Molecular Genetics and Metabolism Reports 38 (2024) 101035

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

Short Communication

# AAV9-based *PMM2* gene replacement augments PMM2 expression and improves glycosylation in primary fibroblasts of patients with phosphomannomutase 2 deficiency (PMM2-CDG)

M. Zhong [a], B. Balakrishnan [a], A.J. Guo [a], K. Lai [a,b,*]

[a] Division of Medical Genetics, Department of Pediatrics, University of Utah Spencer Fox Eccles School of Medicine, USA
[b] Department of Nutrition and Integrated Physiology, University of Utah College of Health, USA

ARTICLE INFO

*Keywords:*
Inherited phosphomannomutase 2 deficiency (PMM2-CDG)
Congenital disorders of glycosylation
AAV9 gene replacement therapy
Primary fibroblast cell model

ABSTRACT

Inherited deficiency of phosphomannomutase 2 (PMM2) (aka PMM2-CDG) is the most common congenital disorders of glycosylation (CDG) and has no cure. With debilitating morbidity and significant mortality, it is imperative to explore novel, safe, and effective therapies for the disease. Our Proof-of-Concept study showed that AAV9-*PMM2* infection of patient fibroblasts augmented PMM2 expression and improved glycosylation. Thus, AAV9-*PMM2* gene replacement is a promising therapeutic strategy for PMM2-CDG patients.

## 1. Introduction

Congenital disorders of glycosylation (CDG) are a group of inherited metabolic disorders characterized by glycoprotein and glycolipid hypoglycosylation [1]. Phosphomannomutase 2 (PMM2) deficiency (aka PMM2-CDG, MIM# 212065), the most common CDG, is caused by recessive pathogenic mutations in the *PMM2* gene [2], which effectively and significantly diminished cellular PMM2 activity, thereby reducing the conversion rate of mannose 6-phosphate into mannose 1-phosphate. The latter is used to synthesize GDP-mannose, a crucial mannose donor for glycan synthesis [3] (Fig. 1A). Patients with PMM2-CDG present with variable features ranging from isolated neurologic involvement to severe multi-organ dysfunction and early death [4,5].

In the absence of curative treatments and a high mortality before two years of age, not to mention the severe morbidity throughout life, it is imperative to explore safe and effective therapeutic strategies [6]. We hypothesized that AAV9-based *PMM2* gene replacement could restore significant PMM2 expression in patient cells and improve the long-term outcome of this disease.

## 2. Materials and methods

### 2.1. Cell culture

PMM2-CDG patient-derived fibroblasts (GM27226, GM27386) were obtained from the Coriell Institute for Medical Research. De-identified control fibroblasts were derived from normal healthy individuals. Cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution.

### 2.2. AAV9-PMM2 infection

AAV9-*PMM2* expression vector was designed by the Lai Lab and synthesized by VectorBuilder Inc. (IL, U.S.A.). Infection of fibroblasts was performed using VectorBuilder's recommended protocol.

### 2.3. PMM2 activity assay

Fibroblasts were assayed for PMM2 activity as previously described [7].

* Corresponding author at: Division of Medical Genetics, Department of Pediatrics, University of Utah Spencer Fox Eccles School of Medicine, 30 N Mario Capecchi Drive, Salt Lake City, UT 84113, USA.
*E-mail address:* kent.lai@hsc.utah.edu (K. Lai).

https://doi.org/10.1016/j.ymgmr.2023.101035
Received 7 December 2023; Accepted 8 December 2023

### 2.4. Immunoblot analysis

PMM2 protein abundance in fibroblasts was evaluated with primary antibody against PMM2 (Proteintech, #10666–1-AP) using our laboratory's published protocol [8]. Assessment of the abundances of glycoproteins ICAM-1 and LAMP1 was performed using primary antibodies against ICAM-1(Santa Cruz Biotechnology, #sc-8439) and LAMP1(Cell Signaling Technology, #3243), respectively. β-actin detected by anti-β-actin antibody (Cell Signaling Technology, #CST-3700) was used as loading control.

