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Case Report

Congenital disorder of glycosylation type Ia in a Chinese family: Function analysis of a novel *PMM2* complex heterozygosis mutation

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ABSTRACT

Congenital disorder of glycosylation type Ia (CDG-Ia) is an autosomal recessive genetic disease caused by a mutation in the phosphomannomutase 2 (PMM2) gene. We have identified a 13-month-old boy who has been diagnosed with CDG-Ia. He displays several characteristic symptoms, including cerebellar hypoplasia, severe developmental retardation, hypothyroidism, impaired liver function, and abnormal serum ferritin levels. Through whole-exome sequencing, we discovered novel complex heterozygous mutations in the PMM2 gene, specifically the c.663C > G (p.F221L) mutation and loss of exon 2. Further analysis revealed that the enzymatic activity of the mutant PMM2 protein was significantly reduced by 44.97% ($p < 0.05$) compared to the wild-type protein.

1. Introduction

Congenital disorder of glycosylation (CDG) is a metabolic disease characterized by a deficiency in protein N-glycosylation modification, affecting multiple systems. Clinical symptoms primarily include damage to multiple organs, neurological movement disorders, and developmental delays [1,2]. CDG is classified into two subtypes: CDG-I and CDG-II. Among these, CDG-Ia (CDG-Ia, OMIM 212065) is more prevalent, with a 25% mortality rate in affected children [3]. Infants with CDG typically exhibit two main categories of symptoms. Firstly, dysplasia-related symptoms include bradykinesia, strabismus, hypotonic tone, cerebellar hypoplasia, inverted nipples, uneven distribution of subcutaneous fat, and feeding difficulties. Secondly, multiple organ damage can lead to impaired liver function, respiratory tract infections, diarrhea, nephrotic syndrome, cardiopathy, coagulopathy, and other complications [4].

CDG-Ia, also known as PMM2 deficiency, is a rare autosomal recessive genetic disease primarily caused by mutations in the phosphomannomutase 2 (PMM2) gene [5]. The PMM2 gene encodes an enzyme called phosphomannomutase 2, PMM2, which plays a critical role in catalyzing the conversion of mannose-6-phosphate into mannose-1-phosphate. Mannose-1-phosphate serves as a precursor to GDP-mannose, a vital component in the biosynthesis of various lipids and glycoproteins [6]. Serum transferrin glycosylation analysis is an important marker for CDG-Ia. The abnormal function of the PMM2 protein leads to either the loss or alteration of the N-oligosaccharide chain on transferrin, resulting in changes to its isoelectric point. As a result, CDG-Ia patients typically exhibit transferrin with one or two missing sugar chains in their serum [7,8]. Consequently, PMM2 plays a crucial role in the process of protein glycosylation. Impairment of protein glycosylation caused by PMM2 deficiency is strongly associated with the observed abnormalities in human multi-system function [9]. The loss of protein glycosylation can contribute to the diverse clinical manifestations commonly seen in CDG-Ia patients.

PMM2 belongs to the HAD-IIB phosphomutase subfamily. Its spatial structure consists of a three-layer α/β sandwich folded core region and a four-helix bundle C1 type cap region (α1-α4), and it contains four conservative loop motifs. The core region comprises six parallel beta-sheets

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surrounded by four spirals, which are adjacent to the cap area [10,11]. Using the SWISS-MODEL database, it has been determined that the catalytically active site of the PMM2 substrate is composed of the 12th and 14th aspartic acid (Asp) residues in the loop1 region. This active site is located in the hydrophobic region between the cap region and the core region. According to the Clinvar database (https://www.ncbi.nlm.nih.gov/clinvar), there are 133 pathogenic variants related to PMM2, with 110 pathogenic mutation sites associated with PMM2-CDG. At least 12 Spanish patients with PMM2-CDG have reported that PMM2 mutations are associated with decreased PMM2 enzyme activity. [12,13]. Therefore, the study of novel mutations is crucial for further understanding the activity of PMM2.

The patient admitted to our hospital presented with severe liver damage and cerebellar hypoplasia, both of which are symptoms of CDG-Ia. Through whole exon sequencing, we discovered a deletion in the second exon of the PMM2 gene in this patient. Additionally, a pathogenic missense mutation (c.663C > G: p.F221L) was found in the eighth exon, resulting in a hybrid variant. To determine the pathogenicity of this mutation, we conducted bioinformatic predictions on the mutant PMM2-F221L. The results indicated that this variation is a deleterious mutation, leading to a decrease in protein structural stability. Furthermore, when comparing the prokaryotic expression of both the wild-type (PMM2-WT) and mutant (PMM2-F221L) proteins, we observed a reduction in protease activity in the mutant protein. Based on these findings, we conclude that this novel mutation is associated with the disease and serves as the causal factor for the patient's congenital glycosylation type Ia condition.

2. Materials and method

2.1. Ethics

The study received approval from the Ethics Committee of Shunde Women and Children's Hospital of Guangdong Medical University. Informed consent forms were signed by the parents of the patient.

