INVITED ARTICLE

New Therapeutic Approaches to Inherited Metabolic Pediatric Epilepsies

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Abstract

Treatment options for inherited metabolic epilepsies are rapidly expanding with advances in molecular biology and the genomic revolution. Traditional dietary and nutrient modification and inhibitors or enhancers of protein and enzyme function, the mainstays of therapy, are undergoing continuous revisions to increase biological activity and reduce toxicity. Enzyme replacement and gene replacement and editing hold promise for genetically targeted treatment and cures. Molecular, imaging, and neurophysiologic biomarkers are emerging as key indicators of disease pathophysiology, severity, and response to therapy.

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Glossary

AADC = aromatic L-amino acid decarboxylase; **AASA** = alpha-aminoadipic semialdehyde; **AAV** = adeno-associated virus; **ASOs** = antisense oligonucleotides; **CFDs** = cerebral folate deficiency syndrome; **CoA** = co-enzyme A; **cPMP** = cyclic pyranopterin monophosphate; **FDA** = Food and Drug Administration; **FRα** = folate receptor-alpha; **GABA** = γ-aminobutyric acid; **GHB** = GABA and γ-hydroxybutyrate; **GLUT-1** = glucose transporter 1; **IMD** = inherited metabolic disorder; **MMA** = methylmalonic acidemia; **MoCo** = molybdenum cofactor; **MRS** = MR spectroscopy; **mtDNA** = mitochondrial DNA; **MTHFR** = methylenetetrahydrofolate reductase; **MTHFS** = methyltetrahydrofolate synthetase; **P5P** = pyridoxal-5-phosphate; **PDE** = pyridoxine-dependent epilepsy; **PKU** = phenylketonuria; **PLP** = pyridoxal-5-phosphate (biologically active pyridoxine); **PNPO** = pyridox(am)ine 5′-phosphate oxidase; **PROSC** = proline synthesis cotranscribed; **SSADH** = succinic semialdehyde dehydrogenase; **SSADHD** = semialdehyde dehydrogenase deficiency; **TMS** = transcranial magnetic stimulation.

An inherited metabolic disorder (IMD) is defined when a clinical condition stems from a confirmed or assumed genetic (inherited or de novo) cause that leads to a primary alteration of a biochemical pathway. The International Classification of Inherited Metabolic Disorders includes 1,450 conditions, many of which are characterized by epilepsy being one of their predominant symptoms. The diagnostic and therapeutic approach to IMDs with epilepsy differs from that of epilepsy in general. An early diagnosis of these conditions may be crucial for dramatically improved outcomes. Treatable metabolic epilepsies are characterized by having a targeted treatment that can prevent or improve the epilepsy and developmental encephalopathy. Although the currently available treatments for many of these diseases remain supportive and symptomatic, the omics revolution has extended to a treatabolome of promising interventions including enzyme replacement therapy and gene therapy using DNA/RNA editing technology.[1] We present a clinical approach to traditional, revised, and potential targeted therapies for inherited metabolic epilepsies and consider complexities such as compensatory effects that present unique challenges.

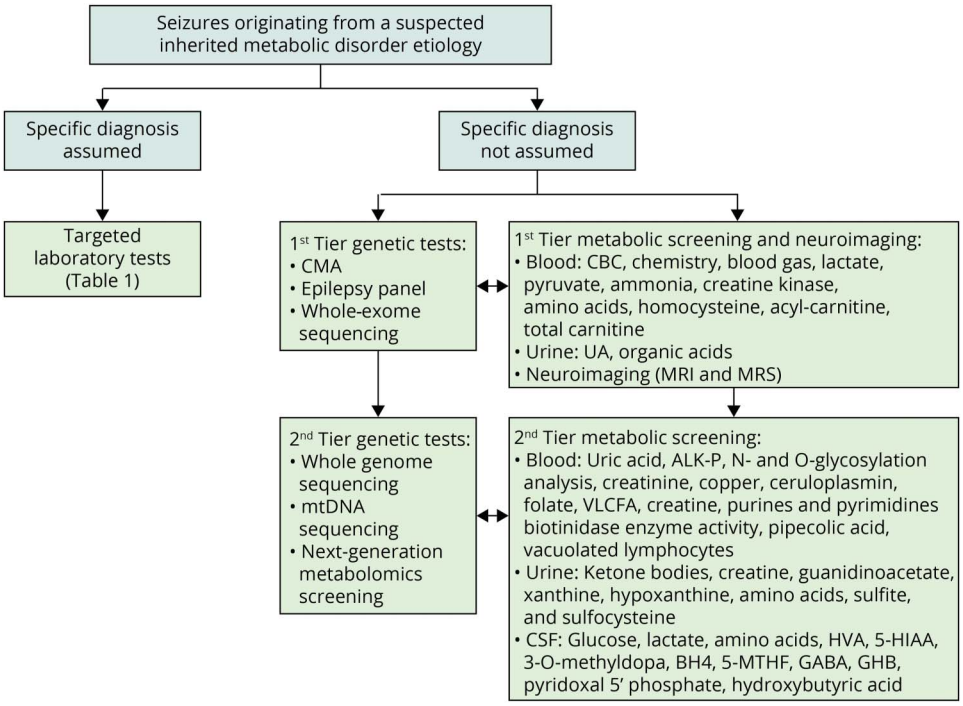
A Practical Classification and Approach to Inherited Metabolic Epilepsies

