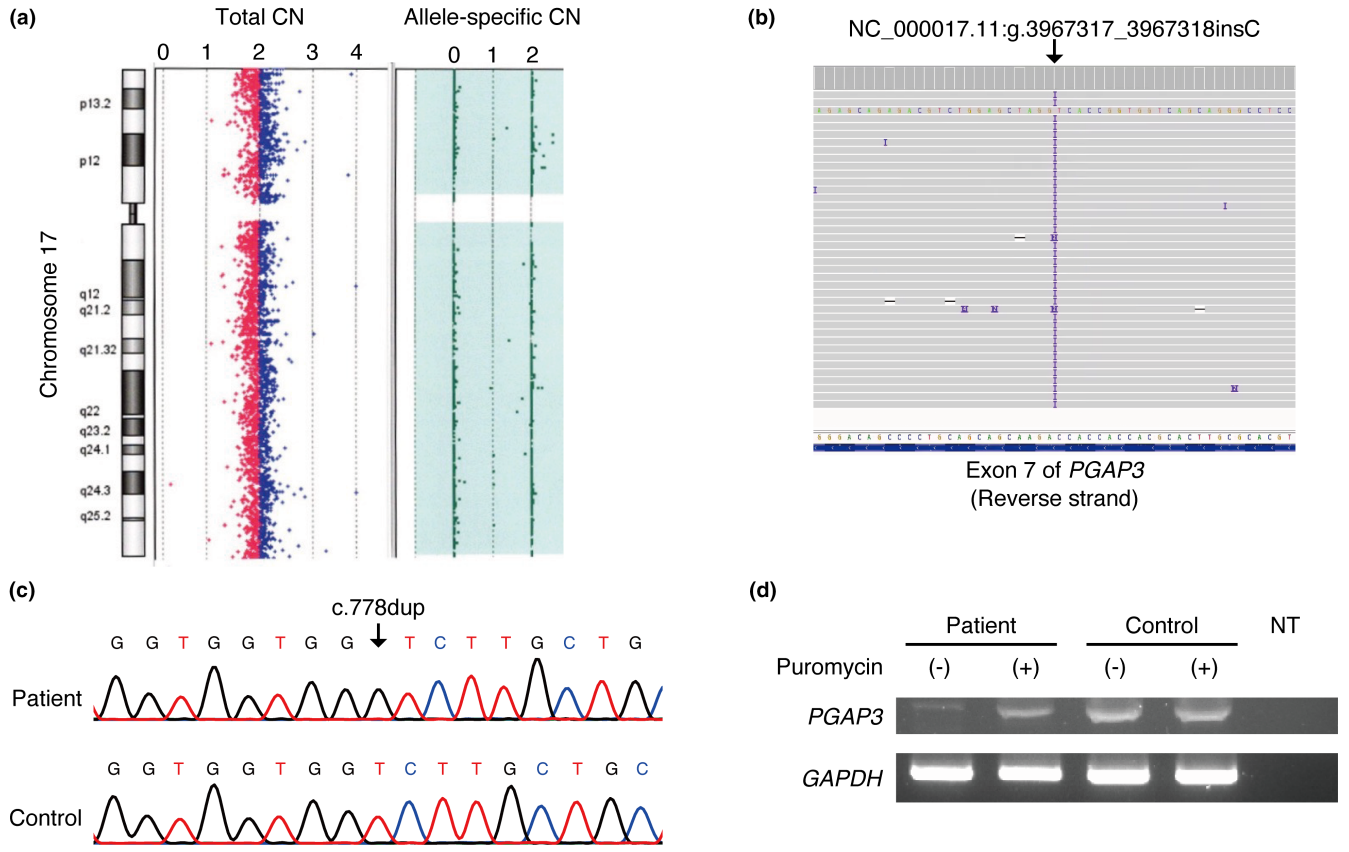

FIGURE 2 Genetic testing results. (a) Copy number (CN) analysis by array comparative genomic hybridization. Total CN of chromosome 17 was 2, while there was region of homozygosity in almost the entire region of chromosome 17. (b) The Integrated Genome Viewer capture of exon 7 of *PGAP3* showed NC_000017.11:g.3967317_3967318insC with a variant allele frequency of 0.95. (c) Sequence chromatograms of exon 7 of *PGAP3*. (d) RT-PCR for *PGAP3* using RNA with or without puromycin incubation. *GAPDH* was used as an internal control. NT, no template.

epilepsy, and other unique facial features. Patients often have increased serum ALP levels. Inherited GPI deficiency due to *PGAP3* deficiency has been reported in approximately 30 patients (Paprocka et al., 2021). Missense, splice, and frameshift variants of the *PGAP3* gene have been reported without any hotspots (Table S1). The current patient had an unreported single nucleotide insertion that became homozygous due to UPiD. A high rate of consanguinity has been reported for inherited GPI deficiency, and no cases of inherited GPI deficiency due to UPiD have been reported prior to this study. The patient in this report had increased serum ALP levels similar to those of patients in previous reports.