2.2. Whole exon sequencing (WES) and variant analysis

Peripheral blood samples were collected from the patient and his parents. Genomic DNA extraction was performed using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA samples were sent to Berry Genomics Co., Ltd. (Beijing, China) for whole-exome sequencing (WES) analysis. The sequencing results were analyzed using various databases, including the Shenzhou Genome Database (https://www.berrygenomics.com), 1000 Genomes (http://browser.1000genomes.org/index.html), Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org/), and Online Mendelian Inheritance in Man (OMIM, https://www.omim.org/), to identify missense mutations, mutation frequencies, and potential diseases associated with the mutations. Additionally, whole-exome sequencing copy number variation (WES-CNV) technology was used to detect exon deletions in the samples.

2.3. RT-PCR and DNA sequencing

To further validate the WES results in the PMM2 gene, Sanger sequencing was employed. The target region was amplified using specific PCR primers (PMM2-F1: 5'-GCTGAAGAAACTCTCCCTGCCCAGT-3′; PMM2-R1: 5'-GCCACCGAATGCCCTATGCT-3′) with genomic DNA (gDNA) as a template. The total blood RNA extraction kit from Tiangen (Beijing, China) was utilized to isolate and purify RNA from the peripheral blood of both the patients and their parents. The extracted RNA was then reverse transcribed into complementary DNA (cDNA) through PCR amplification using PCR-specific primers (PMM2 F2: 5'-CTCTGCCTCTTCGACGTG-3′, PMM2 R2: 5'-GAAAGACATCAAAGCTGAT-3′) targeting the desired region. Finally, the PCR product was sent to Sangon Biotech Co., Ltd. (Shanghai, China) for Sanger sequencing.

2.4. In silico analyses

To evaluate the correlation between mutations in the PMM2 gene and potential diseases, various bioinformatics tools were employed to analyze their evolutionary conservation, pathogenicity, and alterations in the spatial structure of the PMM2 protein. The evolutionary conservation of PMM2 was assessed by comparing the amino acid sequences among different species. We utilized five online tools, namely MutationTaster (http://www.mutationtaster.org/), Deogen2 (https://deogen2.mutaframe.com/), MutationAssessor (http://mutationassessor.org/r3/), FATHMM (http://fathmm.biocompute.org.uk/index.html), and MutPred2 (http://mutpred.mutdb.org/), to evaluate conservation and predict pathogenicity. Furthermore, we performed homology modeling of the PMM2 protein structure using SWISS-MODEL (https://swissmodel.expasy.org/), and visualized the protein structure using VMD software (https://www.ks.uiuc.edu/Research/vmd/).

2.5. Bacterial expression of wild-type and the mutant PMM2 protein

To analyze the activity of the PMM2 protein, we conducted separate synthesis and sequencing of both the PMM2 wild-type and mutant genes. Following this, we constructed prokaryotic expression vectors named pET30a-His-PMM2-WT and pET30a-His-PMM2-F221L, which were then verified by DNA sequencing. Subsequently, the recombinant vectors were introduced into *E. coli* BL21(DE3) cells, and expression was induced using Isopropylthiogalactoside (IPTG). The proteins were purified using Ni affinity chromatography and confirmed through SDS-PAGE analysis.

3. Results

3.1. Clinical manifestations

The patient is a 13-month-old Chinese boy, who is the first child of healthy, unrelated young parents. The baby was born at full term without any complications during pregnancy. At 6 months of age, he was admitted to the hospital due to psychomotor retardation, manifested by his inability to lift his head independently. He does not show any abnormal distribution of subcutaneous fat or inverted nipples, but he does have a larger forehead and ears. The baby prefers to lie down on his back in a supine position.

During the physical examination, it was observed that the baby exhibited low muscle tone (hypotonia), weak visual reaction with a bilateral alternating squint, and infrequent natural grasp. A comprehensive neuropsychological assessment revealed delayed overall development, with a composite score of 3.9 (below the normal range) and a developmental quotient score of 45.35 (out of a total of 100). Electroencephalogram (EEG) results did not show any abnormalities. However, cranial magnetic resonance imaging (MRI) demonstrated severe atrophy of the bilateral cerebellum with vermis hypoplasia (Fig. 1). The patient received acupuncture treatment, intravenous administration of nerve growth factor, and oral administration of cerebroprotein hydrolysate solution for approximately eight weeks. However, these treatments only had limited effectiveness in addressing the physical and cognitive developmental disorders. During the newborn period, the infant was fed with lactose-free cow's milk powder, but experienced prolonged difficulties characterized by spitting up and recurring episodes of diarrhea. However, the symptoms of diarrhea improved following treatment with an amino acid formula. Furthermore, rehabilitation therapy was conducted for a period of five months. Unfortunately, this therapy yielded unsatisfactory results, as evidenced by slow weight and height gain despite adequate food intake (Fig. 2). Laboratory test results indicate that the patient has abnormal liver and thyroid function