A clinically friendly classification scheme for IMDs is based on pathophysiology predominantly implicating small molecules, complex molecules, or defects of energy metabolism. The first category describes disorders in which *Small Molecules* accumulate (such as amino or organic acids or urea cycle intermediates) or are lacking (such as essential amino acids or fatty acids or vitamin dependency states). In the second, the accumulation or deficiency of poorly diffusible *Complex Molecules* (such as glycogen, phospholipids, and glycosaminoglycans) leads to progressive impairment. The latter category includes conditions that result from *Defects of Energy Metabolism,* such as mitochondrial or impairment of membrane carriers of energetic substrates. Clinical clues raising suspicion of an inherited metabolic epilepsy include early life onset, myoclonic seizures or seizures resistant to traditional antiseizure medicines, and other neurologic or systemic features including unexplained irritability, microcephaly, abnormalities of muscle tone, hearing defects, respiratory distress, vomiting, or hepatic dysfunction, in addition to hypoglycemia, lactic acidosis, or hyperammonemia. Notably, seizures can be precipitated by intercurrent illnesses, high protein intake, fasting, or prolonged exercise and follow a swift course that may resolve spontaneously or lead to severe deterioration. There can also be unexplained seizure-free periods that exist despite no antiseizure medication.

Small molecule disorders and disorders of energy metabolism usually present earlier, that is, the neonatal or infantile periods, and their acuity is generally based on the degree of deficiency. However, some IMDs that lead to acute encephalopathies in infancy may manifest instead at older ages with milder phenotypes. Metabolic seizures from Complex Molecule disorders usually arise in older children and adolescents. In these cases, the seizures, especially myoclonic, can be accompanied by dysmorphic features, developmental regression, ataxia, dystonia, rhabdomyolysis, vascular stroke, dysautonomia, or psychiatric symptoms.

After initial clinical assessment, if a specific diagnosis is considered, a targeted evaluation is recommended. A step-wise, tiered diagnostic approach is presented in Figure 1, depending on whether a specific diagnosis is suspected. The overall diagnostic yield of genetic testing is higher than metabolic tests in children with epilepsy.[2] However, basic metabolic tests that require only blood specimens, or dried blood spots and urine ("Tier 1," Figure 1), may direct toward a diagnosis more rapidly than genetic tests (e.g., hypoglycemia and hyperammonemia), allowing rapid initiation of life-saving treatment. In later stages, metabolic biomarkers can also indicate the severity of a condition for which a diagnosis is established. When it is logistically feasible, neuroimaging that includes a brain MRI and MR spectroscopy (MRS) should also be completed early in the diagnosis because it may provide insight to the nature of several IMDs. Depending on accessibility, genetic tests should include a chromosomal microarray analysis and next-generation sequencing methods such as epilepsy panels, whole-exome sequencing, and whole-genome sequencing. Analysis of the mitochondrial DNA (mtDNA) genome should also be considered. Novel technologies enable improved detection of mtDNA genome low-level heteroplasmy, point alterations, and deletions.[3] It if of importance that metabolic tests may also be useful as part of the

Figure 1 Diagnostic Approach to a Child With Suspected Metabolic Epilepsy



ALK-P = alkaline phosphatase; BH4 = tetrahydrobiopterin; CBC = complete blood count; CMA = chromosomal microarray analysis; GABA = γ-aminobutyric acid; GHB = γ-hydroxybutyrate; HVA = homovanillic acid; MRS = MR spectroscopy; mtDNA = mitochondrial DNA; UA = urinalysis; VLCFA = very long chain fatty acids; 3-OMD = 3-O-methyldopa; 5-HIAA = 5-hydroxyindole acetic acid; 5-MTHF = 5-methyltetrahydrofolate.

interpretation of variants of uncertain significance that may result from genetic sequencing-based tests.

Initial management is to provide cardiorespiratory support, reverse catabolic states, lessen the exposure to potential offending nutrients, and attempt to decrease the production and increase the excretion of offending metabolites. In the absence of a specific targeted therapy, antiseizure medications are indicated based on seizure semiology EEG, coupled with awareness of antiseizure medications that may lead to metabolic impairment. For example, valproic acid may interfere with mitochondrial beta-oxidation, cause hyperammonemia, and impede the function of glucose transporter 1 (GLUT-1), which may, respectively, induce or exacerbate epilepsies derived from mitochondrial diseases, urea cycle disorders, and GLUT-1 deficiency.

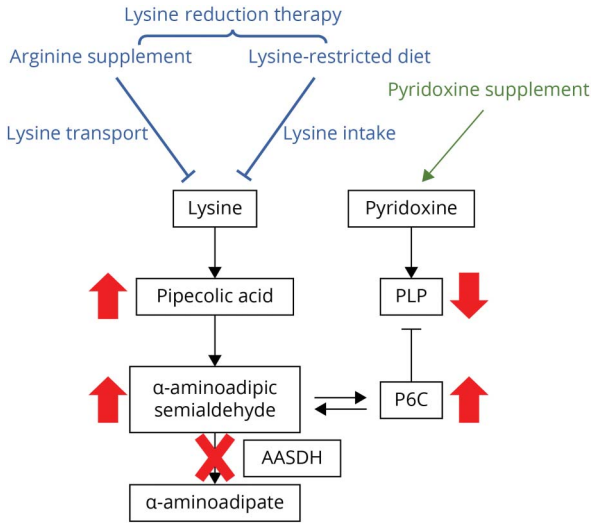
Literature Search

We conducted a systematic literature search of articles in English published in peer-reviewed journals available on "PubMed," "Embase," and "Web of Science" databases that sought to identify the keywords: inborn errors, inherited, metabolic, neurometabolic, epilepsy, seizures, treatment, therapy, management, novel, and update. This was followed by searching for keywords related to the treatment, therapy, or management of every disorder mentioned in this review and adding the keywords gene and enzyme. The citation list of the selected articles was also searched for, using the same keywords. If several publications reported a certain study, the study in which the exposure and outcome information were the most detailed was included.

