A New Missense Mutation in DPM2 Gene is Associated with a Milder Form of DPM2-CDG in Two Chinese Siblings

Peiwei Zhao
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Yanqiu Hu
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Juan Hu
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Yufeng Huang
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Lei Zhang
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Sukun Luo
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Ping Xiong
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Hongmin Zhu
Wuhan Childrens Hospital: Wuhan Women and Children Medical Care Center
Xuelian He (hexuelian2013@hotmail.com)
Wuhan Children's Hospital (Wuhan Maternal and Child Healthcare Hospital), Tongji Medical College, Huazhong University of Science & Technology https://orcid.org/0000-0002-6275-1219

Research Article

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Abstract

Background

Congenital disorders of glycosylation (CDGs) are a genetically heterogeneous group of metabolic disorders caused by abnormal protein or lpid glycoproteins. DPM2 is a subunit of a heterotrimeric complex (dolichol-phosphate-mannose synthase, DPMS), a key enzyme in glycosylation, and only four patients with DPM2-CDG have been reported.

Methods

Whole exome sequencing (WES) was applied to analyze a Chinese family with two daughters with developmental delay, milder intellectual disability, hypotonia and increased serum creatine kinase. In vitro functional study was performed to evaluate the impact of pathogenic genetic mutation .

Results

A homozygous mutation, c.197G > A (p.G66E) in exon 4 of *DPM2* gene (NM_003863) was identified by whole exome sequencing. In vitro functional analysis demonstrated that this variant increased the expression level of DPM2 protein and western blot revealed a significant decrease in ICAM1, a universal biomarker for hypoglycosylation in patients with CDG, suggesting abnormal N-linked glycosylation. We also review the 4 previously reported patients carried homozygous or compound heterozygous mutations of *DMP2* gene, and found that mutations within the region encoding the first domain is correlated with more severe clinical symptoms than ones within the second domain, establishing the possible correlation of genotypes and phenotype based on the localization of the variants.

Conclusions

our study broadens the mutation spectrum of *DPM2* genes, expands the genetic and phenotypic relevance of *DPM2* variants, and emphasizes the need of further functional studies to understand the underlying pathophysiology of the phenotype heterogeneity.

Introduction

Congenital disorders of glycosylation (CDG) are a group of inherited metabolic disorders caused by abnormal glycosylation of proteins or lipids(1). Glycosylation is an essential and the most common cellular process for post-translational modification of proteins and lipids, functioning in cell-cell, macromolecular interactions, protein folding, and protein signaling (2–3), and the patients with glycosylation defects often present with a broad spectrum of complication, usually involving nervous system, liver, heart, muscular, eyes, and immune system(4–6).

Dolichol-phosphate-mannose (DPM) synthase catalyzes the formation of DPM, and plays an important role in N-glycosylation, as well as in C-mannosylation, O-mannosylation, and the formation of glycosyl-

phosphatidylinositol (GPI) anchors(7). DPM2, a hydrophobic protein with 84 amino acids, is a subunit of the heterotrimeric DPM synthase complex, and is required for DPM enzyme activity, possibly by stabilizing the DPM3 protein(8–9).

DPM2-CDG (MIM:615042) is caused by mutations in *DPM2* gene and is an extremely rare CDG type. There is only 4 cases from 3 families reported and three of them with the variant c.68A > G (p.Y23C) died before 3 years old due to severe epilepsy, muscular dystrophy and acute respiratory infections(10–11).

In this study, we report two siblings from a Chinese family with dysmorphic features, developmental delay, milder intellectual disability, hypotonia, strabismus and increased serum creatine kinase. A homozygous mutation c.197G > A(p.G66E) was found in DPM2 gene in these two patients. In addition, we review all reported patients with DPM2-CGD and analyze the correlation between genotypes and clinical presentations of by reviewing literatures.

Material And Methods

Study subject

This study has been approved by the institutional review board of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science & Technology. two siblings with developmental delay, milder intellectual disability were recruited in this study. Upon obtaining informed consent, peripheral venous blood was withdrawn from the patient and both parents. Genomic DNA was extracted from leukocytes of whole blood samples using the QIAamp Blood DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was extracted *using Trizol* reagent (Invitrogen).