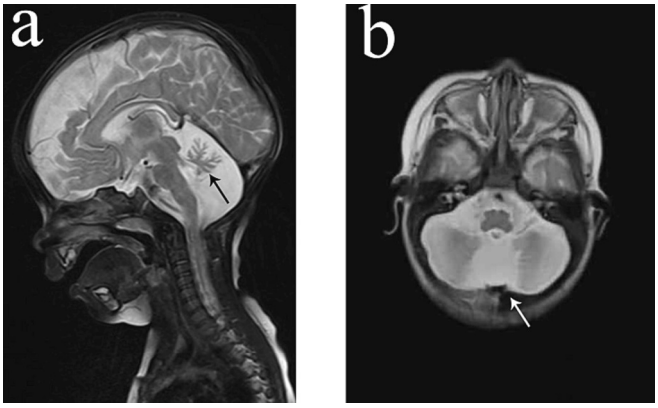

Fig. 1. Magnetic resonance imaging of the patient's brain.
At 6 months of age, (a) T1-weighted mid-sagittal MRI scan of the patient showed severe cerebellar hypoplasia (arrow). Furthermore, (b) there were also noticeable widening and deepening of the sulci (arrows).

(Table 1).

3.2. Identify PMM2 gene mutations by WES

The genomic DNA of the patient and his parents was extracted from venous blood, and whole exon sequencing technology was employed. In exon 8, a missense mutation site at c.663C > G (p.F221L) and loss of exon 2 were identified. It is noteworthy that the child carries both mutations concurrently, as depicted in Fig. 3. The specific mutation c.663C > G has not been found in the Berry Gene Chinese Population Specific Database or the Human Exon Database (ExAC). It is worth mentioning that no cases of heterozygous mutations within this gene have been reported.

3.3. RT-PCR to verify the deletion of the second exon of the PMM2 gene

Based on the test results of WES-CNV detection technology, it was observed that the patient and his father both exhibited a missing copy number of exon 2 in the PMM2 gene. However, the mother's DNA showed a normal copy number of exon 2, as depicted in Fig. 4a. In order to further investigate this genetic variation, specific primers were designed at both ends of the PMM2 gene, as illustrated in Fig. 4b. These primers were then utilized for Reverse Transcription PCR amplification. Analysis of the resulting products on a 1.5% agarose gel revealed the presence of two bands in both the patient and his father's samples. The sizes of these bands were measured to be 529 bp and 417 bp, as shown in Fig. 4c. To determine the composition of these RT-PCR products, the two bands were isolated and subjected to sequence analysis. The analysis indicated that the 417 bp band from both the patient and his father

Table 1
The laboratory test results.

	Results	Reference range
Liver injury		
alanine aminotransferase (U/L)	91.00	9–50
aspartate aminotransferase (U/L)	114.00	15–40
γ-glutamyl transfer peptide enzyme (U/L)	9.00	10–60
total protein (g/L)	51.70	65–85
albumin (g/L)	38.00	40–55
globulin (g/L)	13.70	20–40
Abnormal thyroid function		
thyroid-stimulating hormone (mlU/L)	6.45	0.70–5.79
free Triiodothyronine (pmol / L)	5.45	3.69–8.46
free Thyroxine (pmol / L)	12.91	12.3–22.8

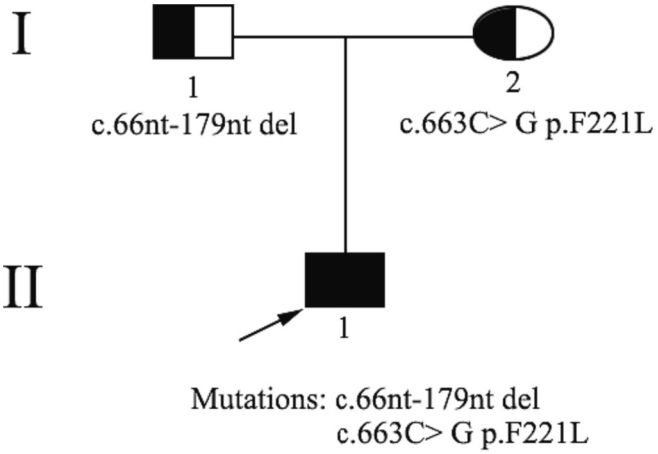

Fig. 3. Family diagram of the patient.
The patient's father carries a heterozygous deletion of exon 2 (c.66 nt–179 nt) in the PMM2 gene. The patient's mother is also a heterozygote, with one copy of a missense mutation (c.663C > G) in the PMM2 gene. The patient (indicated by the arrow) is a compound heterozygote, inheriting pathogenic mutations from both parents.

