Genome Informatics) (the 492 amino acid mGlut1 sequence is SEQ ID NO:78). Mouse *Slc2a1* cDNA (NM 011400) is >97% identical to that of human *SLC2A1*cDNA. (See: Mueckler M et al, 1985; Veggiotti, P et al, 2013; Seidner et al, 1998).

Glut1 deficiency syndrome (Glut1 DS, OMIM 606777) is a rare but debilitating childhood neurological disorder caused by haplo-insufficiency of the *SLC2A1* gene. Glut1 deficiency syndrome is an autosomal-dominant disorder. The most prominent patient phenotype includes infantile seizures, acquired microcephaly, developmental delay and hypoglycoracchia (De Vivo D.C. *et al.* 1991).

Current treatments for the disease include the use of ketogenic diets, as ketone bodies form an alternative source of energy for neurons in the brain. However, the diet involves ingesting large quantities of oils and is reported to have only modest effects on neurobehavioral symptoms. There is an ongoing need for better treatments, especially for gene therapy to restore Glut1 expression in patients.

SUMMARY OF INVENTION

In certain embodiments, the present invention relates to a recombinant adeno-associated vector (rAAV) comprising a nucleic acid sequence comprising a transgene encoding Glut1. In certain embodiments, the Glut1 comprises SEQ ID NO:78 or 79. In certain embodiments, the rAAV further comprises a chicken Beta-actin promoter wherein the rAAV is capable of crossing the blood–brain barrier (BBB). In certain embodiments, the transgene is capable of being expressed in endothelial cells lining the brain microvasculature. In certain embodiments, the rAAV is AAV8 or AAV9. In certain embodiments, the rAAV further comprises miRNA elements selected from the group consisting of SEQ ID NO:48, 56, 59, 64, and 73. In certain embodiments, the rAAV further comprises inverted terminal repeats (ITRs) flanking the miRNA elements.

In certain embodiments, the present invention relates to a composition comprising any of the recombinant AAV's described herein. In certain embodiments, the composition further comprises a pharmaceutical carrier.

mouse Glut1 protein (mGlut1) (Accession No: NP_035530.2; SEQ ID NO:78) sharing 98 % homology. Glut1 exhibits 40% homology with other Gluts.

SLC2A1: Gene encoding the human glucose transporter 1 (hGlut1). Human *SLC2A1* (Accession No: NG_008232.1; gene ID – 6513).

Slc2a1: Gene encoding mouse Glut1 (mGlut1) (Accession No: Genomic #: NC_000070.6; gene ID - 20525).

GLUT1 DS: Glut1 deficiency syndrome

PND: post-natal day

PND3: post-natal day 3

EXAMPLES

Table 1—Recombinant Glut1 plasmids

EGFP-2A-Glut1 Constructs		
Human	pAAV CB6 PI EGFP-2A-hGlut1 (SEQ ID NO: 1) Key Features of Construct 5'ITR (SEQ ID NO: 2) CMV IE enhancer (SEQ ID NO: 3) CB promoter eGFP (SEQ ID NO: 5) 2A-linker (SEQ ID NO: 6) hGlut1 cDNA and 3'UTR (SEQ ID NO: 7) Poly A signal (SEQ ID NO: 8) 3' ITR (SEQ ID NO: 9)	Did not express hGlut1

mouse	pAAV CB6 PI EGFP-2A-mGlut1 (SEQ ID NO: 10) Key Features of Construct 5'ITR (SEQ ID NO: 11) CMV IE enhancer (SEQ ID NO: 12) CB promoter eGFP (SEQ ID NO: 14) 2A-linker (SEQ ID NO: 15) mGlut1 cDNA and 3'UTR (SEQ ID NO: 16) Poly A signal (SEQ ID NO: 17) 3' ITR (SEQ ID NO: 18)	Did not express mGlut1
Glut1-2A-EGFP Constructs		
human	pAAV CB6 PI hGlut1-2A-EGFP (SEQ ID NO: 19) Key Features of Construct 5'ITR (SEQ ID NO: 20) CMV IE enhancer (SEQ ID NO: 21) CB promoter hGlut1 cDNA (SEQ ID NO: 23) 2A-linker (SEQ ID NO: 24) eGFP (SEQ ID NO: 25) Poly A signal (SEQ ID NO: 26) 3' ITR (SEQ ID NO: 27)	Did not express hGlut1
mouse	*pAAV CB6 PI mGlut1-2A-EGFP SEQ ID NO:88*	Did not express mGlut1