Some of children with inherited GPI deficiency caused by *PGAP3* mutations have been reported to have unique facial features, including hypertelorism, a wide nasal bridge, long eye clefts, a tent-shaped mouth, and a cleft lip palate, as observed in the current patient (Abdel-Hamid et al., 2018; Howard et al., 2014). Additionally, MRI abnormalities, such as corpus callosum hypoplasia, Dandy–Walker malformation, and mid-cerebellar hypoplasia, may be observed (Abdel-Hamid et al., 2018). Although there are no reports of a Blake's pouch cyst in patients with inherited GPI deficiency, it may be associated with the Dandy–Walker complex. Psychomotor developmental disabilities are common in patients with inherited GPI deficiency due to *PGAP3* variants (23/23), as is epilepsy (15/23) (Abdel-Hamid et al., 2018). Furthermore, the age at which seizures first appear varies widely from 18 months to 23 years (Knaus et al., 2016). Other reported symptoms include ataxia, sensorineural hearing loss, autism spectrum disorders, sleep disorders, mood disorders, and hypersomnia (Knaus et al., 2016).

The diagnosis of inherited GPI deficiency in this infant was made at 3 months of age, which is much earlier than that in patients in previous reports (Abdel-Hamid et al., 2018). In this patient, an early diagnosis was possible due to comprehensive, detailed, and rapid genome analysis via adaptive sampling using a nanopore sequencer. No convulsions or developmental delays were observed during the outpatient visit at 7 months of age. However, the patient must be monitored for the onset of epilepsy, neurodevelopmental delays, and worsening of hydrocephalus associated with a Blake's pouch cysts.

UPiD of chromosome 17 leads to junctional epidermolysis bullosa with pyloric atresia due to a integrin beta-4 variant (Natsuga et al., 2010), limb-girdle muscular dystrophy R3 due to a sarcoglycan alpha variant (Verebi et al., 2023), and Pompe disease due to a glucosidase alpha acid variant (Labrijn-Marks et al., 2019). To the best of our knowledge, this is the first report of UPiD of chromosome 17 leading to a homozygous pathogenic variant in *PGAP3* resulting in inherited GPI deficiency.

This patient was diagnosed with inherited GPI deficiency that was caused by a biallelic pathogenic variant of *PGAP3* caused by UPiD on chromosome 17. Inherited GPI deficiency is an extremely rare disease, and its symptoms and findings are often nonspecific, making an early diagnosis difficult. However, in this patient, a genetic diagnosis was obtained using microarrays and adaptive sampling and a management and follow-up plan was established at an early stage of life. Inherited GPI deficiency must be considered in patients with unexplained hyperphosphatasemia.

AUTHOR CONTRIBUTIONS

T.M. S.K. and H.T. drafted the initial manuscript. S.K and M.K carried out the experiments. Y.K, H.K., A.I., Y.S., K.K., H.T., N.T., and M.K. revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

FUNDING INFORMATION

This study was supported by the Japan Society for the Promotion of Science (JSPS KAKENHI Grant Number 23K17188) and AMED under Grant Number JP23ek0109549.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

This study was approved by the ethicscommittee of the University of Tokyo Hospital (approval ID: 2022058G)

ORCID

Takeo Mukai https://orcid.org/0000-0001-6648-1271
Hiroki Kitaoka https://orcid.org/0000-0003-0958-5028

REFERENCES

Abdel-Hamid, M. S., Issa, M. Y., Otaify, G. A., Abdel-Ghafar, S. F., Elbendary, H. M., & Zaki, M. S. (2018). PGAP3-related hyperphosphatasia with mental retardation syndrome: Report of 10 new patients and a homozygous founder mutation. *Clinical Genetics*, *93*(1), 84–91. https://doi.org/10.1111/cge.13033

Balobaid, A., Ben-Omran, T., Ramzan, K., Altassan, R., Almureikhi, M., Musa, S., Al-Hashmi, N., Al-Owain, M., Al-Zaidan, H., Al-Hassnan, Z., Imtiaz, F., & Al-Sayed, M. (2018). Delineating the phenotypic spectrum of hyperphosphatasia with mental retardation syndrome 4 in 14 patients of middle-eastern origin. *American Journal of Medical Genetics. Part A*, *176*(12), 2850–2857. https://doi.org/10.1002/ajmg.a.40627