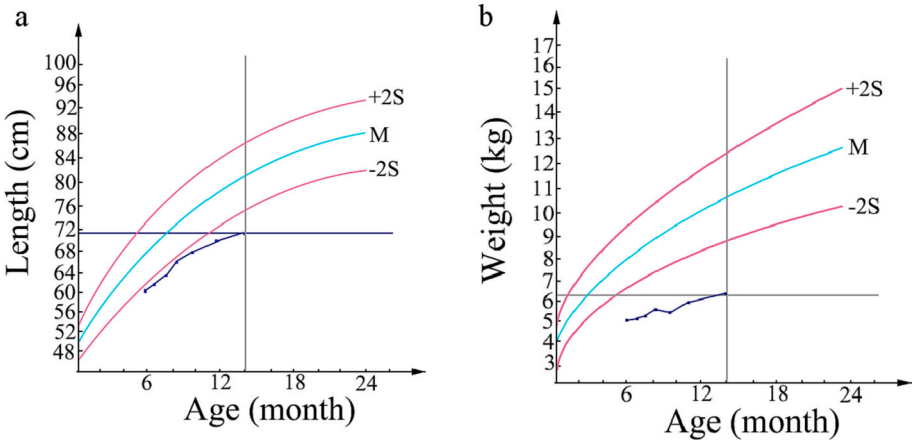

Fig. 2. The patient's weight and growth increase curve.
(a) The patient's height is represented by the blue line, indicating that it is below the average range.
(b) Similarly, the patient's weight is significantly below average, as shown by the blue line. M represents the average value, S represents the standard deviation, +2S represents the value of M + 2S, and —2S represents the value of M-2S.

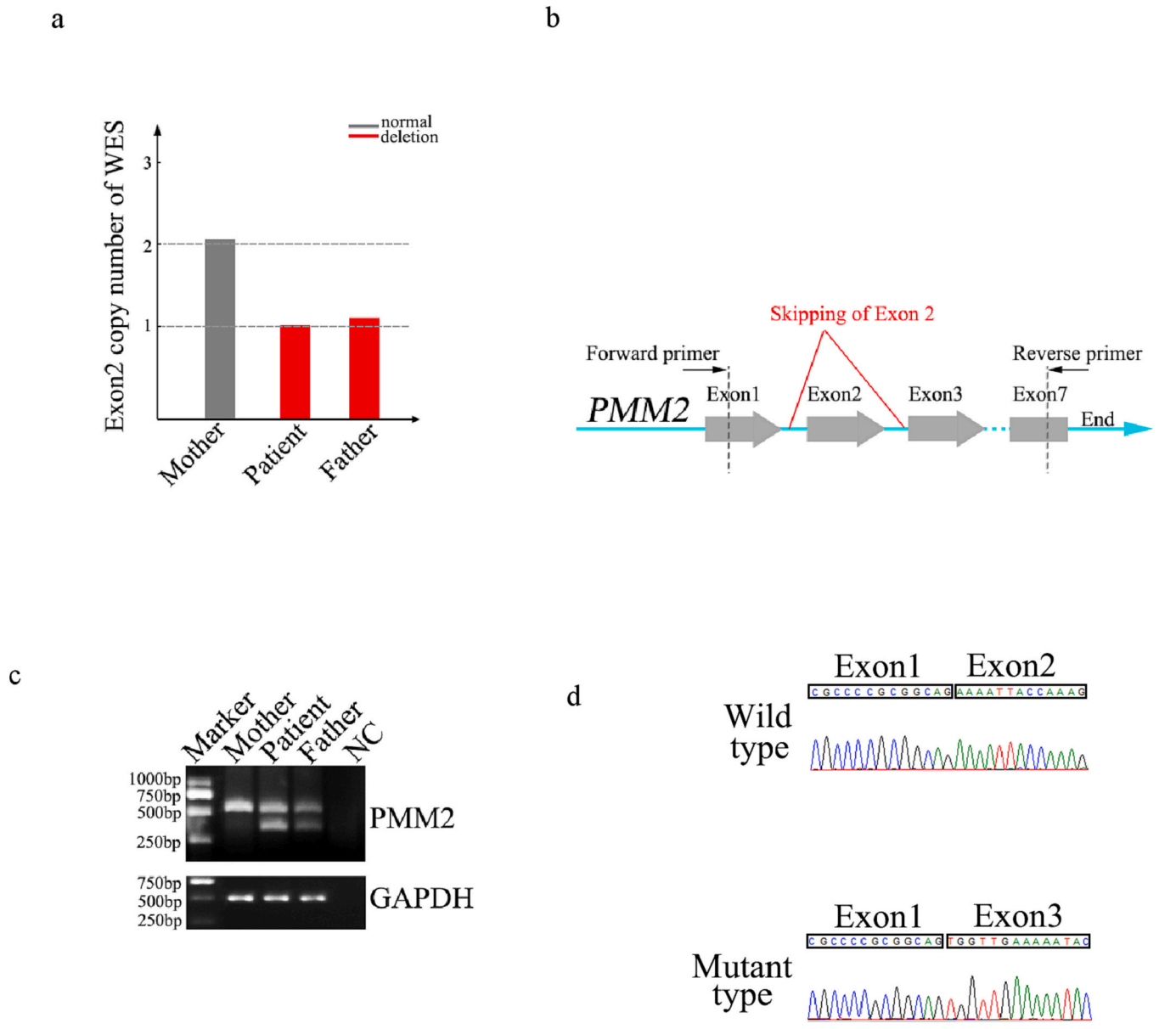

Fig. 4. Verification of deletion of exon 2 of PMM2 gene.
(a) The results obtained from whole-genome exon sequencing of the patient, his father, and his mother revealed a heterozygous deletion of exon 2 in the PMM2 gene in both the patient and his father. (b) To further confirm the deletion, specific primers for the PMM2 gene (PMM2 forward: 5'-CTCTGCCTCTTCGACGTG-3′, PMM2 reverse: 5'-GAAAGACATCAAAGCTGAT-3′) were designed and marked in the schematic diagram. (c) RT-PCR was performed using cDNA from blood as templates, and the PCR products were separated through agarose gel electrophoresis. Both the patient and his father exhibited two PCR products (529 bp and 417 bp). (d) Sequence analysis revealed that the 417 bp band from both the patient and his father lacked exon 2.

lacked exon 2 of the PMM2 gene, as demonstrated in Fig. 4d.

