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Opinion

Tooth Hypoplasia for Differential Diagnosis of Childhood Epilepsy Associated with SLC13A5 Mutations -

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ABSTRACT

Epilepsy remains one of the most common neurological conditions affecting both children and adults. Various genes have been linked to epileptic conditions, which has unveiled, in many cases, mechanistic aspects of epileptogenesis and also lead to development of appropriate treatment strategies. However, neonatal epilepsy continues to be a significant problem, both in terms of understanding the pathogenic mechanisms and designing effective therapies. Early Infantile Epileptic Encephalopathy (EIEE) occur within the first month of life when the brain is most vulnerable and its symptoms least likely to be recognized. Recently, loss-of-function mutations in human SLC13A5, a Na⁺-coupled transporter for citrate in neuronal cells, have been linked to a unique form of EIEE. Patients with SLC13A5 mutations present with delayed motor development, impaired speech and language, and seizures. More importantly, there are significant defects in teeth (hypoplasia) and bone (osteopenia) development, not seen in children with other forms of EIEE. Teeth and bone contain high levels of citrate where it plays an obligatory role in mineralization. It is likely that SLC13A5 is essential for citrate accumulation in these tissues and that loss-of-function mutations in the transporter leads to defective mineralization of bone and teeth. As EIEE can arise from mutations in different genes, the exact genes involved cannot be predicted just based on epileptic phenotype. We propose that tooth hypoplasia is a telltale sign of defective function of the citrate transporter SLC13A5 and that this easily observable phenotype is unique to children with EIEE arising from loss-of-function mutations in this gene. Therefore, tooth hypoplasia could be very useful in the differential diagnosis of EIEE associated with SLC13A5 mutations, thus being of great practical value in the clinics in the selection of appropriate treatment strategies for the affected children.

INTRODUCTION

Epilepsy remains as one of the most common neurological conditions; it often leads to developmental delay in children [1,2]. Pathogenesis of epilepsy involves a variety of genes and environmental factors; but in many cases, mutations in specific voltage-gated or ligand-gated ion channels are linked directly to epilepsy [3-6]. The discovery of genetic mutations in specific genes involved in the onset of epilepsy has improved our understanding of pathogenic mechanisms in the development of epilepsy and also has led, in some cases, to effective treatments. However, neonatal epilepsy remains a serious threat to long-term cognitive health of newborns; it is characterized by epileptic episodes occurring within the first month of life when the brain is most susceptible to damage and requires immediate diagnosis and treatment [7-9]. Affected children present with a wide variety of seizures and behaviors including subtle seizures, tonic seizures, clonic seizures, myoclonic seizures, and non-paroxysmal repetitive behaviors. Additional symptoms include horizontal deviation of the eyes, eyelid blinking or fluttering, sucking, and swimming movements. Despite the significant mortality (15%) and morbidity (30%), 50% of neonates with seizures will return to normal while about one-third of the patients will continue to have epilepsy. Because of the occurrence of seizures early in life and frequent association of this condition with compromised development of brain functions, this class of diseases is identified as Early Infantile Epileptic Encephalopathies (EIEE) [10,11]. Among this group is a recently discovered form of neonatal epilepsy, which results from loss-of-function mutations in SLC13A5, a Na⁺-coupled transporter for citrate expressed in the plasma membrane of specific tissues and cell types, including neurons [12].

SLC13A5 and disease-causing mutations

SLC13A5 is a plasma membrane Na⁺-coupled transporter for citrate that is expressed in the liver, testis, and brain [12-14]. It has been cloned from mouse, rat, and human tissues or cells and functionally characterized [15-17]. There are significant functional differences, especially in substrate affinity and the modulatory effect of lithium, between rodent Slc13a5 and human SLC13A5 [18,19]. In the rat brain, the transporter is expressed mostly in neurons [20]. Astrocytes secrete citrate into extracellular medium and extracellular concentrations of citrate in the brain and cerebrospinal fluid could reach as high as 0.5 mM [21,22]. SLC13A5 in neurons mediates the uptake of citrate from the extracellular medium for subsequent