	Key Features of Construct 5'ITR (SEQ ID NO: 89) CMV IE enhancer (SEQ ID NO:90) CB promoter eGFP (SEQ ID NO:92) mGlut1 cDNA and 3'UTR (SEQ ID NO: 93) Poly A signal (SEQ ID NO: 94) 3' ITR (SEQ ID NO: 95)	
Native Glut1 Constructs		
human	pAAV9-CB6 PI hGlut1 pAAV CB6 PI hGlut1 (SEQ ID NO: 28) Key Features of Construct 5'ITR (SEQ ID NO: 29) CMV IE enhancer (SEQ ID NO: 30) CB promoter hGlut1 cDNA (SEQ ID NO: 32) Poly A signal (SEQ ID NO: 33) 3' ITR (SEQ ID NO: 34)	**Expresses hGlut1**
mouse	pAAV9-CB6 PI mGlut1 pAAV CB6 PI mGlut1 (SEQ ID NO: 35) Key Features of Construct	**Expresses mGlut1**

	5'ITR (SEQ ID NO: 36) CMV IE enhancer (SEQ ID NO:37) CB promoter mGlut1 cDNA (SEQ ID NO: 39) Poly A signal (SEQ ID NO: 40) 3' ITR (SEQ ID NO: 41)	
Glut1-mir122 Constructs		
human	pAAV CB6 PI hGlut1-out3xmiR-122 BS (SEQ ID NO: 42) Key Features of Construct 5'ITR (SEQ ID NO: 43) CMV IE enhancer (SEQ ID NO: 44) CB promoter hGlut1 cDNA (SEQ ID NO: 46) 3'UTR (SEQ ID NO: 47) 3xmiR-122 BS (SEQ ID NO: 48) Poly A signal (SEQ ID NO: 49) 3' ITR (SEQ ID NO: 50)	hGlut1 expression TBD
Human	pAAV CB6 PI hGlut1-in3xmiR-122 BS (SEQ ID NO: 51) Key Features of Construct	hGlut1 expression TBD

	5'ITR (SEQ ID NO: 52) CMV IE enhancer (SEQ ID NO: 53) CB promoter hGlut1 cDNA (SEQ ID NO: 55) 3' UTR and 3xmiR-122 (SEQ ID NO: 56) Poly A signal (SEQ ID NO: 57) 3' ITR (SEQ ID NO: 58)	
mouse	pAAV CB6 PI mGlut1-in3xmiR-122 BS (SEQ ID NO: 59) Key Features of Construct 5'ITR (SEQ ID NO: 60) CMV IE enhancer (SEQ ID NO: 61) CB promoter mGlut1 cDNA (SEQ ID NO: 63) 3'UTR and 3x-miR122BS (SEQ ID NO: 64) Poly A signal (SEQ ID NO: 65) 3'ITR (SEQ ID NO: 66)	mGlut1 expression TBD
mouse	pAAV CB6 PI mGlut1-out3xmiR-122 BS (SEQ ID NO: 67) Key Features of Construct 5'ITR (SEQ ID NO: 68)	mGlut1 expression TBD

	CMV IE enhancer (SEQ ID NO: 69) CB promoter mGlut1 cDNA (SEQ ID NO: 71) 3'UTR (SEQ ID NO: 72) 3xmiR-122BS (SEQ ID NO: 73) Poly A signal (SEQ ID NO: 74) 3'ITR (SEQ ID NO: 75)	
Human EGFP construct		
	pAAV CB6 PI hGlut1-EGFP SEQ ID NO:80 Key Features of Construct 5'ITR (SEQ ID NO: 81) CMV IE enhancer (SEQ ID NO: 82) CB promoter eGFP (SEQ ID NO: 84) mGlut1 cDNA (SEQ ID NO: 85) Poly A signal (SEQ ID NO: 86) 3' ITR (SEQ ID NO: 87)	Expresses hGlut1

Recombinant AAV Construct Development

Four DNA constructs were generated carrying either the murine or human *SLC2A1* gene linked to the nucleotide cassette encoding green fluorescent protein (GFP) reporter as

cycle, but is also thought to provide essential anaplerotic substrates for the cycle as nutrients are eventually broken down to supply the cell's energy requirements. In brief, this experiment will involve treating mutants with (82mg/g) or without triheptanoin until they are administered the AAV9-Glut1 (pAAV9 CB6 PI mGlut1) construct. The different cohorts of mice will then be assessed as described above as a means of predicting the therapeutic outcome of restoring Glut1 expression in older patients on currently available (ketogenic diet) treatments.