3.4. Bioinformatics analysis

By utilizing five bioinformatics software tools (MutationTaster, Deogen2, MutationAssessor, Fathmm, MutPred2), the evaluation of the pathogenicity of the PMM2 F221L mutation demonstrates its detrimental nature (Table S1). Furthermore, a comparative analysis conducted using the Blastp tool revealed a high degree of conservation of the phenylalanine residue at position 221 among various vertebrate species, including Human, Chimpanzee, Mouse, Whit-tufted-ear marmoset, Sperm whale, and Zebrafish.

According to the MutationTaster software, the F221L mutation in the PMM2 protein sequence is predicted to result in the loss of the α-helix formed by amino acids 219 to 222. To further analyze this, the SWISS-MODEL software was employed to model the homology structure, and the VMD software was used to visualize the three-dimensional structure of the PMM2 protein. It was observed that the wild-type PMM2 lacked the α-helix. Subsequently, a comparison analysis between the mutant PMM2 with the helix α9 mutation and the wild-type PMM2 was conducted using VMD software. The analysis revealed that the three-dimensional structures of the mutant and wild-type proteins could not overlap, indicating significant conformational changes resulting from the F221L mutation (Fig. 5).

3.5. PMM2 recombinant protein expression and activity analysis

The amino acid phenylalanine (Phe) at position 221 in the PMM2 protein is highly conserved among vertebrates. In this study, we introduced a mutation by replacing the phenylalanine with leucine (Leu) at