Whole exome sequencing

Whole exome sequencing (WES) and subsequent data analysis were conducted with the help of the third party medical laboratory (Chigene Lab, Beijing China). Candidate gene variants were confirmed by Sanger sequencing using self-designed primers in the patients and their parents. A protein sequence conservative analysis of mutation sites was conducted using MEGA software. The protocol used for WES was the same as described previously(12).

DPM2 plasmids construction and cell transfection

HCT116 cells were grown in DMEM supplemented with 10% fetal bovine serum (Gibco). The DPM2 coding sequence was amplified from HCT116 cells with the oligonucleotides 5'-ATG GCC ACG GGG ACA GAC-3' and 5'-TCA CTG AGC CTT CTT GGT CAC TCTC-3' using Pfu DNA polymerase (TransGen Biotech). The wildtype DPM2 (WT-DPM2) construct was obtained by inserted the amplified fragment into the pcDNA3.1(+) expression plasmid using BamHI and EcoR I restrictions sites.

To generate DPM2 mutated protein (G66E-DPM2), site-directed mutagenesis was performed with the oligonucleotides 5'- GAC GCT CCT GTT TGT GGA ACT GTT CAT CTC -3' and 5'- TCC ACA AAC AGG AGC AGC AGG AGG CCTG -3' using overlap PCR. In addition Y23C-DPM2 was generated as a control. All the positive clones were verified for the correct sequence by Sanger sequencing. HCT116 cells cultured in 6-well plate were

transfected with 1 μg plasmids using Lip3000 (Invitrogen) according to the manufacturer's instructions. Proteins were then resolved by SDS-PAGE on 12% gels (Invitrogen) and electrotransferred onto PVDF membranes.

DPM2 expression analysis using Realtime PCR

Total RNA was extracted from HCT116 cells by using Trizol Reagent (Invitrogen,USA). The first complementary DNA was synthesized from RNA using reverse transcriptase (TAKARA, Dalian). Real-time PCR was performed using SYBR Green PCR Kit (TaKaRa, Dalian) and *GAPDH* as an internal control.

Western blot

Cells were lysed in 1% NP-40 lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% NP-40, and 0.5% sodium deoxycholate) for 30 min on ice, and then centrifugated at 12000rpm for 10 min. Protein concentration was determined by BCA assay (Thermo Fisher Scientific), and 20 ug total protein was separated by 12% SDS-PAGE and subsequently transferred to PVDF membrane. After blocking in 2% BSA (bovine serum albumin), membranes were probed with the following Abs: anti-Flag (Santa Cruz Biotechnology, Inc.), anti-ICAM1(Proteintech, 60299-1-Ig) and anti-GAPDH (Cell Signaling Technology, Inc.), and Bound Abs were detected using secondary Abs (Cell Signaling Technology, Inc) and enhanced chemiluminescence (ECL, Thermo Fisher Scientific).

Immunofluorescence assay

To examine the localization of DPM2, a co-localization analysis of DPM2 with endoplasmic reticulum(ER) marker (PDI) was performed. Cells adhered to poly-L-lysine-coated slides were fixed for 15 min with 3% paraformaldehyde, and then permeabilized for 15 min in 0.1% Triton X-100/PBS (phosphate buffer saline). After blocking for 60 min in 3% BSA/PBS, cells spots were incubated with the anti-DPM2 and anti-PDI antibody(Proteintech, 66422-1-Ig) for 60min. Cells were washed in PBS for 3 times and then incubated with goat anti–rabbit secondary Ab conjugated to Alexa Fluor 488 and Alexa Fluor 555(Cell Signaling Technology, Inc). Cells were then counterstained with DAPI and fluorescent images were acquired on a confocal microscope (Leica Stellaris5) using a oil immersion objective.

Results

Clinical report

The proband was admitted to the Rehabilitation Department in Wuhan Children's Hospital due to developmental delay, milder intellectual disability, hypertonia and strabismus at 11 years old. She is a younger sister of healthy nonconsanguineous parents without relevant family medical history. She was born at 40 weeks of gestation by caesarean section with a birth weight of 3.40kg, birth length of 50cm, and Apgar scores were not available. She was able to walk without support at 3 years old. On examination, she has no obvious facial abnormalities other than strabismus. She has milder intellectual disability, speech delay, dysarthria, and motor retardation. She walks unsteadily, on tiptoe, with the center of gravity of the body in the sacrococcygeal region. She has severe strephenopodia on the right, and need the help of hands to get up down stairs. Her upper limbs are normal and can freely move.