Vitamin Responsive Disorders

The classic vitamin responsive inherited metabolic epilepsy is that of pyridoxine dependency, now parcellated into a variety of conditions, some known and others unexplained, including ALDH7A1 (antiquitin) deficiency, pyridox(am)ine 5′-phosphate oxidase (PNPO) deficiency, proline synthesis cotranscribed (PROSC) gene/pyridoxal 5′-phosphate binding protein deficiency, and congenital hypophosphatasia. While pyridoxine-dependent epilepsy (PDE) was first reported as a single case report in 1954, the underlying metabolic defect, an impairment in lysine catabolism caused by deficiency of alpha-aminoadipic semialdehyde (AASA) dehydrogenase (antiquitin), was reported 50 years later.[4] Furthermore, PNPO deficiency, involving an oxidase needed for the ultimate biosynthesis of the biologically active form of pyridoxine, pyridoxal-5-phosphate (P5P), was elucidated as a disorder typically requiring the administration of P5P.[5] Deficiency of the PLP-binding protein, PROSC, was later recognized as a rare pyridoxine-responsive epileptic encephalopathy.[6] Untreated seizures stemming from these conditions begin early in life and may present dramatically, with prolonged focal, generalized, atonic, and myotonic seizures, status epilepticus, and infantile spasms. Early studies demonstrated that seizures respond to pyridoxine administration in antiquitin deficiency, but evidence of encephalopathy persists. Outcome data in a series of 24 patients treated with pyridoxine showed that over half demonstrate abnormal neurologic examinations, including abnormal IQ (<85) in 75% and progressive ventriculomegaly in nearly half.[7] Consensus guidelines recently published for PDE management recommended a triple-therapy regimen whereby

Figure 2 Pathway Diagram Illustrating PDE Pathology and Its Management



PDE is caused by the deficiency of AASDH (or Antiquitin Gene ALDH7A1), essential for lysine metabolism. The absence of AASDH results in accumulation of lysine metabolites, including pipecolic acid and α-aminoadipic-semialdehyde and δ-piperideine-6-carboxylate, which sequesters pyridoxal-5-phosphate, the biologically active form of pyridoxine. PDE management involves a three-pronged approach (triple therapy), including pyridoxine supplement and lysine reduction therapy through a lysine-restricted diet and arginine supplementation (inhibiting lysine transport). AASDH = α-aminoadipic semialdehyde dehydrogenase (antiquitin); PDE = pyridoxine-dependent epilepsy; PLP = pyridoxal-5-phosphate (biologically active pyridoxine); P6C = δ-piperideine-6-carboxylate.

pyridoxine 15–30 mg/kg/day (maximum 300 mg/day in infants and 500 mg/day in children and adults to avoid peripheral neuropathy and dorsal root ganglionopathy) is supplemented by a lysine-restricted diet and arginine supplementation 200–600 mg/kg/day[8] (Figure 2). These additional interventions are rational given the underlying defect in lysine degradation and the ability of arginine to compete with lysine for intestinal absorption, transport across the blood-brain barrier, and mitochondrial entry. Additional notable recommendations are to monitor plasma amino acids and the intermediate levels, pipecolic-6-carboxylate, and AASA, as well as doubling the pyridoxine dose to 60 mg/kg/day during seizure relapses or febrile illnesses. Data are emerging to support early use of lysine-restricted diet therapy to improve neurodevelopmental outcomes in PDE, based on retrospective assessments and comparisons between early vs late-treated affected siblings.[9] In the case of PNPO deficiency, reliance on dietary therapy, with P5P administered as a food supplement, has led to unforeseen complications because of variations in quality, inactivation due to light exposure, and unregulated dosing, emphasizing the need for pharmaceutical grade therapy[10] (based on pure materials meeting the pharmaceutical standards for manufacturing, as opposed, for example, to substances with "no grade," which are unregulated, or "food grade," which are permitted for human ingestion, but may contain fillers, binders, or other unknown substances).