## 3. Results

We conducted *in vitro* studies by infecting patient fibroblasts with AAV9-based vector expressing the human *PMM2* gene. The patient 1 fibroblast strain was compound heterozygous for R141H and N216I mutations, while patient 2 fibroblast strain was compound heterozygous for R141H and E139K mutations. Patient 1 presented with a range of symptoms, including global developmental delay, oculomotor apraxia, cerebellar hypoplasia, feeding difficulties, abnormal coagulation, muscular weakness, ataxia, strabismus, growth hormone deficiency, eczema, hypothyroidism, iron deficiency, transaminitis, weekly episodes of epistaxis and mild myopia. Patient 2 had developmental delay, muscle weakness, noticeable mild plagiocephaly, dysarthria, strabismus, difficulty chewing and swallowing, severe central sleep apnea with mild hypoxemia. Both patient fibroblast strains expressed residual PMM2 activity (9.22% and 6.85%, respectively of Control) (Fig. 1 B3) and residual immunoreactive PMM2 protein (37.43% and 11.37%, respectively of control) (Fig. 1 B1, 2). Infection of the AAV9-*PMM2* at multiplicity of infection (MOI) of 10,000 augmented PMM2 protein levels by 2.87 and 2.60-fold, respectively (Fig. 1 C1, 2). This was accompanied by an increase in activity 2.50 and 1.67-fold, respectively (Fig. 1 C3). Additionally, augmentation of PMM2 activity in the patient fibroblast strain increased ICAM-1 and LAMP1 expression (Fig. 1D).