Blood biochemistry examinations showed normal liver function, abnormal myocardial function with 2097U/L of creatine kinase (reference: 20 ~ 250U/L) and 58U/L of creatine kinase -MB(0 ~ 25U/L), and there was no obvious abnormality in blood amino acid and urine organic acid analyzed by mass spectrometry. Electroencephalogram examination indicated borderline electroencephalogram in children and occipital background activity was slightly slower. Electrophysiological evaluation showed slowed motor conduction velocity, longer motor latency, and decreased amplitude on the left median and ulnar nerves, but no sensory nerve action potential was detected in the median, ulnar and sural nerves in the right lower limb, suggesting multiple peripheral nerve injury. Brain MRI scan showed slightly longer patchy signal on T1 and T2, high intensity signal in the FLAIR sequence, in the white matter of bilateral parietal lobes considering demyelinating lesions(Fig. 1A).

The elder sister had language and motor retardation durimng childhood, and she has milder intellectual disability and exercise intolerance. Laboratory investigations showed increased creatine kinase (2022U/L) and CK-MB (34U/L), and normal liver and renal functions.

WES Analysis and Putative Pathogenic Mutation Screening

In order to identify the pathogenic gene for a molecular diagnosis and prognosis, trio (including the younger sister and parents) WES was conducted. Bioinformatic analysis was performed to identify candidate variants according to the filtering strategy based on population frequency, variant classification, and variant functional damaging prediction. A homozygous mutation: c.197G > A (p.G66E) in exon 4 of *DPM2* (NM_003863) was found in younger sister, inheriting from her mother and her father, and her older sister was homozygous for this mutation confirmed by Sanger sequencing(Fig. 1B). The variant c.197G > A (p.G66E) is not found in the variation database (i.e. gnomAD, ClinVar, and 1000 genomes). Bioinformatic analysis showed that this site are conserved among different species (Fig. 1C) and the amino acid substitutions was probably damaging, with a high PolyPhen-2 score of 1 and a SIFT tolerance index of 0.006. According to the standards and guidelines recommended by the American College of Medical Genetics and Genomics (ACMG) this mutation was classified as variant uncertain significance.

DPM2 protein expression and subcellular localization

To investigate whether the *DPM2* mutation affects the gene expression of DPM2 in the proband, quantitative real-time PCR was performed by using RNA extracted from PBMCs of the proband and three age-matched normal controls. As shown in Fig. 2A, the gene expression of DPM2 was increased in proband than that of controls. To evaluate the effect of the G66E variant, and also to attempt to clarify the reason why the difference in clinical severity between our patients with G66E and patients with Y23C, four kinds of plasmids, including control (pcDNA3.1), WT-DPM2, Y23C-DPM2 and G66E-DPM2, were transfected into HCT116 cells. After 24 hours, transfected cells were harvested and lysed or fixed for western blot or immunofluorescence staining, respectively. As shown in Fig. 2B and 2C, G66E variant dramatically increased the expressions of DPM2 gene and protein, respectively, whereas Y23C had no significantly change on gene or protein. As reported previously, the patients with Y23C had severe clinical symptoms and expired before 3 years old (12), suggesting that Y23C has devastating effects. Thus, we postulate the protein level is not a critical factor for DPM2 proper function. In order to further figure out the possible cause, immunofluorescence assay was used to examine the localization of mutated DPM2, however, neither Y23C nor E66G altered the cellular localization of DPM2 protein(Fig. 3). Although the protein levels and localization of Y23C-DPM2 were unchanged, ICAM1, a universal biomarker for

hypoglycosylation in patients with CDG(13), was decreased in HCT116 cells(Fig. 2C), which indicates DPM2 regulates glycosylation not by protein levels but by other mechanism.

Literature review of patients with DPM2-CDG

Literature review of DPM2-CDG was conducted by searching for all cases reported from 2012, the year the DPM2-CDG was first reported(12), to 2021 with the keywords "*DPM2* gene" and "congenital disorders of glycosylation". The database included Pubmed, Medline, Clinvar et al. We reviewed 2 articles including 4 cases with DPM2-CDG(12–13), three of which were reported by Rita Barone et al. in 2012. The demographic characteristics and clinical features of these patients (P3-P6), as well as two siblings (P1-P2) we reported, were summarized in Table 1.