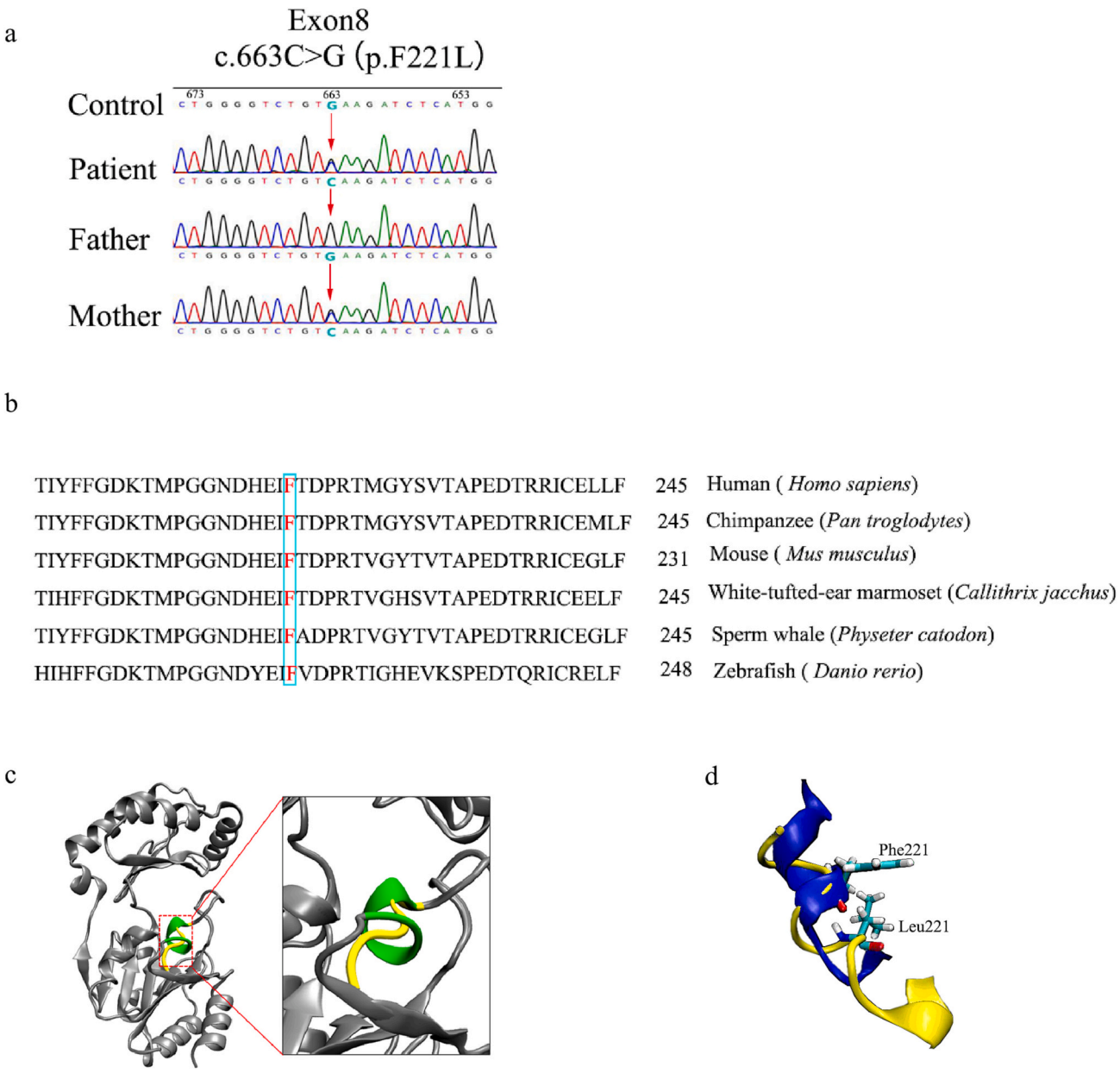

Fig. 5. Analysis of missense mutations of PMM2 (F221L).
(a) Sanger sequencing was performed on the PMM2 gene of the patient, as well as his father and mother. The results revealed that the 663rd cytosine of the 8th exon in both the patient and mother was mutated to guanine (the sequence map is a reverse complementary sequence). This mutation resulted in the substitution of phenylalanine at position 221 with leucine. (b) A comparative analysis of the amino acid sequence of the PMM2 protein across different species showed that the phenylalanine residue at position 221 is highly conserved among vertebrates, as indicated by the boxed region. (c) To predict the consequences of the mutation, MutationTaster software was used. It indicated that the mutation led to the loss of an α-helix structure formed by amino acids 219 to 222 within the PMM2 protein's amino acid sequence. Additionally, structural modeling of the wild-type and mutant PMM2 proteins was performed using SWISS-MODEL software. The wild-type structure is depicted in green within the dotted box, while the mutant structure is shown in yellow. (d) Comparing the PMM2 wild-type and PMM2-F221L (mutant) proteins, conformational changes were observed in helix α9. The wild-type complex is depicted in blue, while the mutant is represented in yellow.

position 221. Phenylalanine is known to be an inert amino acid, while leucine belongs to the hydrophobic amino acid group. Our objective was to investigate the impact of this mutation on the activity of the PMM2 protease. To accomplish this, we successfully expressed and purified both the wild-type and mutant (F221L) PMM2 proteins in *E. coli*. The activity of the PMM2 protease was subsequently assessed using a previously described method. The results demonstrated that the mutant enzyme exhibited significantly lower activity (5.96 ± 0.25 nmol/min ml) compared to the wild-type enzyme (8.64 ± 0.21 nmol/min ml, $p < 0.05$) (refer to Fig. 6).

4. Discussion

In this particular case, the patient is presenting with severe dysplasia, multiple organ damage, and other associated symptoms. Through further analysis using Whole Exome Sequencing (WES), it was determined that the patient's condition was attributed to a mutation in the PMM2 gene. Consequently, the patient was diagnosed with CDG-Ia. The clinical manifestations of CDG-Ia are known to be diverse and typically encompass growth retardation, cerebellar insufficiency, peripheral neuropathy, epilepsy, digestive system disorders, skeletal deformities, and liver damage [14]. The initial diagnosis of this patient revealed

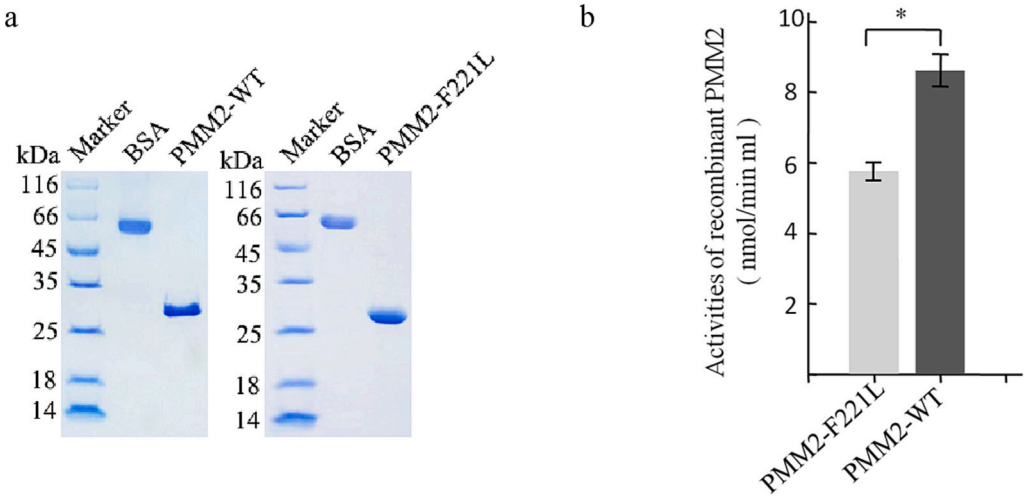

Fig. 6. Analysis of PMM2 recombinant protein expression and activity.
(a) SDS-PAGE was conducted to analyze the expression and activity of the two recombinant proteins of PMM2. Bovine serum albumin was quantified and utilized as an internal control for protein loading. (b) The protease activity of the PMM2-F221L mutant was found to be significantly reduced compared to the wild-type PMM2 protein (*$p < 0.05$).

cerebellar hypoplasia, intestinal malabsorption syndrome, congenital myopathy, liver injury, thyroid dysfunction, and other symptoms that align with the clinical presentation of CDG-Ia [2,15].