Howard, M. F., Murakami, Y., Pagnamenta, A. T., Daumer-Haas, C., Fischer, B., Hecht, J., Keays, D. A., Knight, S. J., Kölsch, U., Krüger, U., Leiz, S., Maeda, Y., Mitchell, D., Mundlos, S., Phillips, J. A., 3rd, Robinson, P. N., Kini, U., Taylor, J. C., Horn, D., … Krawitz, P. M. (2014). Mutations in PGAP3 impair GPI-anchor maturation, causing a subtype of hyperphosphatasia with mental retardation. *American Journal of Human Genetics*, *94*(2), 278–287. https://doi.org/10.1016/j.ajhg.2013.12.012

Knaus, A., Awaya, T., Helbig, I., Afawi, Z., Pendziwiat, M., Abu-Rachma, J., Thompson, M. D., Cole, D. E., Skinner, S., Annese, F., Canham, N., Schweiger, M. R., Robinson, P. N., Mundlos, S., Kinoshita, T., Munnich, A., Murakami, Y., Horn, D., & Krawitz, P. M. (2016). Rare noncoding mutations extend the mutational Spectrum in the PGAP3 subtype of Hyperphosphatasia with mental retardation syndrome. *Human Mutation*, *37*(8), 737–744. https://doi.org/10.1002/humu.23006

Knaus, A., Pantel, J. T., Pendziwiat, M., Hajjir, N., Zhao, M., Hsieh, T. C., Schubach, M., Gurovich, Y., Fleischer, N., Jäger, M., Köhler, S., Muhle, H., Korff, C., Møller, R. S., Bayat, A., Calvas, P., Chassaing, N., Warren, H., Skinner, S., … Krawitz, P. M. (2018). Characterization of glycosylphosphatidylinositol biosynthesis defects by clinical features, flow cytometry, and automated image analysis. *Genome Medicine*, *10*(1), 3. https://doi.org/10.1186/s13073-017-0510-5

Labrijn-Marks, I., Somers-Bolman, G. M., Hoogeveen-Westerveld, M., Kroos, M. A., Ala-Mello, S., Amaral, O., Miranda, C. S., Mavridou, I., Michelakakis, H., Naess, K., Verheijen, F. W., Hoefsloot, L. H., Dijkhuizen, T., Benjamins, M., van den Hout, H. J. M., van der Ploeg, A. T., Pijnappel, W. W. M. P., Saris, J. J., & Halley, D. J. (2019). Segmental and total uniparental isodisomy (UPiD) as a disease mechanism in autosomal recessive lysosomal disorders: Evidence from SNP arrays. *European Journal of Human Genetics*, *27*(6), 919–927. https://doi.org/10.1038/s41431-019-0348-y

Loose, M., Malla, S., & Stout, M. (2016). Real-time selective sequencing using nanopore technology. *Nature Methods*, *13*(9), 751–754. https://doi.org/10.1038/nmeth.3930

Natsuga, K., Nishie, W., Arita, K., Shinkuma, S., Nakamura, H., Kubota, S., Imakado, S., Akiyama, M., & Shimizu, H. (2010). Complete paternal isodisomy of chromosome 17 in junctional epidermolysis bullosa with pyloric atresia. *The Journal of Investigative Dermatology*, *130*(11), 2671–2674. https://doi.org/10.1038/jid.2010.182

Paprocka, J., Hutny, M., Hofman, J., Tokarska, A., Kłaniewska, M., Szczałuba, K., Stembalska, A., Jezela-Stanek, A., & Śmigiel, R. (2021). Spectrum of neurological symptoms in glycosylphosphatidylinositol biosynthesis defects: Systematic review. *Frontiers in Neurology*, *12*, 758899. https://doi.org/10.3389/fneur.2021.758899

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding,

K., Rehm, H. L., & ACMG Laboratory Quality Assurance Committee. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Verebi, C., Caumes, R., Chantot-Bastaraud, S., Deburgrave, N., Orhant, L., Vaucouleur, N., Cuisset, L., Bienvenu, T., Leturcq, F., & Nectoux, J. (2023). How a paternal uniparental isodisomy of chromosome 17 leads to autosomal recessive limb-girdle muscular dystrophy R3. *Neuromuscular Disorders*, *33*(5), 367–370. https://doi.org/10.1016/j.nmd.2023.03.004

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Mukai, T., Kato, S., Tanaka, H., Kuroda, Y., Kitaoka, H., Ito, A., Shitara, Y., Kashima, K., Takami, H., Takahashi, N., & Kato, M. (2024). A case of inherited glycosylphosphatidylinositol deficiency caused by *PGAP3* variant with uniparental isodisomy on chromosome 17. *Molecular Genetics & Genomic Medicine*, *12*, e2452. https://doi.org/10.1002/mgg3.2452