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Commentary

Promise of gene therapy for congenital neurologic disease due to GPI deficiency

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A recent study by Murakami et al.[1] describes a novel genetic treatment for inherited glycophosphatidylinositol (GPI) deficiency (IGD) using adeno-associated virus (AAV)-based gene therapy.[1] *PIGA* is an X-linked gene required for the first step of GPI anchor biosynthesis, a multistep process requiring at least 27 different genes.[2] Disruption of *PIGA* or other genes in the GPI biosynthesis process leads to the loss of approximately 150 different GPI-anchored surface proteins (GPI-APs) in humans.

Complete loss of *PIGA* is incompatible with life due to embryonic lethality. In humans, *PIGA* null mutations have only been observed somatically in the context of a rare blood disease, paroxysmal nocturnal hemoglobinuria (PNH).[3] In PNH, acquired *PIGA* loss is restricted to the hematopoietic lineage and does not interfere with hematopoietic development but, rather, predisposes to complement-mediated hemolytic anemia. In contrast, in IGD disease (*PIGA*-IGD; MIM: 300868 and 301072), *PIGA* mutations are hypomorphic, allowing for partial preservation of PIGA function with reduced (but not absent) levels of GPI-Aps, which protects IGD patients from PNH disease. Male patients, hemizygous for mutant *PIGA*, develop severe congenital disease characterized by profound neurologic abnormalities, developmental delay, intractable seizures, and other malformations and disabilities (Figure 1).[4,5] However, female IGD patients heterozygous for *PIGA* mutations are mosaic due to X inactivation and clinically unaffected.

Historically, modeling *PIGA*-IGD in mice has been difficult. Male mice hemizygous for *Piga* loss ($Piga^{-/}$) die in early embryonic development due to developmental anomalies. Female $Piga^{+/-}$ mice are mosaic for *Piga* loss and die in late embryonic development. Efforts to generate mice modeling patient-specific hypofunctional *PIGA* mutations have been unsuccessful,[1] whether for technical reasons or because of cross-species differences in mutation severity. There are no models of partial *Piga* deficiency; instead, tissue-specific knockouts of *Piga* have been used to model various aspects of *PIGA*-IGD.

Previously, a central nervous system (CNS)-specific knockout of *Piga* was developed, using Cre under the control of the Nestin promoter to model neurologic manifestations of *PIGA*-IGD.[6] In this model, Nestin-driven Cre excises *Piga* starting at embryonic day 11.5 (E11.5) broadly in the nervous system including the developing neurons, astrocytes, and oligodendrocytes. Cre^{+} $Piga^{-/}$ male and $Piga^{+/-}$ female mice die in the early postnatal period (by day 10 and 25, respectively) due to failure to gain weight and severe neurologic abnormalities, including ataxia, tremors, loss of reflexes, and muscle loss.[1,6] Pathological examination showed demyelination particularly affecting areas of high *Piga* expression in development, including the Purkinje cells of the cerebellum and corpus callosum.[6]

To test whether gene therapy can improve the neurologic phenotype of *PIGA* IGD, Murakami et al.[1] and colleagues administered an AAV vector carrying human *PIGA* (h*PIGA*) under the control of a strong ubiquitous CAG promoter to Nestin *Piga* mice. The specific AAV vector (AAV-PHPeB) was selected due to improved ability to cross the blood-brain barrier in mice. Treatment with AAV-PHPeB-hPIGA on postnatal day 1 improved neurologic function and improved survival. AAV-hPIGA-treated Cre^{+} $Piga^{+/-}$ mice had improved myelination and experienced no spontaneous seizures. *hPIGA* was expressed by day 4 post-injection, and in Cre^{+} $Piga^{+/-}$ mice was expressed at levels similar to endogenous mouse Piga (mPiga) on day 25. In Cre^{+} $Piga^{+/-}$ mice that survived to 1 year, *hPIGA* expression exceeded that of *mPiga*. Despite seemingly effective delivery and neurologic improvements, male mice lived about 3 weeks, and female mice survived about 3 months. Of the three AAV-PHPeB-hPIGA-treated female mice who survived to 1 year, all three developed liver tumors, associated with overexpression of the *Rian* gene. Previous studies of AAV delivery in neonatal mice have observed a high frequency of liver tumors, with the majority caused by AAV integration in the *Rian* locus.[7] To what degree this observation is relevant to AAV delivery in human patients remains unknown; notably, no AAV-associated cancers have been reported with the US Food and Drug Administration (FDA)-approved AAV gene therapy for spinal muscular atrophy, with over 3,000 children treated to date.[7,8]