The patient presented with two heterozygous mutations. The first mutation involved a deletion of exon 2, leading to frameshifts and premature termination of translation. Consequently, the protein lost 172 amino acids at its C-terminus, resulting in the loss of PMM2 function. The second mutation was a cytosine to guanine substitution at position 663 of exon 8. This particular mutation caused a substitution of phenylalanine with leucine at position 221 in the PMM2 protein. A comparative analysis of the PMM2 protein's amino acid sequence across different species revealed that phenylalanine residues are highly conserved among vertebrates.

According to the results obtained from MutationTaster software, it is suggested that the mutation disrupts the α-helix structure formed by amino acids 219 to 222 in the PMM2 protein, potentially impacting its function. The DEOGEN2 software prediction indicated that the mutation is deleterious, with a DEOGEN score of 0.963. Furthermore, the use of MutationAssessor, Fathmm, and MutPred2 software also strengthens the association between the mutation and the disease. Based on these findings, it is hypothesized that this specific site plays a crucial role in the functionality of PMM2, and it is believed that the mutation originated from the patient's mother (Fig. 5).

To investigate the impact of the F221L mutation on PMM2 protease activity, we utilized the *E. coli* expression system to successfully express and purify both the PMM2-WT protein and the PMM2-F221L protein. Our findings revealed that the protease activity of PMM2-F221L was significantly lower than that of PMM2-WT protein ($p < 0.05$). PMM2 protein is abundantly expressed in various human systems, including the immune system, muscle system, nervous system, secretory system, and digestive system. N-glycosylation modification of proteins occurs in almost all cells of the human body. However, due to the mutation, the protease activity of PMM2 is reduced, leading to incomplete N-glycosylation modification of glycosylated proteins in multiple organs. This, in turn, affects the structure and function of these glycoproteins. Consequently, patients with the F221L mutation may experience multiple organ disorders, such as developmental delay and digestive system disorders [16,17]. Currently, two types of mutations in the PMM2 gene have been identified in CDG-Ia cases. One type of mutation results in the complete loss of activity of the PMM2 protease, while the other type of mutation results in a decrease in affinity with the substrate and stability of the PMM2 protein, leading to reduced protease activity [18]. The F221L mutation belongs to the latter category. In this specific case, the patient was unable to synthesize a functional PMM2 protein due to the deletion of the second exon of the PMM2 allele inherited from the father. This mutation contributed to the manifestation of clinical symptoms. Importantly, this supports the notion that the mutation is not lethal during the fertilized egg period, as it still allows for some residual PMM2 protein phosphatase activity.

In this study, we combined the clinical symptoms of the patient with gene sequencing technology and discovered novel mutation sites of the PMM2 gene. The findings from this research will have significant implications for genetic testing during pregnancy within this family and will also provide valuable insights for the development of personalized medicine for patients with CDG-Ia.

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CRediT authorship contribution statement

Dan Zhong: Writing – original draft. **Xiujuan Huang:** Writing – original draft. **Taoshan Feng:** Software. **Jieqing Zeng:** Software. **Shanshan Gu:** Software. **Fan Ning:** Software. **Yue Yang:** Data curation. **Jinyuan Zhu:** Data curation. **Yajun Wang:** Data curation. **Riling Chen:** Writing – review & editing. **Guoda Ma:** Writing – review & editing.

Declaration of competing interest

The authors perceive no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2024.101067.