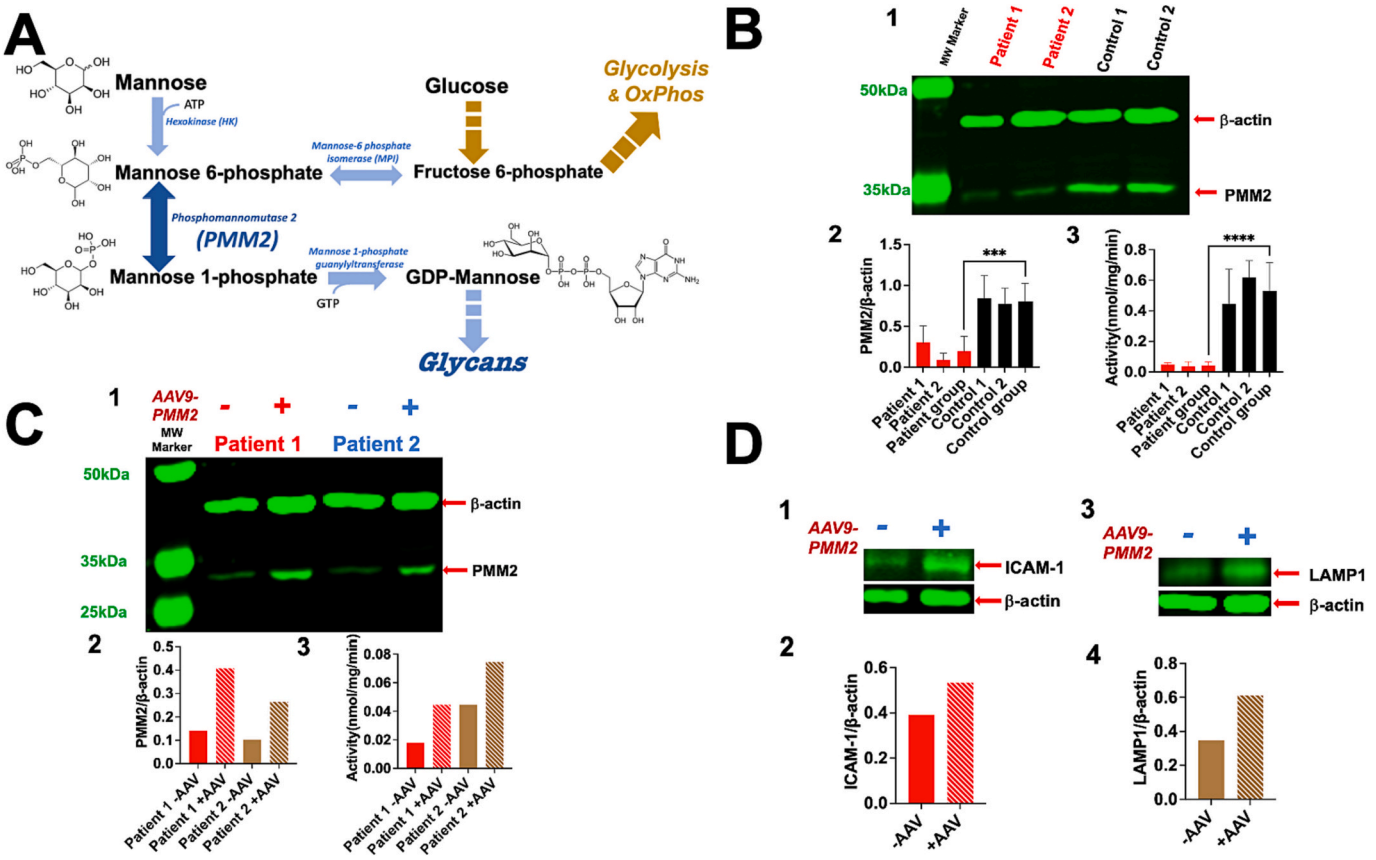


**Fig. 1.** Pharmacokinetic and Pharmacodynamic studies of AAV9-*PMM2* in patient-derived fibroblast cell models.
(A) Role of phosphomannomutase 2 (PMM2) in glycan biosynthesis.
(B) Characterization of PMM2 expression in PMM2-CDG patient-derived and control fibroblasts.
(1) Assessment of PMM2 protein expression through immunoblot analysis.
(2) Comparative quantification of PMM2 protein levels normalized to β -actin protein abundance.
(3) Evaluation of PMM2 enzymatic activity. Each sample represents three independent replicates of the experiment. ***, $P < 0.001$. ****, $P < 0.0001$.
(C) Evaluation of PMM2 expression in AAV-treated and untreated PMM2-CDG patient-derived fibroblasts.
(1) Assessment of PMM2 protein expression through immunoblot analysis.
(2) Comparative quantification of PMM2 protein levels normalized to β -actin protein abundance.
(3) Evaluation of PMM2 enzymatic activity post-infection. Each sample represents pooled extracts prepared from three separate 10-cm plates of cells treated independently in the specified manners.
(D) Evaluation of glycosylation in AAV-treated and untreated PMM2-CDG patient-derived fibroblasts.
(1) Assessment of ICAM-1 protein expression through immunoblot analysis.
(2) Comparative quantification of ICAM-1 protein levels normalized to β-actin protein abundance.
(3) Assessment of LAMP1 protein expression through immunoblot analysis.
(4) Comparative quantification of LAMP1 protein levels normalized to β-actin protein abundance. Each sample represents pooled extracts prepared from three separate 10-cm plates of cells treated independently in the specified manners.

Interestingly, supplementation of the patient cells with 1 mM D (+) mannose did not appear to improve the infection efficiency (data not shown).

## 4. Discussion

With an estimated prevalence of 1:20,000 in some studies [9], PMM2-CDG is the most prevalent CDG. Therefore, it is not surprising that different therapeutic strategies have been tested to address the great unmet needs of patients. These approaches include, but are not limited to, the use of phosphomannose isomerase inhibitors [10], aldose reductase inhibitors [11], pharmacological chaperones [12], and acetazolamide supplementation [13]. None of these approaches restore near-normal PMM2 activity in patient cells. The recent Food and Drug Administration (FDA)-approved gene-based therapies like Zolgensma®[14]and Hemgenix® [15] indicated that AAV-mediated gene replacement therapy could be a promising modality for PMM2-CDG. Although gene-based therapy like Zolgenma® is an intuitively sound approach for many recessive genetic disorders, we were originally concerned if it would work for CDG because cell receptors for AAV vectors are glycoproteins that could be so severely altered due to hypoglycosylation in PMM2-CDG that AAV infection is no longer feasible. Consequently, it is crucial to test the viability of such approach in pilot Proof-of-Concepts (POC) studies in patient cells early in the therapeutic development programs. Our results shown here are not only reassuring, but they also demonstrate that AAV9-mediated *PMM2* replacement augmented PMM2 expression and improved glycosylation in the patient cells (Fig. 1), thus paving the way for *in vivo* preclinical studies.