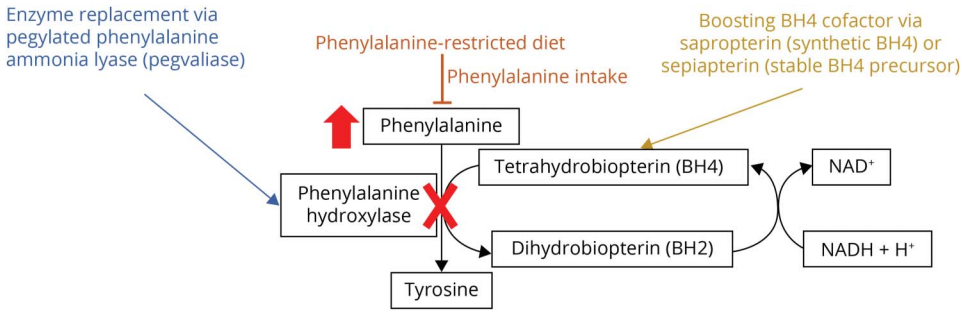
Cerebral folate deficiency syndrome (CFDs) refers to a group of conditions characterized by a cerebral deficiency of the physiologically active form of folate, 5-methyltetrahydrofolate, in the presence of normal levels of peripheral total folate. The clinical phenotype commonly includes neurodevelopmental deficits, megaloblastic anemia, leukoencephalopathy, and myoclonic seizures.[11] Primary etiologies of CFDs include function alterations of the *FOLR1* gene coding for folate receptor-alpha (FRα) and IMDs in which there is a deficiency of methylenetetrahydrofolate reductase (MTHFR), dihydrofolate reductase, and 5,10 methyltetrahydrofolate synthetase (MTHFS). Folate deficiency can also be secondary to other IMDs, such as mitochondrial disorders (especially Kearns-Sayre syndrome) and serine deficiency, and due to FRα autoantibodies that hinder the transport of folate into the cerebrum. Treatment approaches for the primary causes of CFDs include folinic acid, betaine in the case of MTHFR deficiency, and 5-methyltetrahydrofolate and intramuscular methylcobalamin in the case of MTHFS deficiency.[11]

Complementary to classic vitamin-sensitive disorders, new vitamin-responsive neurometabolic disorders continue to be identified. While classic biotinidase deficiency, with a phenotype of developmental impairment, hypotonia, focal or generalized tonic-clonic seizures, ataxia, and cutaneous lesions, including alopecia, responds to biotin dosing of 10 mg daily, holocarboxylase synthetase deficiency and biotin responsive basal ganglia disease are also biotin responsive. Furthermore, the latter was identified as a thiamine transportopathy and renamed biotin-thiamine responsive basal ganglia disease.[12] Other diagnoses have emerged simulating these conditions phenotypically and biochemically, for example, thiamine phosphokinase deficiency as biotin-responsive basal ganglia disease,[13] and sodium-dependent multivitamin transporter deficiency, responsive to biotin and pantothenic acid.[14]

Transport Defects

Transportopathies are an important and potentially treatable group of neurometabolic conditions and range from cerebral folate to creatine, manganese, and, as noted, thiamine transporter deficiencies.[15-18] GLUT-1 deficiency, a prototype of brain energy failure with phenotypic heterogeneity largely associated with age at onset, can manifest with various types of seizures, including absence, focal, generalized myoclonic, clonic, tonic, and nonconvulsive status epilepticus. Treatment for GLUT-1 deficiency includes a set of future interventions beyond ketogenic diet. Triheptanoin, a seven-carbon triglyceride and anaplerotic agent, was proposed as an alternative therapy to the ketogenic diet because it is metabolized to acetyl-co-enzyme A (CoA), thus priming the tricarboxylic acid cycle, as well as propionyl CoA, a source of C3 ketone bodies. Despite initial promising reports, triheptanoin has not been effective thus far in phase 3 clinical GLUT-1 deficiency clinical trials. Small molecules and biologics to increase transport expression, such as alpha lipoic acid (thioctic acid) or knockdown of insulin-like

Figure 3 Pathway Diagram Illustrating PKU Pathology and a Variety of Viable Therapeutic Approaches



PKU is caused by the deficiency of phenylalanine hydroxylase, an enzyme responsible for the conversion of phenylalanine (Phe) to tyrosine, leading to pathologic accumulation of brain Phe and CNS damage. Classical PKU treatment paradigm involves the dietary restriction of Phe intake. Pharmacologic boosting of phenylalanine hydroxylase cofactor BH4 through sapropterin or sepiapterin is responsive in certain patients with PKU. Enzyme replacement therapy (pegylated phenylalanine ammonia lyase) is available for adult patients with uncontrolled blood Phe levels. BH4 = tetrahydrobiopterin; PKU = phenylketonuria.

growth factor 1, are under investigation as well as viral vector-mediated gene replacement.[19]

Aminoacidopathies

Untreated individuals with phenylketonuria (PKU) develop profound intellectual deficiency, seizures (generalized tonic-clonic, myoclonic, and epileptic spasms), spasticity, and microcephaly. However, well-treated classical PKU individuals can develop normally. PKU illustrates the combination of classical and newer metabolic therapeutic approaches. The mainstay of treatment remains nutritional intervention, using a phenylalanine (phe)–restricted diet. Other approaches include supplementation with large neutral amino acids (other than phe) that block phe transport into the brain and neutral amino acid transporter inhibitors that inhibit phe intestinal absorption and reuptake by the proximal kidney. Although some patients respond to sapropterin, a synthetic formulation of the cofactor tetrahydrobiopterin, its precursor sepiapterin may be biologically more active and is in development.[20] Furthermore, enzyme replacement efficacy has been enhanced by pegylation, the process of attaching polyethylene glycol moieties to create synthetic replacement enzymes. For example, the enzyme phenylalanine ammonia-lyase is formed from recombinant bacteria. The pegylated form, pegvaliase, keeps the enzyme from breaking down and decreases immunogenicity. Pegvaliase is effective in reducing blood phe levels along with sustained improvements in neuropsychiatric outcomes and was approved by the Food and Drug Administration (FDA) in 2018 as a novel enzyme therapy for adults with PKU and uncontrolled phe levels[21] (Figure 3). Finally, there have been successful preclinical trials implementing mRNA-based gene therapy,[22] and there are ongoing gene replacement clinical trials for PKU (ClinicalTrials.gov; NCT04480567 and NCT03952156).