metabolism. In the mitochondria, citrate enters the citric-acid cycle to produce ATP; in the cytoplasm, citrate is used in fatty acid and cholesterol synthesis, glutamate and GABA synthesis, acetylcholine synthesis, and post-translational modification of non-histone proteins [12]. Interestingly, deletion of the transporter in mice did not result in any obvious neurological pathology or phenotype; in fact, the Slc13a5-null mice showed a metabolic phenotype of mice with caloric restriction [23]. The knockout mice were resistant to diet-induced obesity and metabolic syndrome. These beneficial effects arising from the deletion of the transporter have been replicated using different approaches [24,25]. The caloric-restriction phenotype of the knockout mice seems to agree with the original description of this transporter in *Drosophila* where heterozygous disruption of the transporter was found to enhance lifespan, thus justifying the naming of the transporter "INDY" (I'm Not Dead Yet) [26,27]. These mice do, however, show evidence of defective enamel and bone development, thus attesting to the potential function of this transporter in teeth and bone [28]. The metabolic phenotype seen in Slc13a5-null mice seems to stem primarily from the biological functions of the transporter in the liver and has no relevance to the role of the transporter in the brain. In stark contrast to the apparent lack of neurological dysfunction in Slc13a5-null mice, loss-of-function mutations in SLC13A5 have been found to cause severe epilepsy early in life in humans [29-34]. The presence of neonatal clonic seizures and convulsive seizures later in life aids in distinguishing SLC13A5-associated epileptic patients from more common genetic neonatal epileptic encephalopathies. Accordingly, the disease caused by mutations in SLC13A5 is classified as one of the several forms of Early Infantile Epileptic Encephalopathy (EIEE). There are at least 50 different types of EIEE and the SLC13A5-associated EIEE is identified as EIEE25 (Gene/Locus Mendelian Inheritance in Man # 608305; Phenotype Mendelian Inheritance in Man # 615905). Kohlschutter-Tonz syndrome is another disease with infantile epilepsy and it is associated with mutations in ROGDI, a gene coding for a protein of unknown function [35]; it has recently shown that mutations in SLC13A5 are found in patients with this syndrome who are negative for mutations in ROGDI [36].

Clinical and metabolic presentation of patients with loss-of-function mutations in SLC13A5

In addition to epilepsy, these patients exhibit various symptoms of defective neurological development, including motor-development delays, high fever sensitivity, variable cognitive delays, limited

language skills, and seizures (Table 1). It has to be noted, however, that the affected children are born with the birth weight and Apgar scores in the normal range. Despite these normal parameters, the onset of epilepsy is within 24 hours of birth in most cases, and is refractory to most of the commonly used antiepileptic drugs, including ketogenic diet. Many of the affected children have short stature, exhibit developmental delays, fail to thrive, and suffer from mild to severe intellectual disability. Brain MRI detects subtle abnormalities such as punctate white matter lesions, blurring of gray-white matter junctions, and gliotic scarring. EEG is abnormal. ¹H-MRS shows reduced levels of N-Acetyl Aspartate (NAA) and decreased NAA/choline and NAA/creatinine ratios. A most recent report documents significant metabolic changes in these affected children [34]. Citrate levels in the plasma and Cerebro Spinal Fluid (CSF) are elevated, even though urinary excretion of citrate remains mostly unaltered. CSF contains elevated levels of 2-methylcitrate and isocitrate, but decreased levels of β -hydroxybutyrate. Interestingly and inexplicably, the urinary excretion of the steroid derivative 16 α -hydroxy-DHEA-3-sulfate is markedly elevated.

Possible molecular mechanisms underlying epileptogenesis associated with SLC13A5 mutations