Optimization of Glut1 constructs for clinical trials

Although no untoward effects of *slc2a1* expression in Glut1 DS mice have been observed to date, preliminary studies on a small sample of wild-type mice administered the construct pAAV9 CB6 PI mGlut1 vector (SEQ ID NO:35) indicated lowered CSF and blood glucose levels. This unexpected event could result from increased *slc2a1* expression in liver, a preferred AAV9 target organ, and consequently enhanced transport of glucose into this tissue. The net result is a fall in circulating glucose which is reflected in a hypoglycemic state. In anticipation of such an event, the hGlut1 construct (pAAV CB6 PI hGlut1) will be modified to preclude its expression at high levels in liver – an organ for which AAV9 has a particularly high tropism (Zincarelli et al, 2008; Pacak et al, 2006). To do so, binding sites (BS) for miRNA – miR-122 (expressed specifically in hepatocytes) will be introduced into constructs. This strategy has been successfully implemented previously (*See* Xie et al, 2011) and takes advantage of miRNA-mediated endonucleolytic cleavage of target mRNAs, thus restricting the expression of the transcript to tissues of interest. Such constructs shown in Fig. 1 D and Table 1 (pAAV CB6 PI hGlut1-in3xmiR-122 BS, pAAV CB6 PI hGlut1-out3xmiR-122 BS, pAAV CB6 PI mGlut1-in3xmiR-122, pAAV CB6 PI mGlut1-out3xmiR-122) will be tested in a subset of mice side-by-side with the original (unmodified) Glut1 expressing vectors, examining each for Glut1 expression levels and therapeutic efficacy. An increased tendency of the original construct to cause hypoglycemia will indicate the benefit of using the new Glut1-miRNA-BS constructs in subsequent experiments and trials.

To optimize expression of the test constructs described herein not just as a means of reducing viral titers during the manufacturing process, but also to address safety concerns associated with large concentrations of the virus, the *SLC2A1* and *slc2a1* genes will be evaluated using a codon optimization process using freely available software.

In addition, consensus Kozak sequences will be introduced into constructs as needed. Thus, any of the constructs or elements described in

CLAIMS

1. A recombinant adeno-associated vector (rAAV) comprising a nucleic acid sequence comprising a transgene encoding Glut1 and a chicken Beta-actin promoter and wherein the rAAV is capable of crossing the blood-brain barrier.

2. The recombinant AAV of claim 1, wherein the transgene encoding Glut1 is capable of being expressed in endothelial cells lining the brain microvasculature.

3. The recombinant AAV of claim 1, wherein the AAV is AAV8 or AAV9.

4. The recombinant AAV of claim 1, wherein the Glut1 comprises SEQ ID NO:78 or 79.

5. The recombinant AAV of claim 1, further comprising miRNA elements selected from the group consisting of SEQ ID NO:48, 56, 59, 64, and 73.

6. The recombinant AAV of claim 5, wherein the recombinant vector further comprises inverted terminal repeats (ITRs) flanking the miRNA elements.

7. A composition comprising the recombinant AAV of claim 1.

8. The composition of claim 7, further comprising a pharmaceutical carrier.

9. A kit comprising a container housing comprising the composition of claim 7.

10. The kit of claim 9, wherein the container is a syringe.

11. Use of the recombinant AAV vector of claim 1 for the manufacture of a medicament for treating Glut1 deficiency syndrome.

12. The use of claim 11, wherein the AAV is AAV8 or AAV9.

13. The use of claim 11, wherein the Glut1 comprises SEQ ID NO:78 or 79.

14. The use of claim 11, further comprising miRNA elements selected from the group consisting of SEQ ID NO:48, 56, 59, 64, and 73.

15. The use of claim 14, wherein the recombinant vector further comprises inverted terminal repeats (ITRs) flanking the miRNA elements.

16. The use of claim 11, wherein the recombinant AAV is comprised within a composition further comprising a pharmaceutical carrier.

17. An effective amount of the recombinant AAV vector of claim 1 for use in treating Glut1 deficiency syndrome.

18. The effective amount of the recombinant AAV vector for use of claim 17, wherein the AAV is AAV8 or AAV9.

19. The effective amount of the recombinant AAV vector for use of claim 17, wherein the Glut1 comprises SEQ ID NO:78 or 79.

20. The effective amount of the recombinant AAV vector for use of claim 17, further comprising miRNA elements selected from the group consisting of SEQ ID NO:48, 56, 59, 64, and 73.

21. The effective amount of the recombinant AAV vector for use of claim 20, wherein the recombinant vector further comprises inverted terminal repeats (ITRs) flanking the miRNA elements.

22. The effective amount of the recombinant AAV vector for use of claim 17, wherein the recombinant AAV is comprised within a composition further comprising a pharmaceutical carrier.