Homocystinuria can be caused by impairments of cystathionine β-synthase (a vitamin B_6-dependent enzyme), methylenetetrahydrofolate reductase, or methionine synthase but also by defects in metabolic pathways that result in methylmalonic acidemia (MMA) and by deficiencies of vitamins B6, B_{12}, and folate. These metabolic imbalances may lead to ectopia lentis, osteoporosis, brittle and thin skin, thromboembolism, stroke, intellectual disability, and seizures (generalized tonic-clonic, focal, and infantile spasms). Depending on the etiologic abnormality, dietary treatment aims to reduce hyperhomocysteinemia. Individuals with cystathionine β-synthase deficiency, the most common type, may be vitamin B_6 responsive. For those who are not, treatment consists of a methionine-restricted diet with cysteine supplementation. The methylator betaine may also lower homocysteine levels.[23] Chemical modification of human cystathionine β-synthase is the basis of a novel enzyme replacement therapy. Preclinically, it has shown a capacity to reduce the homocysteine levels and the clinical symptoms,[24] and there are ongoing clinical trials (ClinicalTrials.gov; NCT03406611) assessing its efficacy. In addition, preclinical studies of an adeno-associated virus (AAV)–based gene therapy have shown an ability to increase the enzymatic activity of cystathionine β-synthase and lead to clinical improvement.[25]

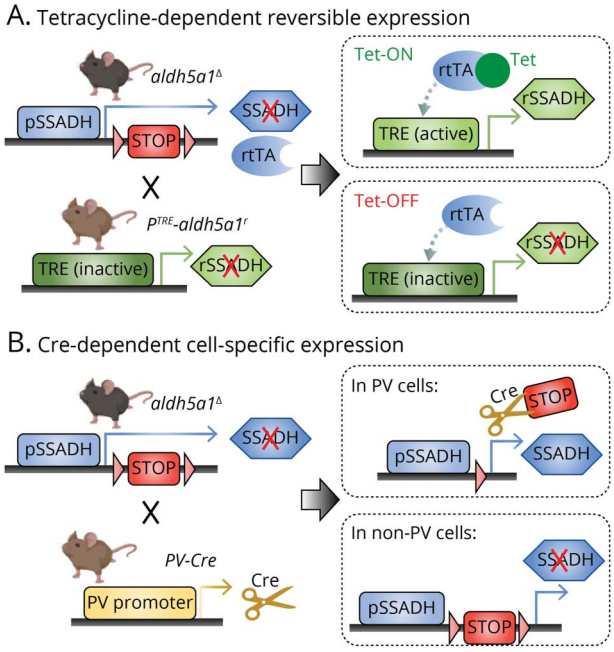
Organic Acidemias

There are several genetic defects that can lead to MMA, which can be isolated or joined by increased homocysteine because both are processed by enzymes that require vitamin B_{12}. Isolated MMA results from defects in methylmalonyl-CoA mutase and, depending on the residual protein function, can present early or late in life. The clinical phenotype also varies in the severity of developmental delay, megaloblastic anemia, and recurrent seizures. Therapy is based on preventing or reversing catabolism, restriction of precursors, intermediate toxic metabolite scavenging, and supplementing deficient cofactors and products. Liver transplantation is optional when conventional treatment fails.[26] In recent years, several preclinical genetic-based therapeutics for MMA have resulted in favorable outcomes,[27] and there are ongoing clinical trials investigating these approaches (ClinicalTrials.gov; NCT04581785, NCT04899310, and NCT05506254).

Lysosomal Storage Diseases

The lysosomal storage disease late infantile neuronal ceroid lipofuscinosis 2 results from autosomal recessive pathogenic variants of the *TPP1* gene encoding the enzyme tripeptidyl

Figure 4 Dual Molecular Strategy Studying SSADH (*aldh5a1*) Restoration in Mice



(A) Tetracycline-dependent SSADH expression is achieved through breeding of (1) a mouse expressing an inactive rtTA in lieu of normal SSADH (*aldh5a1*$^{\Delta}$) and (2) a mouse expressing a recombinant SSADH under an inactive TRE promoter (*P*^{TRE}-*aldh5a1*^{r}). The resultant mouse is responsive to tetracycline (Tet) administration, resulting in bidirectional recombinant SSADH expression toggling between Tet-ON or Tet-OFF configurations. (B) Cre-dependent cell-specific SSADH expression is achieved through breeding *aldh5a1*$^{\Delta}$ mouse with Cre-expressing mouse regulated by a cell-specific promoter, for example, PV-Cre. The resultant mouse reactivates the endogenous *aldh5a1* gene expression only in desired cell types and therefore is an ideal tool to investigate cell-specific enzyme restoration effects for symptom improvement. Relevant promoters might be included in the eventual AAV vector design. AAV = adeno-associated virus; rtTA = reverse tetracycline–controlled transactivator; SSADH = succinic semialdehyde dehydrogenase; TRE = tetracycline response element.

peptidase 1 and is associated with severe myoclonic epilepsy, retinopathy, and cognitive regression. Disease onset is between age 2 and 4 years. Recombinant human TPP1 proteoenzyme therapy known as cerliponase alfa was approved by the FDA in 2017 for intrathecal administration through intraventricular delivery,[28] with resultant stabilization of disease.