Table 1
Clinical characteristics of patients with DPM2-CDG

Patients	Present study (P1)	Present study (P2)	P3(12)	P4(12)	P5(12)	P6(13)
Sex	F	F	F	M	M	M
Age of onset	11Y	20Y	after birth	after birth	after birth	23Y
Country	China	China	Italy	Italy	Italy	India
Year of publish			2012	2012	2012	2021
Dysmorphic features	yes	yes	yes	yes	yes	no
Microcephaly	no	no	yes	yes	yes	yes
Developmental delay	yes	yes	severe	yes	yes	yes
Feeding difficulties	no	no	yes	yes	yes	no
Hypotonia	hypertonia		severe	severe	severe	yes
Seizure	no	no	intractable seizures	Treatment-resistant epilepsy	Treatment-resistant epilepsy	no
Congenital heart disease	no	NA	no	no	no	yes
Hepatobiliary abnormalities	no	no	hepatomegaly, elevation of serum transaminases	na	na	no
Skeleton	Contracture of ankle joint	no	no	congenital contractures of the joints and scoliosis.	congenital contractures of the joints and scoliosis.	
Recurrent infection	yes	no	yes	yes	yes	no
Hearing abnormality/ Ophthalmological	strabismus	no	Optic atrophy	No visual tracking, strabismus	No visual tracking	
Serum creatine kinase	elevated	elevated	elevated	elevated	elevated	elevated
EEG	Abnormal EEG	NA	bursts of paroxysmal multiple spikes	NA	NA	NA

Patients	Present study (P1)	Present study (P2)	P3(12)	P4(12)	P5(12)	P6(13)
Brain MRI	bnormal signal of bilateral parietal white matter	NA	loss of cerebral periventricular and subcortical white matter, without overt cerebellar atrophy	mild cerebellar hypoplasia with severe vermis hypoplasia	NA	
Outcome	Alive, 11 year	Alive, 20 year	died at 3 years	died at 16 months	died at 7 months	Alive, 20 year
Allele 1	c.197G > A (p.G66E)	c.197G > A (p.G66E)	c.68A > G (p.Y23C)	c.68A > G (p.Y23C)	c.68A > G (p.Y23C)	c.139C > T(p.R47X)
Allele 2	c.197G > A (p.G66E)	c.197G > A (p.G66E)	c.4-1G > C	c.68A > G (p.Y23C)	c.68A > G (p.Y23C)	c.173G > A(p.G58D)

As shown in Table 1, there were 3 male patients and 3 female patients, aged from 2 months to 23 years. The main clinical manifestations of the patients were progressive microcephaly, severe visual defect, intractable epilepsy, muscular dystrophy, liver involvement, and decreased coagulation factors. These three patients reported by Rita Barone et al. in 2012 expired at very early age due to acute respiratory infections. The forth patient was an 23 years old adult male with truncal hypotonia, hypertonicity, congenital heart defects, intellectual disability, and generalized muscle wasting(13). In this study we reported two cases of DPM2-CDG with much milder symptoms which broadened the phenotypic spectrum of the disease.

There are 4 germline mutations (Y23C, c.4-1G > C, R47X, G58D) have been reported before. Patients with Y23C and c.4-1G > C mutation have severe symptoms and patient with compound heterozygous mutations(R47X and G58D) had relatively mild symptoms. Two patients in this study carried G66E mutation, and the clinical presentation was much less severe than that of the previously described patients.

Discussion

Glycosylation is an essential biological process for various protein or lipid modifications, and many human disorders of glycosylation pathways have now been identified; they include defects in synthetic pathways for N-linked oligosaccharides, O-linked oligosaccharides, shared substrates, glycophosphatidylinositol (GPI) anchors and dolichols(1–2). In this study, we identified a novel variant in DPM2 gene by using WES in two Chinese siblings with mild developmental delay, hypotonia and increased creatine kinase. Bioinformatic analysis and further in vitro experiment suggested that this variant was likely pathogenic.

DPM2 is the subunit of the heterotrimeric dolichol-phosphate-mannose (DPM) synthase complex (DPM1, DPM2, and DPM3), which catalyses the synthesis of dolichol-P-mannose and plays a critical role in N-glycosylation as well as O-mannosylation (14–16). DPM2 stabilizes the synthase complex(17). Our study showed that G66E increased DPM2 protein level whereas another variant Y23C had no effect on protein expression, and both variants did not change the cellular localization of mutant proteins. However, the tyrosine amino acid at 23 in the first transmembrane domain is highly conserved during evolution, and is essential for proper function of DPM2 (18). Thus, we speculate that protein expression level is not the critical factor in maintain the proper function of DPM2 in the process of glycosylation, and further study is needed to understand the detailed mechanism of DPM2 in modulating glycosylation and the effects of variants in *DPM2* gene on glycosylation.