References

[1] T. MARQUARDT, J. DENECKE, Congenital disorders of glycosylation: review of their molecular bases, clinical presentations and specific therapies, Eur J Pediatr 162 (2003) 359–379.
[2] C.R. Ferreira, R. Altassan, D. Marques-Da-Silva, R. Francisco, J. Jaeken, E. Morava, Recognizable phenotypes in CDG, J. Inherit. Metab. Dis. 41 (2018) 541–553.
[3] J. Jaeken, H. Stibler, B. Hagberg, The carbohydrate-deficient glycoprotein syndrome. A new inherited multisystemic disease with severe nervous system involvement, Acta Paediatr. Scand. Suppl. 375 (1991) 1–71.
[4] H. Stefanits, V. Konstantopoulou, M. Kuess, I. Milenkovic, C. Matula, Initial diagnosis of the congenital disorder of glycosylation PMM2-CDG (CDG1a) in a 4-year-old girl after neurosurgical intervention for cerebral hemorrhage, J. Neurosurg. Pediatr. 14 (2014) 546–549.
[5] A. Erlandson, C. Bjursell, H. Stibler, B. Kristiansson, J. Wahlstrom, T. Martinsson, Scandinavian CDG-Ia patients: genotype/phenotype correlation and geographic origin of founder mutations, Hum. Genet. 108 (2001) 359–367.
[6] T. Pascreau, F. Saller, E.P. Bianchini, D. Lasne, A. Bruneel, C. Reperant, F. Foulquier, C.V. Denis, P. De Lonlay, D. Borgel, N-glycosylation deficiency reduces the activation of protein C and disrupts endothelial barrier integrity, Thromb. Haemost. 122 (2022) 1469–1478.
[7] C. Lam, D.M. Krasnewich, PMM2-CDG, in: M.P. Adam, D.B. Everman, G.M. Mirzaa, R.A. Pagon, S.E. Wallace, L.J.H. Bean, K.W. Gripp, A. Amemiya (Eds.), GeneReviews(®), University of Washington, Seattle, Seattle (WA), 1993. Copyright © 1993-2024, University of Washington, Seattle. GeneReviews is a registered trademark of the University of Washington, Seattle. All rights reserved.
[8] V. Westphal, G.M. Enns, M.F. Mccracken, H.H. Freeze, Functional analysis of novel mutations in a congenital disorder of glycosylation Ia patient with mixed Asian ancestry, Mol. Genet. Metab. 73 (2001) 71–76.
[9] J. Jaeken, Congenital disorders of glycosylation, Handb. Clin. Neurol. 113 (2013) 1737–1743.
[10] A. Briso-Montiano, F. Del Caño-Ochoa, A. Vilas, A. Velázquez-Campoy, V. Rubio, B. Pérez, S. Ramón-Maiques, Insight on molecular pathogenesis and pharmacochaperoning potential in phosphomannomutase 2 deficiency, provided by novel human phosphomannomutase 2 structures, J. Inherit. Metab. Dis. 45 (2022) 318–333.
[11] N.R. Silvaggi, C. Zhang, Z. Lu, J. Dai, D. Dunaway-Mariano, K.N. Allen, The X-ray crystal structures of human alpha-phosphomannomutase 1 reveal the structural basis of congenital disorder of glycosylation type 1a, J. Biol. Chem. 281 (2006) 14918–14926.
[12] A.I. Vega, C. Pérez-Cerdá, D. Abia, A. Gámez, P. Briones, R. Artuch, L.R. Desviat, M. Ugarte, B. Pérez, Expression analysis revealing destabilizing mutations in phosphomannomutase 2 deficiency (PMM2-CDG): expression analysis of PMM2-CDG mutations, J. Inherit. Metab. Dis. 34 (2011) 929–939.
[13] P. Yuste-Checa, A. Gámez, S. Brasil, L.R. Desviat, M. Ugarte, C. Pérez-Cerdá, B. Pérez, The effects of PMM2-CDG-causing mutations on the folding, activity, and stability of the PMM2 protein, Hum. Mutat. 36 (2015) 851–860.
[14] M. Schiff, C. Roda, M.L. Monin, A. Arion, M. Barth, N. Bednarek, M. Bidet, C. Bloch, N. Boddaert, D. Borgel, A. Brassier, A. Brice, A. Bruneel, R. Buissonnière, B. Chabrol, M.C. Chevalier, V. Cormier-Daire, C. De Barace, E. De Maistre, A. De Saint-Martin, N. Dorison, V. Drouin-Garraud, T. Dupré, B. Echenne, P. Edery, F. Feillet, I. Fontan, C. Francannet, F. Labarthe, C. Gitiaux, D. Héron, M. Hully, S. Lamoureux, D. Martin-Coignard, C. Mignot, G. Morin, T. Pascreau, O. Pincemaille, M. Polak, A. Roubertie, C. Thauvin-Robinet, A. Toutain, G. Viot, S. Vuillaumier-Barrot, N. Seta, P. De Lonlay, Clinical, laboratory and molecular findings and long-term follow-up data in 96 French patients with PMM2-CDG (phosphomannomutase 2-congenital disorder of glycosylation) and review of the literature, J. Med. Genet. 54 (2017) 843–851.
[15] D. Marques-Da-Silva, V. Dos Reis Ferreira, M. Monticelli, P. Janeiro, P.A. Videira, P. Witters, J. Jaeken, D. Cassiman, Liver involvement in congenital disorders of glycosylation (CDG). A systematic review of the literature, J. Inherit. Metab. Dis. 40 (2017) 195–207.
[16] J.N. Arnold, M.R. Wormald, R.B. Sim, P.M. Rudd, R.A. Dwek, The impact of glycosylation on the biological function and structure of human immunoglobulins, Annu. Rev. Immunol. 25 (2007) 21–50.
[17] V. Westphal, G. Srikrishna, H.H. Freeze, Congenital disorders of glycosylation: have you encountered them? Genet. Med. 2 (2000) 329–337.
[18] S. Kjaergaard, F. Skovby, M. Schwartz, Carbohydrate-deficient glycoprotein syndrome type 1A: expression and characterisation of wild type and mutant PMM2 in E. Coli, Eur. J. Hum. Genet. 7 (1999) 884–888.