Yet, we are aware that patients with PMM2-CDG suffer liver disease and neurological deficits, which could not be fully modeled in patient fibroblasts. However, we believe that construction of immortalized patient cell lines of liver and central nervous system (CNS) origins is outside the scope of this study, not to mention how achievability it will be. Moreover, we would like to emphasize that the focus this Short Communication is to test whether any residual PMM2 activity in patient cells will be sufficient for AAV9 infection. As we now demonstrate that it is feasible, we will proceed to *in vivo* testing in animal models of the disorder in the future, so that we can address the tissue-specific phenotypes more effectively. Furthermore, we have chosen in this study the AAV9 serotype because of its documented ability to cross the blood-brain barrier, which has led to successful, non-invasive intravascular delivery in animal models of several diseases [16,17]. In fact, the Zolgensma® [14] gene therapy approved by FDA to treat Spinal Muscular Atropy (SMA), a neurological disease, also employed AAV9 as the delivery agent. Therefore, we are confident that the AAV9-*PMM2* vector we designed and tested in this pilot study will allow us to further evaluate the efficacy of this modality in alleviating the neurological disease of PMM2-CDG in our ongoing studies.

## 5. Conclusion

AAV9-based *PMM2* gene replacement is a promising therapeutic strategy to tackle the unmet medical needs for the patients with PMM2-CDG.

## Source of funding

Intramural grant support from Department of Pediatrics, University of Utah Spencer Fox Eccles School of Medicine (to KL).

## CRediT authorship contribution statement

**M. Zhong:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Writing – original draft. **B. Balakrishnan:** Formal analysis, Methodology, Writing – review & editing. **A.J. Guo:** Data curation, Methodology. **K. Lai:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

## Declaration of Competing Interest

The authors declare no conflict of interest.

## Data availability

Data will be made available on request.