The exact molecular mechanisms by which the functional deficiency of SLC13A5 causes epilepsy and impairs neuronal development and function remain to be elucidated. However, three major hypotheses have been proposed to explain the neurological

deficits: the cytoplasmic citrate deficit hypothesis, the interneuron energy hypothesis, and the zinc chelation hypothesis [12]. Of important note here is the lack of any overt epileptic phenotype in Slc13a5-null mice; the absence of suitable animal models is a major roadblock for a better understanding of the molecular pathways that contribute to epilepsy and encephalopathy associated with SLC13A5 mutations. This also raises the question as to why mice with the deletion of the transporter do not exhibit epileptic and encephalopathic phenotype. There are two potential explanations for this discrepancy. First, the marked functional differences between mouse Slc13a5 (a high-affinity, low-capacity transporter) and human SLC13A5 (a low-affinity, high-capacity transporter) could underlie the phenotypical variations between Slc13a5-null mouse and children with loss-of-function mutations in SLC13A5. Second, even though all of the mutations found in SLC13A5 lead to inactivation of the transporter function, the possibility that these mutations impart changes in the protein with a gain of hitherto unknown function cannot be ruled out. However, the findings that several different mutations spanning different regions in SLC13A5 (Y82C, T142M, G219R, Y227M, S427L, L488P, L492P, D524H) have been identified in patients with EIEE25, all with an almost identical clinical phenotype, favor the first explanation. Some of these mutations lead to truncation of the protein due to premature stop codon (R333X, T341X); it seems therefore very unlikely that all these diverse mutations would lead to a gain of some unknown, but identical, function in the protein.

Tooth hypoplasia/hypodontia and defective enamel as a clinical phenotype in differential diagnosis of epilepsy arising from SLC13A5 mutations

The clinical features in children with SLC13A5-associated EIEE include not only early onset epilepsy and delayed neurological development but also significant defects in tooth development such as hypodontia, teeth hypoplasia, widely spaced teeth, gingival hyperplasia, and amelogenesis imperfecta (i.e., lack of mature enamel) [29-31]. If relevant, note here is the presence of mutations in this transporter in some patients with Kohlschütter-Tonz syndrome, which is also characterized by epilepsy, encephalopathy, and amelogenesis imperfecta [36]. The same is true with Slc13a5-null mice which show a multitude of features related to defective tooth development, including absence of mature enamel, aberrant enamel matrix, and fragile teeth with predisposition to tooth abscesses [28]. Interestingly, these mice also show decreased bone mineral density and impaired bone formation [28], and decreased growth and shorter body length, suggestive of defective skeletal development [23]. Very little is known on possible bone defects in children affected with SLC13A5-associated EIEE. Citrate is an important component for the structure and formation of bone and teeth; ~70% of citrate in the body is present in bone and teeth, the concentration of citrate in these tissues being at least two orders of magnitude higher than in plasma [37,38]. Citrate is essential for formation and stabilization of apatite crystals in bone [39,40]. Interestingly, despite the fact that bone and teeth contain enormous amounts of citrate, how these tissues accumulate this important metabolite remains mostly unexplored. There are conflicting reports in published literature with regard to the source of citrate in bone. Franklin et al [41]. recently reported a detailed study on the source of citrate in the bone-forming cells (osteoblasts), which concluded that osteoblasts generate citrate intracellularly via truncated citric acid cycle; the plasma membrane citrate transporter SLC13A5 is not expressed in these cells and therefore osteoblasts do not take up citrate from plasma. In contrast,

Table 1: Clinical presentation of patients with SLC13A5 mutations.

General	
Birth weight	Normal range
Apgar score	Good
Development	Short stature Developmental delay; Failure to thrive Mild to severe intellectual disability
Epilepsy	
Onset	Most cases within 24 hours after birth
Response to drugs	Refractory to most antiepileptic drugs Most cases non-responsive to ketogenic diet
Neuro-imaging	
MRI	Punctate white matter lesions Punctate white matter lesions
¹ H-MRS	Reduced NAA/choline ratio Reduced NAA/creatinine ratio Absence of elevated lactate peak
EEG	Abnormal
Metabolic profile	
Citrate	Increased levels in plasma and CSF Normal levels in urine
2-Methylcitrate	Increased levels in CSF
Isocitrate	Increased levels in CSF
β -Hydroxybutyrate	Decreased levels in CSF
16 α -Hydroxy-DHEA-3-sulfate	Elevated levels in urine
Dental phenotype	
Development	Hypoplasia/hypodontia
Enamel	Defective