Purine/Pyrimidine Metabolism Disorders

The outlook for type A molybdenum cofactor (MoCo) deficiency improved dramatically with the substitution of the intermediate cyclic pyranopterin monophosphate (cPMP), the molecule after the enzymatic block. MoCo deficiency and isolated sulfite oxidase deficiency are related disorders affecting xanthine and sulfite metabolism and present as severe neonatal onset epileptic encephalopathies with progressive encephaloclastic processes. Molybdenum-dependent enzymes include sulfite oxidase, xanthine oxidase, nitrate reductase, and nitrogenases. Laboratory features of MoCo deficiency are hypouricemia and hypouricosuria, with elevated urine sulfites, S-sulfocysteine, xanthine, and hypoxanthine. Imaging shows lesions of the globus pallidi and subthalamic nuclei, cerebral infarctions, pontocerebellar hypoplasia, and progressive encephalomalacia. While there are 3 types of MoCo deficiencies, type A represents a block in the catabolism of GTP to cPMP. Accordingly, cPMP substitution led to rapid clinical and laboratory responsiveness in 11 neonates with type A MoCo deficiency with dramatic improvement in parenchymal imaging compared with the natural history of the condition and was followed by FDA approval in 2021.[29]

In another treatable disease example, the pyrimidine uridine is a key therapeutic in deficiency of a trifunctional protein acronymized as CAD: carbamoyl phosphate synthetase, aspartate transcarbamylase, and dihydroorotase. This enzyme catalyzes the first 3 of the six-step pyrimidine synthesis pathway and, when deficient, causes a lethal global developmental disorder associated with epilepsy (mostly generalized, including absence seizures) and anemia with anisopoikilocytosis having onset in the first year of life. As pyrimidines can be recycled from uridine, its replacement is a rational therapy that reverses the neurologic and hematologic manifestations of CAD deficiency.[30]

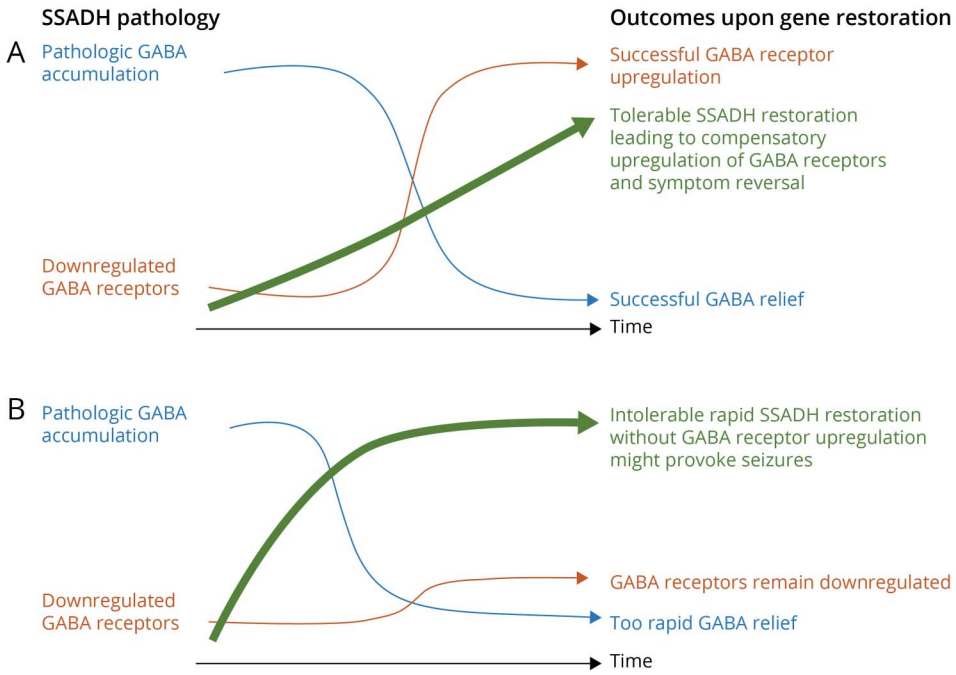
Mitochondrial Diseases

Mitochondrial diseases commonly affect tissues with high energy utilization, such as the brain, and therefore, their multisystem clinical presentation often includes seizures. Numerous mtDNA or nuclear alterations lead to a heterogeneous phenotype. Epilepsy, a poor prognostic factor, is frequently associated with conditions such as pyruvate dehydrogenase deficiency, mitochondrial encephalomyopathy, lactic acidosis, stroke-like episodes, myoclonic epilepsy with ragged red fibers syndrome, *POLG*-related disorders (e.g., Alpers-Huttenlocher syndrome, myoclonic epilepsy myopathy sensory ataxia, and spinocerebellar ataxia with epilepsy), *RARS2* alterations (that also lead to pontocerebellar hypoplasia type 6), and disorders of impaired mtDNA maintenance such as coenzyme Q_{10} synthesis defects. Current management of mitochondrial epilepsies is challenging and commonly involves attempts of multiple conventional antiseizure medications, ketogenic diet, and vagus nerve stimulation with limited efficacy. Thiamine, riboflavin, biotin, niacin, and coenzyme Q_{10} supplementation may have some benefit in cofactor deficiency states.[31] However, there have been recent developments of therapeutic approaches for mitochondrial diseases. Acipimox, bezafibrate, and omaveloxolone have the capacity to increase the cellular concentration of NAD^+ and improve oxidative metabolism of individuals with mitochondrial depletion syndromes.[32] Other compounds which enhance the functionality of the oxidative phosphorylation mechanism are antioxidants such as cysteamine and other medications such as nab-sirolimus and dichloroacetate.[33,34] Investigational treatment approaches include cell replacement therapy, mitochondrial augmentation therapy, hypoxic therapy, gene therapy, mtDNA base editing, and mitochondrial replacement therapy.[35]