DPM2-CDG is extremely rare and only 6 cases from 4 families (Table 1), and a total of 5 variants (c.139C > T, c.173G > A, c.197G > A, c.4-1G > C and c.68A > G) were found. DPM2 is composed of 84 amino acids and containis two transmembrane domains. The Y23C (c.68A > G) is located in the first transmembrane domain whereas the mutations, E66G (197G > A), G58D(c.173G > A), and Y47X(c.139C > T), are located in the second transmembrane domain. Three patients, first reported in 2012 and died before 3 years old, carried a homozygous Y23C or compound heterozygous of Y23C and c.4-1G > C(12). Another DPM2-CDG patient reported by Silvia et al(13), and our patients showed much milder clinical phenotypes and had mutations in second ransmembrane domain. According to these information, we infer that the first transmembrane domain of DPM2 is more important than the second one in stabilizing the complex, more patients are needed to analyze the correlation the genotype to phenotype.

In addition, although electrophysiological evaluation did not found significant muscle damage and muscle biopsy was refused, the young sister had difficulties in movement, especially in going up and down stairs, and the elder sister had exercise intolerance, and both had elevated creatine kinase. These evidences suggested the siblings suffered muscle damage. As mentioned above, in addition to N-glycosylation, DPM synthase has a critical role in O-mannosylation, the defect in latter contributes to dystroglycanopathies, a subgroup of the congenital muscular dystrophies(19). Therefore, it is likely that E66G affects the O-mannosylation by interfering the function of DPM2, and more evidence is needed to support this hypothesis.

In conclusion, we identified and characterized a novel DPM2 mutation, c.197G > A (E66G) in two Chinese siblings with a milder form of CDG compared to patients with Y23C mutation. By reviewing literatures, we found that mutations within the region encoding the first domain is correlated with more severe clinical symptoms than ones within the second domain. Our study broadens the mutation spectrum of *DPM2* genes, establish the possible correlation of genotypes and phenotypes, and emphasizes the importance of WES in assisting with the diagnosis of rare diseases. Our study also raises many questions regarding to the possible mechanism which need further study.

Declarations

Consent for publication

Written consent was obtained from the parents of patients for the publication.

Availability of data and materials

All data and material are available from one of corresponding authors, Xuelian He, who can be contacted by email on the email address (hexuelian2013@hotmail.com).

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Authors' contributions

Study concepts: Xuelian He, Hongmin Zhu, Ping Xiong

Study design: Peiwei Zhao,Yanqiu Hu, Xuelian He

Literature research: Peiwei Zhao,Yanqiu Hu, Yufeng Huang

Clinical information collection: Juan Hu, Ping Xiong

Data acquisition: Peiwei Zhao,Yanqiu Hu, Hongmin Zhu

Data analysis/interpretation: Peiwei Zhao,Yanqiu Hu, Ping Xiong, Lei Zhang

Manuscript preparation: Xuelian He, Peiwei Zhao, Sukun Luo

Manuscript editing: Xuelian He

Manuscript final version approval: Ping Xiong, Hongmin Zhu, Xuelian He

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Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study has been approved by the institutional review board of Wuhan Children's Hospital, Tongji Medical College, Huazhong University of Science & Technology (NO.2020R006-F01). Written informed consent for the study was obtained from all study participants.

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Figures

Figure 1

(A) MRI shows abnormal signal foci in the white matter of the brain, and demyelination change in proband.(B) Sanger sequencing of DPM2 mutations in the family of this study; (C) Conservation analysis of DPM2 protein among different species. The position of the mutations at amino acids 66 is indicated in red and highly conserved throughout all indicated species; (D) Scheme of the distribution of DPM2 mutations, The mutations in red were reported in present study.

Figure 2

The expression level of DPM2 in PBMCs of the patient 1 and normal control(A). (B) The expression level of DPM2 in HCT116 cells transfected with wildtype or mutant DPM2, RNA level. (C) the protein expression level of DPM2 and ICAM1 in HCT116 cells. NC:normal control, P1: patient 1.

Figure 3

DPM2 protein expression and subcellular localization in HCT116 cells.