Why wasn't gene therapy more effective? One key reason is the severity of neurologic abnormalities caused by loss of GPI-anchored proteins in early CNS development. Among GPI-anchored proteins integral to normal neuronal development are proteins integral to neuron development and function, including contactin 1 (CNTN1; a cell adhesion molecule and Notch1 ligand necessary for the normal interactions between

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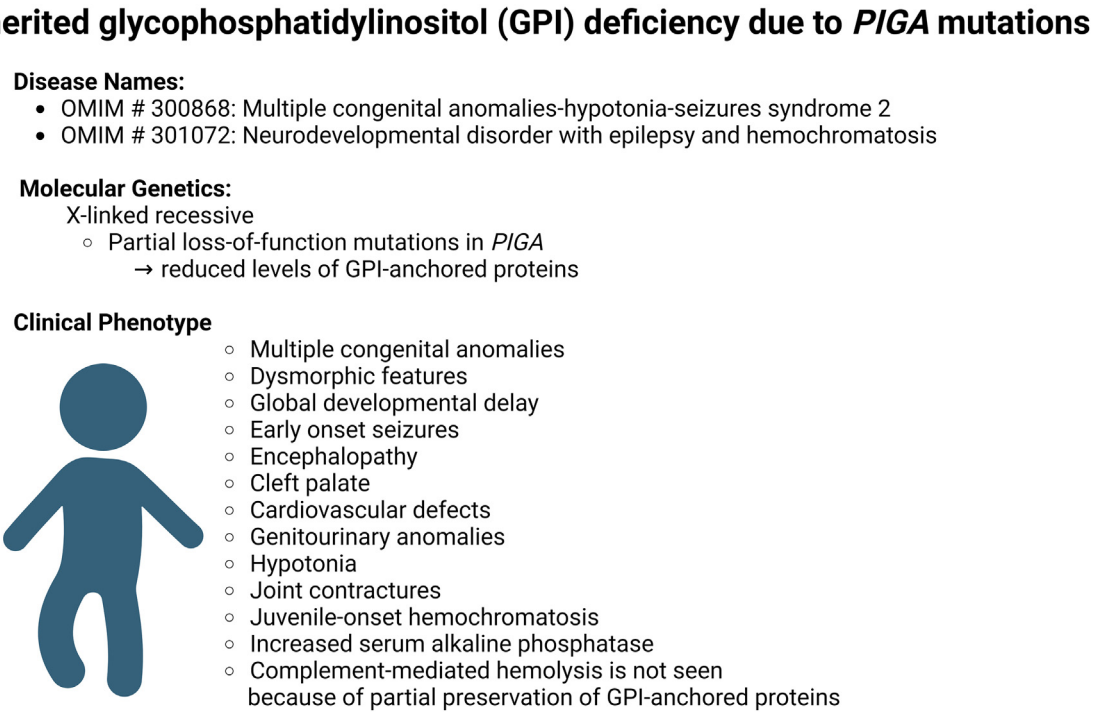

Figure 1. Graphical summary of the molecular genetics and clinical phenotype of X-linked inherited glycophosphatidylinositol (GPI) deficiency caused by loss-of-function *PIGA* mutations.

axons and glia), voltage-dependent calcium channel complex subunits (CACNA2D1–CACNA2D4), glial cell line-derived neurotrophic factor receptor, folate receptor, and an alkaline phosphatase (TNAP) that mediates metabolism of a GABA synthase cofactor B6 and other B vitamins. An abrupt, complete loss of these crucial molecules leads to profound CNS developmental abnormalities that are likely too severe to be reversed by postnatal PIGA delivery. Indeed, treated mice surviving to 17 days had gross structural CNS abnormalities of the corpus callosum and cerebellum.