Pediatric Neurotransmitter Disorders

Gene therapy is rapidly advancing in pediatric neurotransmitter disorders. The clinical conditions stratify between those affecting the synthesis, recycling, and degradation of the

Figure 5 Derisking SSADH Restoration



SSADH is characterized by pathologic GABA accumulation and compensatory GABA receptor downregulation. In scenario A, gradual enzyme restoration is strategized to achieve hyperGABAergic relief, gradual upregulation of GABA receptors, and symptomatic improvement. In scenario B, overly rapid enzyme restoration may be deleterious because of an abrupt decrease in GABA levels, lack of GABA receptor upregulation, and exacerbation of seizures. GABA = γ-aminobutyric acid; SSADH = succinic semialdehyde dehydrogenase.

biogenic amines, leading to the synthesis of the catecholamines and serotonin vs γ-aminobutyric acid (GABA). Aromatic L-amino acid decarboxylase (AADC) deficiency affects dopamine and serotonin synthesis. It results in a profound extrapyramidal syndrome with hypotonia, dystonia, ptosis, and oculogyric crises, in addition to autonomic, cognitive, and mood dysfunction. Although relatively rare, generalized or focal onset seizures may also occur. Direct parenchymal administration of AAV-mediated AADC gene therapy has demonstrated safety, efficacy, and restoration of enzymatic activity in 7 children aged 4–9 years into the substantia nigra and ventral tegmental area[36] and in 26 patients aged 1–8 years into the putamen.[37]

Succinic semialdehyde dehydrogenase deficiency (SSADHD) is ultra-rare but the most common inherited disorder of GABA metabolism. Biallelic pathogenic variants in the *ALDH5A1* gene lead to the pathologic accumulation of GABA and γ-hydroxybutyrate (GHB), a neuroactive byproduct that serves as a hallmark of diagnosis in physiologic fluids. Patients typically manifest profound neurodevelopmental impairment with intellectual disability and prominent defect in expressive language, hypotonia, obsessive-compulsive disorder, and in half, generalized, or focal onset epilepsy. Experience with conventional antiseizure medications has mostly shown success with lamotrigine, carbamazepine, oxcarbazepine, or levetiracetam, either as monotherapy or in combination with clobazam.[38] Valproic acid, which has an inhibitory influence on semialdehyde dehydrogenase (SSADH), is not advised, and vigabatrin, an irreversible inhibitor of GABA transaminase, has shown conflicting evidence of success.[39] Early studies of $GABA_A$ receptor expression and function in the SSADHD animal model using radiolabeled binding, immunohistochemistry, and hippocampal slice electrophysiology showed downregulation of activity.[40] Similarly, binding and immunoblot studies in the murine model showed decreased $GABA_B$ receptor-mediated function.[41] This compensatory downregulation of $GABA_A$ and $GABA_B$ receptor activity was then demonstrated in clinical studies using flumazenil PET[42] and paired-pulse transcranial magnetic stimulation (ppTMS[43]). These compensatory properties led to the concept that an "on-demand" model of enzymatic restoration is necessary to identify the safe and effective rate of enzyme restoration in advance of clinical trials.[44]

Biomarkers in Neurometabolic Disease

Metrics of disease severity and target engagement by novel therapies are emerging as important needs in neurometabolic disease. The use case for such objective measures is especially apparent for gene therapies where serum and CSF drug concentrations are not relevant. Neurometabolic biomarkers can be coarsely divided into molecular, imaging, and neurophysiologic metrics that reflect either disease severity or treatment efficacy. For neurometabolic diseases, blood, urine, and CSF molecular biomarkers are disease hallmarks and can reflect disease severity as well as success of treatment. Anatomic imaging biomarkers by MRI can show abnormalities in many of the above listed disorders, although the mechanistic link from disease to MRI findings such as cerebral atrophy or

T2 basal ganglia hyperintensity is often unclear. By contrast, MRS can provide noninvasive measures of metabolite deficiencies or of the build-up of reactants in cases of primary or secondary enzyme deficiencies (e.g., GABA[45]).

Complementary to molecular and imaging biomarkers, neurophysiologic measures provide functional CNS measures. By conventional EEG, seizure susceptibility can be estimated in the neurometabolic epilepsies, and response to treatment can be apparent in real time to certain extent in some disorders such as PDE. By EEG recorded with a provoking stimulus, novel measures such as the auditory steady state response can provide insight into network functions, particularly those that are dependent on intact corticothalamic GABAergic inhibition.[46]

Another set of emerging biomarkers can be obtained by transcranial magnetic stimulation (TMS) of the motor cortex. In TMS, small intracranial electrical currents are induced by a fluctuating extracranial magnetic field. When such noninvasive cortical stimulation is delivered to the motor cortex, cortical excitability can be measured by recording the size of the elicited motor evoked potential which can be recorded by surface electromyogram electrodes placed usually on the hand muscles. A range of TMS protocols reflect either aggregate excitability or, selectively, GABAergic cortical inhibition or glutamatergic cortical excitation. Given the vulnerability of the GABA signaling system to many metabolic disturbances in the abovementioned syndromes, one such family of TMS protocols, termed ppTMS, has emerged as a means to detect deficiencies in cortical inhibition and potentially response to therapy.[47]

Genetically Targeted Interventions

The genomic revolution affords opportunities to target a specific gene, nucleic transcripts, or polypeptides for more precise treatment of otherwise very challenging disorders to ameliorate or reverse. The typically early age at onset, effects of progression over time, and resistance to traditional therapies render seizures stemming from IMDs among the most refractory to successful therapeutic intervention. In addition, physiologically driven compensatory responses, while inadequate to meet the challenges of the underlying disorder, render treatments even more complicated. Receptor downregulation, for example, in response to a chronically elevated level of an endogenous neurotransmitter or metabolite may lead to undesirable secondary and downstream effects of interventions targeting the metabolic error.