## References

[1] J. Jaeken, Congenital disorders of glycosylation, Handb. Clin. Neurol. 113 (2013) 1737–1743, https://doi.org/10.1016/b978-0-444-59565-2.00044-7.
[2] R. Altassan, R. Péanne, J. Jaeken, R. Barone, M. Bidet, D. Borgel, S. Brasil, D. Cassiman, A. Cechova, D. Coman, et al., International clinical guidelines for the management of phosphomannomutase 2-congenital disorders of glycosylation: diagnosis, treatment and follow up, J. Inherit. Metab. Dis. 42 (1) (2019) 5–28, https://doi.org/10.1002/jimd.12024.
[3] V. Sharma, M. Ichikawa, H.H. Freeze, Mannose metabolism: more than meets the eye, Biochem. Biophys. Res. Commun. 453 (2) (2014) 220–228, https://doi.org/10.1016/j.bbrc.2014.06.021.
[4] M. Schiff, C. Roda, M.L. Monin, A. Arion, M. Barth, N. Bednarek, M. Bidet, C. Bloch, N. Boddaert, D. Borgel, et al., 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 (12) (2017) 843–851, https://doi.org/10.1136/jmedgenet-2017-104903.
[5] S. Kjaergaard, M. Schwartz, F. Skovby, Congenital disorder of glycosylation type Ia (CDG-Ia): phenotypic spectrum of the R141H/F119L genotype, Arch. Dis. Child. 85 (3) (2001) 236–239, https://doi.org/10.1136/adc.85.3.236.
[6] A. Gámez, M. Serrano, D. Gallego, A. Vilas, B. Pérez, New and potential strategies for the treatment of PMM2-CDG, Biochim. Biophys. Acta Gen. Subj. 1864 (11) (2020), 129686, https://doi.org/10.1016/j.bbagen.2020.129686.
[7] E. Van Schaftingen, J. Jaeken, Phosphomannomutase deficiency is a cause of carbohydrate-deficient glycoprotein syndrome type I, FEBS Lett. 377 (3) (1995) 318–320, https://doi.org/10.1016/0014-5793(95)01357-1.
[8] B. Balakrishnan, W. Chen, M. Tang, X. Huang, D.D. Cakici, A. Siddiqi, G. Berry, K. Lai, Galactose-1 phosphate uridylyltransferase (GalT) gene: a novel positive regulator of the PI3K/Akt signaling pathway in mouse fibroblasts, Biochem. Biophys. Res. Commun. 470 (1) (2016) 205–212, https://doi.org/10.1016/j.bbrc.2016.01.036.
[9] E. Schollen, S. Kjaergaard, E. Legius, M. Schwartz, G. Matthijs, Lack of hardy-Weinberg equilibrium for the most prevalent PMM2 mutation in CDG-Ia (congenital disorders of glycosylation type Ia), Eur. J. Hum. Genet. 8 (5) (2000) 367–371, https://doi.org/10.1038/sj.ejhg.5200470.
[10] V. Sharma, M. Ichikawa, P. He, D.A. Scott, Y. Bravo, R. Dahl, B.G. Ng, N.D. Cosford, H.H. Freeze, Phosphomannose isomerase inhibitors improve N-glycosylation in selected phosphomannomutase-deficient fibroblasts, J. Biol. Chem. 286 (45) (2011) 39431–39438, https://doi.org/10.1074/jbc.M111.285502.
[11] S. Radenkovic, A.N. Ligezka, S.S. Mokashi, K. Driesen, L. Dukes-Rimsky, G. Preston, L.F. Owuocha, L. Sabbagh, J. Mousa, C. Lam, et al., Tracer metabolomics reveals the role of aldose reductase in glycosylation, Cell Rep. Med. 4 (6) (2023), 101056, https://doi.org/10.1016/j.xcrm.2023.101056.
[12] M. Monticelli, L. Liguori, M. Allocca, G. Andreotti, M.V. Cubellis, beta-Glucose-1,6-bisphosphate stabilizes pathological Phophomannomutase2 mutants in vitro and represents a Lead compound to develop pharmacological chaperones for the Most common disorder of glycosylation, PMM2-CDG, Int. J. Mol. Sci. 20 (17) (2019) 4164, https://doi.org/10.3390/ijms20174164.
[13] A.F. Martinez-Monseny, M. Bolasell, L. Callejon-Poo, D. Cuadras, V. Freniche, D. C. Itzep, S. Gassiot, P. Arango, D. Casas-Alba, E. de la Morena, et al., AZATAX: acetazolamide safety and efficacy in cerebellar syndrome in PMM2 congenital disorder of glycosylation (PMM2-CDG), Ann. Neurol. 85 (5) (2019) 740–751, https://doi.org/10.1002/ana.25457.
[14] T. Ogbonmide, R. Rathore, S.B. Rangrej, S. Hutchinson, M. Lewis, S. Ojilere, V. Carvalho, I. Kelly, Gene therapy for spinal muscular atrophy (SMA): a review of current challenges and safety considerations for Onasemnogene Abeparvovec (Zolgensma), Cureus 15 (3) (2023), e36197, https://doi.org/10.7759/cureus.36197.
[15] D. De Wolf, K. Singh, M.K. Chuah, T. VandenDriessche, Hemophilia gene therapy: the end of the beginning? Hum. Gene Ther. 34 (17–18) (2023) 782–792, https://doi.org/10.1089/hum.2023.112.
[16] M. Vyas, N.M. Deschenes, K.J.L. Osmon, Z. Chen, I. Ahmad, S. Kot, P. Thompson, C. Richmond, S.J. Gray, J.S. Walia, Efficacy of adeno-associated virus serotype 9-mediated gene therapy for AB-variant GM2 Gangliosidosis, Int. J. Mol. Sci. (2023) 24 (19), https://doi.org/10.3390/ijms241914611.
[17] J. Saraiva, R.J. Nobre, L. Pereira de Almeida, Gene therapy for the CNS using AAVs: the impact of systemic delivery by AAV9, J. Control. Release 241 (2016) 94–109, https://doi.org/10.1016/j.jconrel.2016.09.011.