Because human *PIGA*-IGD patients have only partial GPI-AP deficiency, their CNS defects are less severe, and postnatal delivery of *PIGA* would be more likely to meaningfully improve neurologic function. A recent proof-of-principle study used a mouse model of partial GPI-AP deficiency caused by mutations of the *PIGO* gene demonstrated improvements after postnatal *PIGO* delivery.[9] Encouragingly, despite modest levels of transgene expression after gene delivery, there were clear improvements in various neurologic phenotypes as well as weight and muscle mass. These results suggest that early gene therapy could be effective in improving neurologic phenotypes in patients with partial GPI-AP deficiency.

However, unanswered questions remain. Normal regulation of *PIGA* expression is largely unexplored, and whether forced overexpression of *PIGA* could lead to detrimental effects *in vivo* is unknown. Murakami et al.[1] noted that tissues expressing *hPIGA* in Cre^{+} $Piga^{+/-}$ mice had a compensatory reduction in expression of endogenous *mPIGA*, suggesting the existence of a feedback loop regulating *PIGA* expression. Despite effective *hPIGA* delivery, several GPI-APs were not restored after h*PIGA* delivery. This may be caused by varying requirements for optimal expression of different GPI-APs, which may involve a specific developmental window, competition with other GPI-APs, or requirements for optimal stoichiometry between PIGA to other proteins in the GPI biosynthesis pathway. As this study involved mice being treated with human *PIGA*, species-specific differences in PIGA function could play a role; however, *in vitro*, hPIGA can be substituted for *mPiga* to reconstitute GPI biosynthesis.[10] Future studies evaluating the level of gene correction needed for phenotypic improvement and the relationship between *PIGA* and various GPI-AP expression across tissues will be informative.

In conclusion, the results of Murakami et al.[1] highlight the opportunities for effective gene therapy for *PIGA*-IGD and underscore the importance of early correction of CNS defects (Table 1). Early therapy will require improved clinical recognition of IGD to enable prompt diagnosis. Gene therapy vectors must be effectively delivered to the CNS, while delivery to other organs may be beneficial for other systemic manifestations of IGD. AAV is expected to be lost over time due to dilution of the episomal vector with diminishing GPI-AP expression and clinical benefit. The choice of the vector, dose, delivery, tropism, and promoter all need to be carefully considered, including potential for

Table 1. Considerations for effective gene therapy for *PIGA*-IGD

Goals of therapy	Improvement of neurologic function and other systemic complications of *PIGA*-IGD
Optimal timing of therapy	early, neonatal, or *in utero*
Required tissue target	CNS
Other desired tissue delivery	systemic, to other affected organs (e.g., cardiovascular)
Gene replacement	*PIGA*
Evidence of successful gene delivery	restoration of adequate GPI-AP levels in the affected organs
Optimal duration of gene replacement	life-long
Potential risks and toxicities	insertional mutagenesis AAV related toxicities hepatitis dysregulated PIGA and GPI-AP expression (risks unknown)

insertional mutagenesis. Future studies exploring gene editing or potential regulatory elements of *PIGA* may offer alternative therapeutic strategies to increase *PIGA* expression, particularly if the efficiency of *in vivo* gene correction could be improved. The study by Murakami et al.[1] and advances in gene therapy offer new hope of future breakthroughs and a better future for IGD patients and their families.

DECLARATION OF INTERESTS

D.E.S. is a consultant for Poseida Therapeutics and Biomarin Pharmaceuticals and receives licensing royalties from Spark Therapeutics.

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