Gene therapy achieves a therapeutic benefit by altering the biological function of an individual's genetic code. Gene therapy can be used by in vivo or ex vivo methods. In vivo refers to the direct injection of a vector that encodes the gene of interest or gene editing tools into the circulation or to a specific tissue. Its main limitations involve off-target biodistribution and immunogenicity elicited against the vector.[48] Ex vivo pertains to the genetic modification of an individual's own cells outside the body, by gene editing or supplementation, followed by autologous engraftment of those cells. Integrating methods (whereby the transgenic DNA permanently exists in the cell genome and continues to future cell progeny) can be achieved by modifying the host genome, for example, with a lentiviral vector. Conversely, nonintegrating methods can be applied with vectors such as the AAV. The main complications arising from these modalities are that some cells are difficult to culture and do not have a reasonable engraftment rate, and in the case of hematopoietic stem cells, conditioning through chemotherapy is necessitated, which can lead to adverse effects that are associated with immunosuppression.[49]

Antisense Oligonucleotides

Antisense oligonucleotides (ASOs) are short (16–24 nucleotide), single-stranded nucleic acids designed to bind mRNA by sequence complementarity. Their mechanisms of action are to knock down target mRNAs, for example, to suppress a mutated, toxic gene or alter mRNA splicing, for example, to rescue a mis-spliced gene. Genetic alterations amenable to ASO therapy create an interfering splice site, weakening but not destroying a required splice site. In metabolic therapies, a proof-of-concept was demonstrated in CLN7, an ultra-rare form of neuronal ceroid lipofuscinosis.[50] ASO therapy is in development for other metabolic disorders, including 6-pyruvoyl-tetrahydrobiopterin synthase deficiency; Canavan disease; CDG Ia; Fabry disease; Gaucher disease; methylmalonic and propionic acidemia; mucopolysaccharidoses I, II, and III; Niemann-Pick type C; PKU; Pompe disease; primary hyperoxaluria; and pyridoxine-dependent epilepsy.[51]

Preclinical Studies in Gene Therapy

Animal (mainly mouse) disease models play key roles in targeted therapy development, particularly for testing pipeline therapeutics and developing clinically relevant biomarkers. For example, clinical biomarkers under investigation in the current SSADHD Natural History Study,[45] including biochemical, neuroimaging, and electrophysiologic data, have clear counterparts in murine studies. Elevated GABA and GHB are biochemical, clinical hallmarks of SSADHD[52] and are elevated in the SSADH knockout mouse model. Furthermore, a proof-of-concept study using AAV vectors in mice to restore the enzyme led to successful GABA and GHB reduction.[53] Other findings in patients using EEG,[45] MRI,[54] and TMS measures[55] can be further developed using animal models. In SSADHD (and likely many other syndromes), preclinical models also offer an opportunity to design therapies that accommodate compensatory changes that accompany a primary metabolic deficit. For example, recent work for developing SSADH-targeted therapy uses an inducible and reversible genetic rescue strategy in mice.[44] This versatile animal model system combines Tet-on/Tet-off methodology[56] and Cre-dependent recombination capability[57]

allowing "on-demand" enzymatic restoration (Figure 4). Using this model, investigators can titrate enzyme replacement to accommodate compensatory GABA receptor downregulation in SSADHD. That is, a mouse model enables explicit testing of whether seizures are provoked by SSADH restoration, which, if too rapid, can lower ambient GABA levels in the setting of depressed GABA receptors and thus provoke seizures (Figure 5). In addition, cell-restricted restoration efficacy might allow further fine tuning of gene therapy vector design lowering toxicity or long-term risk, including oncogenic potential.[58] Genetically modified mice expressing Cre recombinase in specific cell types[59] can be used to investigate relevant cell types in the brain that are ideal for targeted gene restoration. Furthermore, specific brain structures including subcortical and hippocampal regions relevant for SSADH expression might be interrogated using Cre lines that exhibit brain structure specificity.[60] Favorable results from these genetic cross experiments will give insights into the design of AAV vectors including the use of cell-specific promoters for targeted expression and the consideration of relevant delivery methods for maximized efficacy with minimal off-target effects. Careful mouse disease model design enables the derisking of an eventual clinical therapy.

Conclusions

Given recent preclinical and clinical diagnostic and therapeutic advances, we are solidly in the era where symptom relief prospects and targeted therapeutics are realistic for many patients with epilepsies caused by IMDs. There are ongoing improvements in treatments based on diets, provision of cofactors or their precursors, and stimulation of alternative metabolic pathways that result in fewer toxic metabolites. Metrics of biochemical, neurophysiologic, and imaging modalities emerge as biomarkers that can determine pathophysiologic processes, disease severity, and response to therapy.

Furthermore, novel and precise therapies, such as wild type or modified enzyme replacement, gene editing or expression, and correction of oligonucleotide missplicing, provide an exciting curative potential for these unique conditions.

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