MRI, magnetic resonance imaging; ¹H-MRS, proton magnetic resonance spectroscopy; EEG, electroencephalogram; NAA, N-acetyl aspartate; CSF, cerebrospinal fluid; DHEA, dehydroepiandrosterone.



two different groups of investigators have reported transcriptome profile of bone tissue, one during mineral matrix formation [42] and the other in a mouse line with defective bone mineralization [43]; both reports have provided evidence for the expression of Slc13a5 mRNA in bone. In the report by Mantila Roosa et al. [42], several transporters were found to be upregulated in bone during matrix formation, and this includes the plasma membrane citrate transporter Slc13a5 and also the mitochondrial citrate transporter Slc25a1. In fact, Slc13a5 is upregulated at a much greater level than Slc25a1. In the report by Liu et al [43], microarray analysis of the bone tissue from wild type mice and from X-linked hypophosphatemic rickets mice, which is caused by inactivating mutations in Phex (phosphate-regulating gene with homologies to endopeptidases on the X chromosome), revealed marked down regulation of Slc13a5 in the mutant mouse, associated with defective mineralization of bone. Similarly, Pemberton et al [44] have conducted microarray gene expression analysis to identify the genes that were related to tooth development; this was done by comparing the gene expression patterns in developing molar tooth in mice between postnatal day 1 and postnatal day 10. This study revealed that Slc13a5 is upregulated during tooth development. These findings documenting up regulation of Slc13a5 in bone during matrix formation and in tooth during development associated with enamel formation, and the down regulation of Slc13a5 in bone with defective mineralization strongly suggest an essential role for the transporter in bone and tooth mineralization. This agrees with the various defects in tooth development and in bone mineral density observed in Slc13a5-null mice [28]. The expression and function of Slc13a5 in bone are further supported by another report in which the transporter was upregulated during differentiation of osteoblastic cell line into osteocytes in response to vitamin D [45]. In the teeth, enamel is formed by ameloblasts; consistent with the defective formation of mature enamel in Slc13a5-null mice [28], the expression of Slc13a5 is increased during pre-ameloblast differentiation into enamel-forming secretory ameloblasts [46].

CONCLUSION

SLC13A5 is a plasma membrane transporter for citrate that mediates the Na⁺-coupled active entry of citrate from plasma into cells. This process must play an important role in neuronal function as the transporter is expressed in neurons and loss-of-function mutations in the transporter cause early onset epilepsy and defective neuronal development (EIEE25). The affected children also exhibit defective tooth development noticeable as hypodontia, decreased enamel, and widely spaced teeth, and also might have decreased bone mineral density. The association of SLC13A5 mutations with compromised mineralization in bone and teeth strongly indicate an essential role for the entry of extracellular citrate via SLC13A5 into the appropriate cell types in these tissues for their optimal development. This notion is supported by the strong evidence in the literature for the expression of Slc13a5 in cell types involved in the mineralization of bone and enamel. As early-onset epilepsy with encephalopathy occurs as a result of mutations in several genes, it is difficult in the clinics to identify the affected children in whom the disease is caused specifically by loss-of-function mutations in SLC13A5. As the defective tooth development, which is easily observable in the form of hypodontia and decreased enamel, is a unique feature in patients with SLC13A5 mutations, this phenotype could serve as a simple but highly useful biomarker for the differential diagnosis of EIEE25. In most cases, epilepsy in patients with EIEE25 is refractory to treatment with traditional anti-epileptics. Therefore, an effective means to differentiate EIEE25

patients from others with early-onset epilepsy and encephalopathy using the easily noticeable clinical phenotype of hypodontia and defective enamel would be of great practical value in the clinics. It is likely that, similar to the findings in Slc13a5-null mice, patients with EIEE25 also suffer from decreased bone mineralization and bone density, but detection of such changes in bone morphometrics might not be as easy as the detection of the defective tooth development phenotype. The potential use of tooth hypoplasia in the differential diagnosis of EIEE25 patients is amenable for testing and validation, which would require determination of the concordance rate for tooth hypoplasia and mutations in SLC13A5 in a cohort of patients with EIEE. As specific treatments become available for patients with EIEE25 in the future, an easy and reliable differential diagnosis method would facilitate the choice of appropriate treatment options for these patients.

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